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Effect of drying temperature on the functional properties of biodegradable CMC-based film for potential food packaging

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Abstract

The purpose of this study was to investigate the functional properties (thickness; water vapor permeability (WVP); film microstructure, tensile strength (TS) and biodegradability) of carboxymethyl cellulose (CMC)/gelatin (gel)/chitosan (chi) biocomposite film as influenced by different drying temperature (25 and 60 °C). Seven formulations (CMC/gel/chi) prepared were control (100/0/0), formulations A (80/20/0), B (80/0/20), C (80/10/10), D (60/20/20), E (60/30/10), and F (60/10/30). Different drying temperature resulted in different time taken for the film to dry. Results revealed that formulation F was optimal due to its high tensile strength and low WVP rate which support its biodegradability for both drying conditions. FTIR assay revealed a strong carboxyl group for CMC, which contributed to high biodegradability results (85.3 vs. 85.50%) for room vs. oven dried specimens, respectively. Such desirable characteristics demonstrated that film F holds remarkable potential as edible films material with enhanced positive impacts on the environment and community.

Introduction

The purpose of food packaging is to preserve product quality while minimizing product–packaging interactions. Recently, a wide variety of packaging materials have been employed to interact with food that offer the desired effects (Silveira *et al*., 2007). Current studies have also revealed that plastics is widely used as food packaging materials due to its low cost and availability (Prashanth and Tharanathan, 2007). However, increasing usage of plastic as packaging materials lead to serious environmental problem due to its total non-degradable properties (Aider, 2010). Growing environmental awareness demands that packaging materials becoming more environmental friendly. Consequently, biodegradability has become a functional requirement for the 'now environmentally conscious' industry (Siracusa *et al*., 2008). The review of the literature also confirmed the growing interest in the development of food packaging films from environmentally friendly polymer blends (Dutta *et al*., 2009).

Carboxymethyl cellulose (CMC) is the most common polysaccharide used for film blending primarily because it has high viscosity and is non-toxic as well as non-allergenic. The numerous hydroxyl and carboxylic groups in CMC contribute to high water binding and moisture sorption properties (Siracusa, 2012). The addition of CMC to cassava starch films increased tensile strength and reduced elongation at the break of the blended film (Tongdeesoontorn *et al*., 2011). CMC is an alternative polymer used for blending with gelatin due to its excellent viscosity, high shear stability, biocompatibility and availability (Wiwatwongwana and Pattana, 2010). It is also filler used in biocomposite film production to increase mechanical and barrier properties because of its polymeric structure and high molecular weight (Almasi *et al*., 2010).

However, edible films made from CMC have weaknesses such as lower tensile strength. Hence, CMC enhancement with characteristics found in hydrocolloids is called for. Gelatin is one of the most popular biopolymers extracted from animal collagen and the addition of gelatin for film blending entails some modification of its functional properties (Bergo and Sobral, 2007). Gelatin and collagen is the major protein component of animal skin, bones and connective tissue, including fish and poultry (Cheng *et al*., 2003). It is a tasteless and colorless solid derived from fibrous protein via acid, alkaline or enzymatic hydrolysis. Gelatin is widely used for medicine, cosmetics, food industries, packaging and many other applications. Extensive studies exist on

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gelatin's capacity to form films used as outer wraps to protect food against light and oxygen (Liu *et al*., 2012).

Blending gelatin with other proteins or polysaccharides such as alginate, chitosan and hyaluronic acid improves the biodegradable properties of such films (Wiwatwongwana and Pattana, 2010). Chitosan is used in film blending is due to its short-term biodegradability and biocompatibility with human tissue. It also provides antimicrobial and antifungal activities and is non-toxic to humans (Aider, 2010). Chitosan films are flexible, durable, strong, and tough as well as hard to break and are moderately permeable to both water and oxygen. The mechanical properties of chitosan films also compares favorably with commercial polymers of medium strength such as cellulose (Jo *et al*., 2005; Martínez-Camacho *et al*. 2010). In addition, chitosan films are selectively permeable to gasses (CO_2 and O_2).

To date, little data exists regarding films produced by blending CMC with gelatin and chitosan. Therefore, the authors investigated to optimize parameter for CMC-based films blended with gelatin and chitosan for use as potential food packaging. The variables studied were the effect from different drying temperatures (25 vs. 60^0C) on functional properties such as film thickness, water vapor permeability (WVP), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), tensile strength (TS) and biodegradability.

