

Effect of sorbitol at different concentrations on the functional properties of gelatin/carboxymethyl cellulose (CMC)/chitosan composite films

¹Bakry, N.F., ²Isa, M.I.N. and ^{1*}Sarboon, N.M.

¹School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

²School of Fundamental Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

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Abstract

This study investigated consequent functional effects (mechanical and physical) on Gelatin/CMC/Chitosan composite films from the addition of sorbitol. With glycerol as a plasticizer, solutions for Gelatin/CMC/Chitosan composite films containing graduated sorbitol concentrations (0%, 5%, 10%, 15%, 20%, 25% and 30%), were cast on a petri dish and oven dried at 45°C. The fabricated films were then characterized for tensile strength, elongation at break (EAB) and puncture resistance (mechanical properties); as well as film thickness, water vapor permeability (WVP), thermal properties, light transmittance and transparency (UV and visible light transmission), biodegradability, and X-ray diffraction (physical properties). Results indicated that by increasing sorbitol concentration, melting point and tensile strength decreased overall ($p < 0.05$). However, films with 25% sorbitol content increased tensile strength due to antiplasticization. EAB increases with sorbitol concentration for 5% and 10% however shows no trends for addition of 15%-20% due to phase segregation as plasticizer exceeds compatibility limit of film. Water vapor permeability, light transmittance, transparency and amorphous consistency all increased as sorbitol concentrations increased ($p < 0.05$). Sorbitol also decreased glass transition (T_g) (range: 42.6–43.2°C). Using the soil burial method, there were no significant difference ($p > 0.05$) in weight loss (%) as sorbitol concentrations increased. The addition of 10% sorbitol to the Gelatin/CMC/Chitosan composite films under study demonstrated satisfactory mechanical properties and low water vapor penetration. Film C (10% sorbitol) demonstrated sufficient mechanical properties with 1.51 MPa for tensile strength, 5.05% for EAB, 6.36N for puncture force in terms of flexibility and low WVP at 3.83 g.mm/h².Pa compared to all other formulations. CE/g DW) and capacities ($2.00 \pm 0.01 \mu\text{mol TEAC/g DW}$ and $0.84 \pm 0.01 \mu\text{mol TEAC/g DW}$) amongst four species of seaweed.

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Introduction

Edible film is defined as a thin, continuous layer of comestible coating/wrapping material that enhances the shelf life of food and improves the quality of most processed food as a mechanical barrier that also inhibits mass transfer (Gennadios, 2002). Generally, edible films and coating materials derive from various renewable sources such as carbohydrates, proteins and lipid. Edible films made from proteins are superior and preferred because of their enhanced mechanical and barrier properties (Jongjareonrak, 2006). Various proteins have been used to produce edible films, including gelatin, corn and zein (Park *et al.*, 1994); whey (McHugh and Krochta, 1994) and soy protein (Ghorpade *et al.*, 1995).

Gelatin stems from the thermal denaturation of collagen and has a characteristically high content

of glycine, proline and hydroxyproline. Gelatin is profitably used as a gelling agent for food dispersion systems, drug encapsulation and sundry pharmaceuticals, but mainly for biodegradable film packaging (Rivero *et al.*, 2010). Numerous studies on gelatin based films concern derivatives of pig skin (Sobral *et al.*, 2001); fish skin (Jongjareonrak *et al.*, 2006); and bovine skin (Gómez-Estaca *et al.*, 2009). However, stand-alone gelatin films are brittle and susceptible to cracking due to the polymer's resilient cohesive energy density (Jongjareonrak, 2006; Rivero *et al.*, 2010). In addition, these films are thermally unstable and provide an inadequate barrier to water due to their hydrophilic nature, and have disappointing mechanical properties (Mu *et al.*, 2012). Hence, crosslinking agents and plasticizers have been added for the improvement of these less than passable functional properties.

*Corresponding author.

Email: norizah@umt.edu.my

Tel: + 609 668 4968; Fax: + 609 668 4949

Crosslinking as a viable method to improve the mechanical and barrier strength of protein films incorporates adjuncts in film forming solution that increase tensile strength while reducing solubility in water and as well as oxygen permeability (Wihodo and Moraru, 2013). To date, several attempts to enhance the less than desirable properties of protein films include the addition of crosslinking agents such as formaldehyde, dehydroalaline, chitosan (Pereda *et al.*, 2011), and dialdehyde polysaccharide carboxymethyl cellulose (CMC) (Mu *et al.*, 2012).