Material and Methods

Materials

Commercial carboxymethyl cellulose (CMC), in addition to bovine gelatin (type B, bloom value = ~225 g), chitosan (partial deacetylated, medium molecular weight) and glycerol (molecular weight = 92.09), were supplied by Sigma Aldrich Company Ltd., Poole, Dorset, BH12 4QH, UK.

Production of carboxymethyl cellulose (CMC)-based films

Film solutions were constantly stirred until completely solubilized and then cast onto petri dishes and dried at room (25 ºC) and oven (Thermo Scientific Haraeus 6000 series, Fisher Scientific Inc, USA) (60 ºC) temperatures. Film samples were then tested for functional properties such as film thickness, water vapor permeability (WVP), tensile strength (TS) and biodegradability. Further examinations by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (X-RD) followed. Seven film formulations prepared were control (100/0/0); A (80/20/0); B (80/0/20); C (80/10/10); D (60/20/20); E (60/30/10) and F (60/10/30). The amount of plasticizer was fixed at 30% glycerol for each formulation.

Functional properties of carboxymethyl cellulose (CMC)-based films

Determination of film thickness

Film thickness was measured with a precision digital micrometer (Digimatic Indicator, Mitutoyo Corporation, Japan) to the nearest 0.0001 (±5%) at five random locations on the film: four at edge regions and one within the central area. Means from five separate measurements were recorded.

Determination of water vapor permeability (WVP)

Water vapor permeability (WVP) was measured by using the modified ASTM method described by Gontard *et al.* (1992). The films were sealed onto a cup containing silica gel at 0 percent relative humidity with silicone vacuum grease and a rubber band to hold the films in place. The cups were then measured for initial weight and placed in desiccators containing distilled water at 30ºC, followed by repeat weighing at 1 h intervals over 8 h period. Measurements were done twice and the WVP was calculated according to the method described by McHugh *et al.* (1993) as follows:

$$\text{WVP (g m}^{-1}\text{s}^{-1}\text{Pa}^{-1}) = \text{wxA}^{-1}\text{t}^{-1}\ (P_2 - P_1)$$

Where:
w is the weight gain of the cup (g);
x is film thickness (m);
A is exposed area of film (m^2);
t is the time of gain (s);
$(P_2 - P_1)^{-1}$ is the difference in vapor pressure across the film (Pa).

Determination of fourier transform infrared spectroscopy (FTIR)

Infrared spectra were recorded using a Thermo Nicolet 380 Spectrometer (Fisher Scientific Inc, USA) with a deuterated triglycerine sulphate (DTGS) detector. FTIR spectrum was used to measure the functional group that arose from the blending of film materials. The sample holder comprised a Multi-ounce horizontal attenuated total reflectance (HATR) plate of zinc selenite (ZnSe) crystal. The plate was cleansed with acetone and a background spectrum (without sample) was collected. Film samples were then affixed to the plate and spectra recorded. Resolutions varied between 4000 to 650 cm^{-1} over 32 scans. A single beam spectrum for each sample was ratioed against an ambient air single beam background spectrum before conversion to absorbance units. Each analysis was repeated three times.

Determination of X-ray diffraction (XRD)

A Rigaku X-Ray Diffractometer (Rigaku Corp, USA) took x-ray diffraction (XRD) measurements with a copper source using 40 kV and 30 mA current at room temperature as described by Bergo and Sobral (2007). XRD was used to measure the crystalline and amorphous characteristic of the film. Each sample was cut into 4 x 4 cm^2 and mounted onto a glass slide before placement in the diffractometer chamber for measurement. Each assay was repeated twice with an angle diffraction range of $2\Theta = 3 – 80^o$. Each scan took about 30 minutes.

Determination of tensile strength (TS)

Tensile strength (TS) was determined by using a texture analyzer (TA.TX Plus, Stable Micro System, Surrey, UK) following the ASTM method 882-97 (ASTM 1997). Film strips of 10 x 100 nm were prepared using a cutting blade and placed in the AT/G probe's grip attached to the texture analyzer with a 5 kg loaded cell. The initial gap between upper and lower parts of the grip was set at 20 mm. Film strips were stretched by moving the headspace at 50 mm/min until rupture. Each measurement was repeated three times.

Determination of film biodegradability

Biodegradability was analyzed by burying an equal mass of each dried film sample in soil according to the method described by González *et al*. (2011), with some modification. Films from each formulation were cut into rectangular pieces (3 x 3 cm^2), buried in 100 g of soil and then placed in an area with enough sunlight to ensure aerobic conditions for degradation. Measurements were repeated twice and changes in film weight were recorded daily for five days. The percentage of weight loss was calculated according to the following formula:

$$\text{Weight loss (\%)} = \frac{\text{(Initial weight – final weight)}}{\text{(Initial weight)}} \times 100$$

Statistical analysis

Minitab 14.0 software was used for all statistical analyses. Significant differences were calculated with the two-way ANOVA and means were compared via Tukey's method (confidence level $p < 0.05$).

Results and Discussion

Film formation

The films control (100/0/0), A (80/20/0), B (80/0/20), C (80/10/10), D (60/20/20), E (60/30/10), F (60/10/30) (CMC/gelatin/chitosan) took 20, 25, 23, 29, 40, 42, and 37 days to completely dry at room temperature, respectively (Table 1). The control (100% CMC) dried quickest (20 days); formulation B (80% CMC/20% chitosan) dried by day 23. The measurement of the completeness of the drying process has been determined as it is easily peeled off from the petri dish without any left residues (Tongdeesoontorn *et al*., 2011). As the chitosan component increased to 30% (formulation F), drying time increased to 37 days. Formulation C (10% gelatin) took 29 days to dry. As the gelatin component increased to 20% (formulation A), drying time decreased to 25 days. When both gelatin and chitosan components were at 20% (formulation E), drying time increased to 40 days. Drying time continued to increase to 42 days as gelatin incorporation increased to 30%.

These results indicated that films with a higher CMC component dried faster (from 20–29 days), which correspond to the study made by Tongdeesoontorn *et al.* (2011) that showed a drying rate between 21 and 30 days for CMC based films. Drying time continued to increase as the percentage of CMC decreased with increasing percentages of gelatin and chitosan in the mix. However, films with a higher proportion of gelatin dried slower compared to those with higher proportions of CMC and chitosan. The slow drying rate was likely due to gelatin's high water content Gomez-Guillen *et al.* (2011). Formulation E (60/30/10) had the slowest drying rate (42 days), likely due to its having the highest percentage of gelatin. According to Bergo and Sobral (2007), among polymers, protein has the lowest solid content as well as high water content, which likely accounts for the prolonged drying time of film E.

The oven-dried films (60ºC) took only 2 days to dry completely. This may be due to the increased temperature as well as controlled relative humidity and air circulation in a vacuum oven (Mayachiew and Devahastin, 2008). Kristo *et al.* (2008) opined that high temperature speeds molecular vibration; hence also, the evaporation of any free water in solution, which then serves to explain the more rapid drying process. High temperature increased molecular vibrations between CMC, gelatin and chitosan molecules, which allowed the oven-dried films to desiccated more rapidly compared to films dried at room temperature.

Functional properties

Film thickness

The determination of a film's thickness is important when estimating the barrier properties of a packaging system (Thomazine *et al*., 2005). The observed thicknesses of the CMC-based blended films obtained was ranged from 0.66–0.75 mm. The control film (100% CMC) had the greatest thickness value (0.75 mm) for room and oven-dried processing. Formulation A (80% CMC/20% gelatin) had a thickness of 0.70 mm for both conditions. Thickness readings for formulation B (80% CMC/20% chitosan) increased to 0.73 and 0.71 mm for room vs. oven conditions, respectively. Thicknesses for formulation C (gelatin and chitosan content 10% each) decreased from 0.72 to 0.66 mm for room vs. oven-dried conditions, respectively. Readings for formulation D (CMC 60%) also decreased from 0.70 to 0.68 mm for room and oven dried conditions, respectively. Formulation E (gelatin and chitosan 30% and 10%, respectively), had the lowest thickness values of 0.67 and 0.69 mm for room and oven-dried conditions, respectively. Thickness values for formulation F (chitosan component 30%) were 0.71 and 0.73 mm for room and oven dried conditions, respectively.

These results showed no significant differences ($p > 0.05$) between drying conditions on film thickness results. The data analysis showed that the variation between film thicknesses did not affected by the different in drying conditions. Therefore, it can be concluded that the variation in the reading of film thicknesses was merely affected by the ratio of the materials used in film blending. It is proven that film thickness increased as the percentage of CMC and chitosan components increased, perhaps due to the higher solid contents of CMC and chitosan compared to gelatin. These findings agreed with those of Tharanathan (2003) who concluded that CMC's high solid content contributed to the structure of its thick film. This also explains the highest thickness value obtained for the control formulation (100/0/0). Film thickness also decreased as the percentage of gelatin increased, likely due to gelatin's higher water content. Bergo and Sobral (2007) found that pigskin gelatin possessed high water content, which contributed to a film's thin structure and high water permeability. Yakimets *et al*. (2005) found that gelatin possesses high intensity peaks at 3650 cm^{-1} (OH^{-}), which further indicates that higher water content likely contributes to thin film structure.

Water vapor permeability (WVP)

Water vapor permeability (WVP) values observed for carboxymethyl cellulose (CMC)-based films blended with gelatin and chitosan are shown in Table 1. The determination of a polymer film's barrier properties is crucial when estimating the shelf life of a product-package (Siracusa, 2012). Low WVP refers to the low amount of water vapor passing through the film is, therefore it could reduce the potential food spoilage, whereas high WVP refers to the high amount of water vapor passing through the film, in which it will promote food spoilage easily due to high moisture in the film. Good film packaging is expected to have a low water vapor permeability. Water is the main cause of food spoilage, therefore, film with low WVP can reduce the possible food deterioration by acting as a water barrier to the food (Tharanathan, 2010). Results obtained showed that control had 0.27 and 0.19 mm/Hg WVP readings for room vs. oven conditions, respectively. Formulation A (80% CMC/20% gelatin) had 0.21 and 0.18 mm/Hg WVP for room vs. oven conditions, respectively. Formulation B (80% CMC/20% chitosan) had the lowest WVPs of 0.05 vs. 0.07 mm/Hg for room vs. oven conditions, respectively. WVP showed increments as the gelatin component increased and the chitosan component decreased. The incorporation of 10% gelatin and 10% chitosan in formulation C increased WVP from 0.05 to 0.16 and from 0.07 to 0.23 mm/Hg for room vs. oven-dried conditions, respectively. Formulation D's (20% gelatin) WVP values increased from 0.16 to 0.18 mm/Hg and from 0.23 to 0.25 mm/Hg for room and oven dried conditions, respectively. Formulation E (30% gelatin) had the highest WVPs for both room and oven dried conditions, compared to others, of 0.31 and 0.34 mm/Hg, respectively. However, film F showed lower rate of WVP (0.09 and 0.11 mm/Hg) for room vs. oven dried conditions, respectively. Formulation F had the highest content of chitosan (30%) with 10% gelatin. The addition of chitosan decreased the free volume of the polymeric matrix in gelatin crosslinks, therefore, the diffusion rate of water molecules through the film will be decreased, thus lowering the film WVP (Cao *et al*., 2007).

Results showed that there was no significant differences ($p > 0.05$) on the film's WVP as affected by different drying conditions. The data analysis showed that the variation between film WVP did not affected by the different in drying conditions. It can be concluded that the variation in the reading of film's WVP was contributed by the ratio of the materials used in film blending. This is because, the WVP result patterns showed that the WVP increases as the percentage of gelatin increases, and decreases as the percentage of chitosan rises for both room temperature and oven dried conditions, respectively. According to Bergo and Sobral (2007), gelatin possesses higher water content than either CMC or chitosan. Gelatin also has good gas permeability. However, its hydrophilicity constitutes a major drawback as the crosslinking of gelatin membranes reduces the desirable barrier property of moisture resistance (Martucci and Ruseckaite, 2009). Sousa *et al.* (2009) posited that compounds with high hydrogen bonding capabilities generate gelatin (bovine) films that are more susceptible to water vapor, while compounds with hydrophobic groups constitute excellent moisture barriers. This explains the high WVP in films with a higher proportion of gelatin compared to other formulations. Moreover, although chitosan also has good film forming ability, its cationic characteristic contributes to high water permeability and limits its application (Durango *et al.*, 2006; Vásconez *et al.*, 2009). Vargas *et al.* (2009) showed that blending chitosan with other polymers or substances, like oleic acid, could improve its WVP. This further explains that the blending of gelatin, chitosan and CMC improves the film water vapor permeability. Hence, it can be concluded that the higher solid content of CMC and chitosan contributed to low water permeability. This finding also correlated to the study of Ghanbarzadeh *et al.* (2010) which described polysaccharides, including CMC and chitosan, as possessing high solid content contributing to the crystalline nature of a compact structure that decreases the permeability of blended films.

Fourier transform infrared spectroscopy (FTIR)

Figures 1 and 2 show the FTIR spectra range (4000–650 cm^{-1}) for specimens of CMC-based films blended with gelatin and chitosan and dried under room and oven conditions. Infrared spectroscopy identified film structures produced by molecular interactions between CMC, gelatin and chitosan. FTIR results revealed similar functional groups for each formulation under both drying conditions without the formation of new peaks. However, peak intensity differed for each formulation due to different component percentages (CMC, gelatin and chitosan). Peak intensity also differed for room and oven dried conditions, reflecting the effect of temperature.

Each formulation presented similarly structured functional groups for all assayed films. Six characteristic transmission bands were held in common. These included the hydrogen bonding stretching region (3315 cm^{-1}) (Zaïdi *et al.*, 2011); C-H stretching associated with methane ring hydrogen atoms (2920 cm^{-1}) (Su *et al.*, 2010); COO^{-1} assigned to carboxyl group stretching (1590 cm^{-1}) (Su *et al.*, 2010); hydroxyl stretching in plane (1410 cm^{-1}) (Chai and Isa 2013); C-H stretching in symmetric of CMC (1320 cm^{-1}) (Chai and Isa, 2013); and C-O stretching on the polysaccharide skeleton (1110 cm^{-1} and 1040 cm^{-1}) (Taleb *et al.*, 2012).

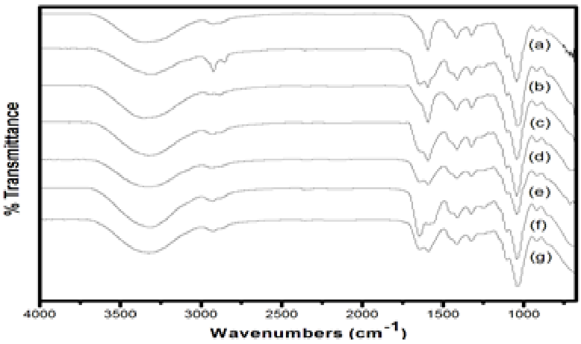

Figure 1. FTIR spectrum of cmc-blended films, oven dried at (60ºC)
Where (a) control, (b) cmc–A, (c) cmc–B, (d) cmc–C, (e) cmc–D, (d) cmc–E, (g) cmc–F.
Where a–g is formulation control (100% CMC),–A (80/20/0), –B (80/0/20), –C (80/10/10), –D (60/20/20), –E (60/30/10), –F (60/10/30).

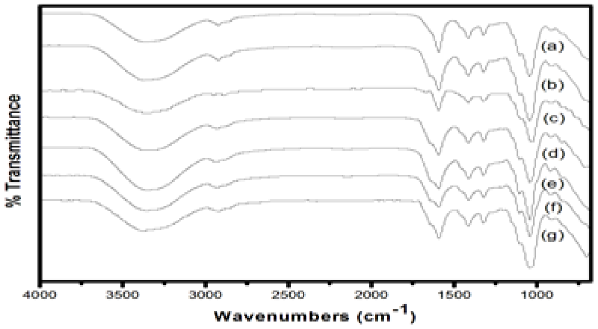

Figure 2. FTIR spectrum of cmc-blended films dried at room temperature (25ºC)
Where (a) control, (b) cmc–A, (c) cmc–B, (d) cmc–C, (e) cmc–D, (d) cmc–E, (g) cmc–F.
Where a–g is formulation control (100% CMC), –A (80/20/0), –B (80/0/20), –C (80/10/10), –D (60/20/20), –E (60/30/10), –F (60/10/30).

With the addition of gelatin and chitosan, the peak intensity for H^{+} at 3315 cm^{-1} increased gradually as the percentage of gelatin and chitosan increased for both room and oven dried samples. The H^{+} band's intensity also increased for both room and oven dried samples as the percentage of CMC decreased, which implies that the blending of CMC with gelatin and chitosan increases the deprotonation of CMC-based films. Increased deprotonation further explains increased WVP as the percentage of incorporated CMC rises. This trend corresponds with a study made by Chai and Isa (2013) whose work also indicated that deprotonation increased as the CMC component increased, which also explains why the highest WVP

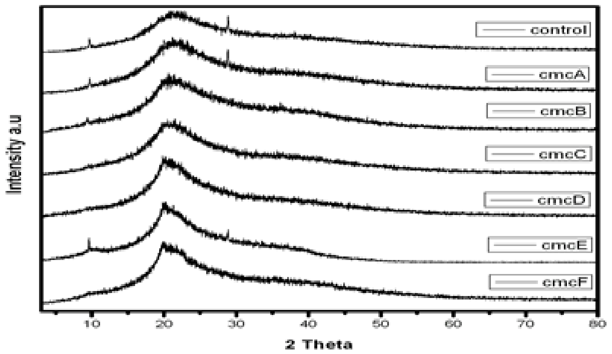

Figure 3. XRD spectrum of cmc-blended films, oven dried at (60ºC)

Where (a) control, (b) cmc–A, (c) cmc–B, (d) cmc–C, (e) cmc–D, (d) cmc–E, (g) cmc–F.
Where a–g is formulation control (100% CMC), –A (80/20/0), –B (80/0/20), –C (80/10/10), –D (60/20/20), –E (60/30/10), –F (60/10/30).

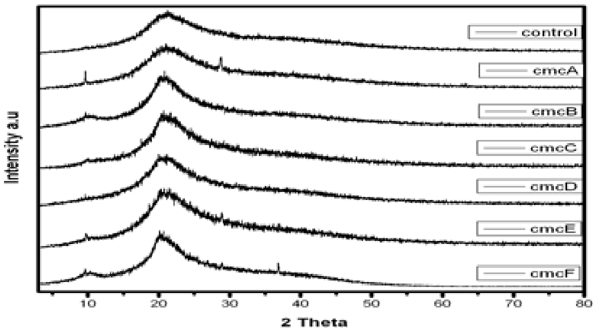

Figure 4. XRD spectrum of cmc-blended films dried at room temperature (25°C)

Where (a) control, (b) cmc–A, (c) cmc–B, (d) cmc–C, (e) cmc–D, (d) cmc–E, (g) cmc–F.
Where a–g is formulation control (100% CMC), –A (80/20/0), –B (80/0/20), –C (80/10/10), –D (60/20/20), –E (60/30/10), –F (60/10/30).

was found in formulation E (highest component at 30%). Peak intensity at 1590 cm^{-1} (COO^{-}) shifted to 1600 cm^{-1} for film A (20% gelatin), and to 1650 cm^{-1} for film E (30% gelatin/10% chitosan). These shifts reflected interactions between the CMC carboxyl group and amino acids of both chitosan and gelatin showing differences for each formulation, respectively. These results agree with Bourtoom and Chinnan (2008) who showed that interactions between CMC, gelatin and chitosan demonstrated peaks from 1590 cm^{-1} to 1650 cm^{-1}. Such interactions likely explain both the high water vapor permeability and the more rapid biodegradation for formulations A and E.

X-ray diffraction (XRD)

X-ray diffraction is used to observe the microstructure of the packaging film materials in terms of crytallinity and amorphousness properties of film produced. X-ray diffraction patterns (from 2ϴ = 3–80º) for carboxymethyl cellulose (CMC)-based films blended with gelatin and chitosan and dried under room vs. oven conditions are shown in Figures 3 and 4, respectively. XRD patterns revealed equivalent peaks for each formulation under both drying conditions. However, new peaks with rising gelatin and chitosan component ratios were observed. Nevertheless, the intensity of these peaks differed according to the amounts of CMC, gelatin and chitosan incorporated. Different drying temperatures had no effects on XRD patterns and revealed the same patterns and peaks under either condition.

XRD analysis revealed complex formations in polymer matrices with the addition of gelatin and chitosan, which indicated that the films held amorphous characteristics, except for formulation F. The differences between formulations A through E and formulation F was found in the crystalline structure of film F, which also had the highest chitosan component (30%). These results corresponded with findings obtained by Bergo and Sobral (2007), who showed that the incorporation of chitosan in gelatin film exhibited two crystal peaks at 11.5º and 18º, respectively. The present study found that chitosan powder in a crystalline state as the two main diffraction peaks (2ϴ = 11° and 20°) observed in X-ray diffraction patterns. These findings agreed with Nunthanid *et al.* (2001) and Xu *et al.* (2005), where both groups found two crystalline peaks (2ϴ = 11° and 20°) as chitosan was incorporated in gelatin films. The decreasing crystalline structure observed in composite films has been mainly attributed to broken hydrogen bonds in chitosan molecules (Liu *et al.*, 2012), which implies that hydrogen bonding between gelatin and chitosan lends itself to their greater compatibility (Aider 2010).

In contrast, formulations A through E possessed strong bands at 2ϴ = 20°, indicating the amorphous characteristic of these films. Broad patterns demonstrate an amorphous structure with molecular miscibility and interactions between components. Amorphous films exhibit lower water vapor permeability with excellent mechanical attributes, and Zhong and Xia (2008) also demonstrated improved gas barrier properties. The control formulation (100% CMC) exhibited the lowest intensity x-ray pattern at 21° under either drying condition. However, peak intensity increased as gelatin and chitosan components also increased. Both figures demonstrate that peaks (2ϴ = 20°) from formulation A (20% gelatin) were the most intense compared to the control formulation (Figures 3 and 4). These peaks continued to increase their intensity as the amount of incorporated gelatin

Table 1. Drying period, tensile strength, water vapor permeability and film weight loss of CMC-based film blended with gelatin and chitosan at different drying temperature

Film	Drying Period (days)		Tensile Strength (N/mm)		Water Vapor Permeability (mm/Hg)		Film Weight Loss (%)		Film Thickness (mm)	
	RT (25⁰C)	OD (60⁰C)	RT (25⁰C)	OD (60⁰C)	RT (25⁰C)	OD (60⁰C)	RT (25⁰C)	OD (60⁰C)	RT (25⁰C)	OD (60⁰C)
Control	20	2	10.0 (.54)^b	22.5 (.11)^b	0.27 (.01)^a	0.19 (.03)^{ab}	48.81 (.07)^b	55.29 (.93)^b	0.75 (.11)^a	0.75 (.27)^a
A	25	2	65.5 (.41)^a	80.0 (.71)^a	0.21 (.02)^a	0.18 (.01)^{ab}	71.18 (.29)^{ab}	75.39 (.52)^a	0.70 (.22)^a	0.70 (.06)^a
B	23	2	38.5 (.56)^{ab}	42.5 (.41)^a	0.05 (.01)^b	0.07 (.02)^b	40.09 (.58)^b	57.20 (.92)^{ab}	0.73 (.19)^a	0.71 (.32)^a
C	29	2	16.0 (.41)^b	9.0 (.44)^b	0.16 (.05)^{ab}	0.23 (.03)^a	54.99 (.10)^{ab}	65.38 (.06)^{ab}	0.72 (.54)^a	0.70 (.48)^a
D	40	2	9.0 (.77)^b	10.0 (.82)^b	0.18 (.03)^{ab}	0.25 (.03)^a	51.74 (.44)^b	56.28 (.51)^b	0.69 (.43)^a	0.68 (.27)^b
E	42	2	54.5 (.82)^a	65.5 (.00)^a	0.31 (.12)^a	0.34 (.10)^a	81.26 (.79)^a	78.95 (.08)^a	0.67 (.22)^b	0.69 (.12)^b
F	37	2	40.3 (.95)^{ab}	39.7 (.70)^a	0.09 (.03)^b	0.11 (.05)^b	85.35 (.74)^a	85.50 (.27)^a	0.71 (.56)^a	0.73 (.64)^a

Values as means ± SD for three determinations.
(a–b) indicate means with significant difference (p < 0.05) between the column.
Ratio of composite blended film (gelatin/CMC/chitosan) where control (100/0/0); formulations: –A (80/20/0),–B (80/0/20), –C (80/10/10), –D (60/20/20), –E (60/30/10), –F (60/10/30).

Abbreviations:
RT= room temperature
OD=oven dry

increased to 30% in formulation E. However, peak intensity (2Θ = 20°) clearly increased as chitosan was incorporated as seen for films B and F, which contained 20% and 30% chitosan, respectively. This may have been due to the presence of H^+ in the samples, which also accommodated observation on water vapor permeability values. With higher H^+ presence, the films became more permeable to water, thus, increasing water vapor permeability as also observed by Bigi *et al.* (2004) and Bigi *et al.* (2001). Peak intensity for each formulation showed the same x-ray patterns under both drying conditions as well. These findings lead to conclusion that drying conditions had little to no effect on XRD patterns.

Tensile strength

Table 1 shows the tensile value results for specimens of CMC-based films blended with gelatin and chitosan and dried under room and oven conditions. Tensile value estimates both strength and stretchability, which are two of the most important criteria qualifying food-packaging materials (Arvanitoyannis *et al*. 1998). The control formulation (100% CMC) yielded 10.0 vs. 22.5 N/mm^2 (room vs. oven) tensile values, respectively. Tensile values increased remarkably to 65.5 vs. 80.0 N/mm^2 (room vs. oven) for film A (80% CMC/20% gelatin). Film B yielded tensile values of 38.5 vs. 42.5 N/mm^2 (room vs. oven), representing a decrement after the addition of 20% chitosan. Tensile values for film C continued this decrease at 16.0 vs. 9.0 N/mm^2 (room vs. oven) as the percentage of gelatin and chitosan decreased to 10% each. Film D had the lowest tensile values of 9.0 vs. 10.0 N/mm^2 (room vs. oven) as the percentage of gelatin and chitosan increased to 20% each. However, the tensile values for film E increased to 54.5 vs. 65.5 N/mm^2 (room vs. oven), reflecting effects from the increased gelatin (30%) component. Tensile values for film F decreased to 40.3 vs. 39.7 N/mm^2 (room vs. oven) with its components of 30% chitosan and 10% gelatin.

The present results indicated that there was no significant different (p>0.05) on the film tensile strengths as affected by different drying conditions. From the data analysis obtained, the difference reading between film tensile strength did not affected by the different in drying conditions. Therefore, it can be concluded that the variation in the reading of film tensile strength was affected by the ratio of the materials used in film blending. Table 1 shows that tensile strengths increased as the gelatin component of the blend increased, and decreased with increasing amounts of CMC and chitosan. These results possibly derive from gelatin's high water content and the high solid content of CMC and chitosan, respectively. These findings also agreed with Bergo and Sobral (2007), who suggested that the high water content of gelatin films contributed to tear strength and stretchability. Khan *et al*. (2012) showed that tensile strength decreased in blended films as the chitosan component increased. Blending CMC films with gelatin appears to increase the film's stretchability due to the lower solid content compared to CMC and chitosan film (Sobral *et al*., 2001). In addition, increased percentages of CMC and chitosan resulted in lower tensile strength due to their combined high solid content that contributed to a more compact, crystalline and brittle film structure (Tongdeesoontorn *et al*., 2011).

Biodegradable test

Table 1 shows biodegradability results for all CMC based films. For a period of one week observation for any variation in weight loss, which provides direct evidence of film biodegradation (Tongdeesoontorn *et al*., 2011). The degradation rate was showed increased by 50% within three days under room (25ºC) and oven dried (60ºC) conditions for all formulations. The rate of biodegradation increased with the addition of both chitosan and gelatin. The control (100% CMC) sample presented the lowest degradation rate for both drying conditions; room (48.81%) vs. oven-dried (55.29%). The lower degradation rate was possibly due to CMC's compact structure and high solid content. This finding agreed with Campos, Gerschenson, and Flores, (2010) who showed that CMC's high solid content contributed to its stronger, compact structure. Formulation F (30% chitosan) had the highest degradation rate for both room vs. oven temperatures at 85.35% and 85.50%, respectively.

The addition of chitosan gradually increased the rate of degradation. Comparing formulation C (10%) vs. B (20%), the degradation rate increased from 50 to 80%, respectively, possibly because of chitosan's crystalline nature. This finding corresponded with a study by Nunthanid *et al.* (2001) which also suggested that the compact, brittle structure of chitosan enhanced the film's biodegradability. Additional gelatin also increased the biodegradation rate from 55 to 70 and 80% for formulations C, A and E (10, 20 and 30% gelatin, respectively) (Table 1). These results were likely due to gelatin's hydrophilic nature and water content, which facilitated greater moisture loss. Sobral *et al.* (2001) observed that films with higher proportions of gelatin and chitosan degraded faster due to the hydrophilic structure and heterogeneous mixture of water-soluble proteins as confirmed by the present work.

Conclusion

In conclusion, the different in drying condition did not significantly affect the functional properties of carboxymethyl cellulose (CMC) based films blended with gelatin and chitosan. However, interactions between CMC, gelatin and chitosan are such that gelatin and chitosan incorporation greatly influenced the functional properties of CMC-based blended films. The addition of gelatin appears to improve the tensile strength of CMC-based blended films by increasing their tensile value while enhancing biodegradability as well. Formula F's CMC-gelatin-chitosan ratio of 60:10:30 proved optimal and promises high potential for food packaging because of maximized biodegradability, high tensile value and low water vapor permeability.

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