CMC, an anionic linear polysaccharide derived from cellulose, is used as a viscosity modifier (thickener) for the stabilization of various food product emulsions. It absorbs water and moisture formation and the hydrogel has a wide range of applications because of its excellent properties such as high water content and superior biodegradability (Tongdeesoontorn *et al.*, 2009). Blending gelatin with CMC improved polymer viscosity, high shear stability, biocompatibility and mechanical properties in term of modulus elasticity (Biswal and Singh, 2004; Wang *et al.*, 2007; Wiwatwongwana and Pattana, 2010).

Chitosan derives from chitin by deacetylation in the presence of alkali, and is commonly described in terms of deacetylation and average molecular weight (Muzarelli, 1996). Film composites comprising gelatin, chitosan and plasticizing agents have demonstrated improved strength and water vapor resistance (Rivero *et al.*, 2009). Pereda *et al.* (2011) reported that the bi-layer of a gelatin/chitosan film increased elongation at break (EAB) by 40% compared to pure chitosan film.

Plasticizers are commonly added to protein derived polymer films to reduce protein-protein chain interaction and stabilize the film's network (Jongjareonrak, 2006). Although plasticizers improve flexibility, they also increase a film's gas and water vapor permeability. Sobral *et al.* (2001) observed that sorbitol had considerable influence on puncture force and deformation in both bovine hide and pig skin gelatin films, and also increased water vapor permeability with increasing concentrations. This due to sorbitol are hexabasic alcohol and have isometric compound thus it is compatible to water soluble polymer and protein (Sobral *et al.*, 2001; Barreto *et al.*, 2003; Jongjareonrak, 2006).

Given the absence in the study on the effect of various concentrations of different types of hydrophilic plasticizers on mechanical properties and physical properties of Gelatin/CMC/Chitosan edible film. This study could highlight the most fitting formulation of composite Gelatin/CMC/Chitosan

Table 1. Polymer film formulations

Film blends	Composition (g/100 ml water)				Sorbitol (g)
	Gelatin	CMC	Chitosan	Glycerol	
A	2.4	0.8	0.8	1.2	0
B	2.4	0.8	0.8	1.2	0.2
C	2.4	0.8	0.8	1.2	0.4
D	2.4	0.8	0.8	1.2	0.6
E	2.4	0.8	0.8	1.2	0.8
F	2.4	0.8	0.8	1.2	1.0
G	2.4	0.8	0.8	0	1.2

Film Formulations: (A) Control, 0% sorbitol; (B) 05% sorbitol; (C) 10% sorbitol; (D) 15% sorbitol; (E) 20% sorbitol; (F) 25% sorbitol; (G) 30% sorbitol. Films A-F: 30% glycerol was added except for Film G.

edible film with the preferable types of hydrophilic plasticizers in term of improved mechanical and physical properties.

Thus, this paper relates our attempts to improve the functional properties of Gelatin/CMC/Chitosan composite films with glycerol as plasticizer, by adding different concentrations of sorbitol to the film's initial formulation (Suderman *et al.*, 2016).

Material and Methods

Materials

Bovine gelatin, type B, Carboxymethyl cellulose-sodium salt (CMC), and chitosan were obtained from sigma-Aldrich. Glycerol and D (-); and Sorbitol (plasticizers) were obtained from Amresco and R and M Chemicals, respectively.

Methods

Film fabrication

Film fabrication was followed Suderman *et al.* (2016). The amount of sorbitol (%) has been decided based on the study by Thomazine *et al.* (2005) and Sobral *et al.* (2001) with slight modifications on the ratio of plasticizer:material used. The amount of glycerol used in the film formulation is as suggested by Suderman *et al.* (2016) with the similar film composition CMC/gelatin/chitosan composite film. Table 1 lists the different formulations.

Seven samples were prepared:

- (A) Control, comprising Gelatin/CMC/Chitosan plasticized with 30 %glycerol without sorbitol);
- (B) Gelatin/CMC/Chitosan plasticized with 30% glycerol plus 5% sorbitol;
- (C) Gelatin/CMC/Chitosan plasticized with 30% glycerol plus 10% sorbitol;
- (D) Gelatin/CMC/Chitosan plasticized with 30% glycerol plus 15% of sorbitol;

(E) Gelatin/CMC/Chitosan plasticized with 30% glycerol plus 20% sorbitol;

(F) Gelatin/CMC/Chitosan plasticized with 30% glycerol plus 25% sorbitol;

(G) Gelatin/CMC/Chitosan with 30% sorbitol without glycerol.

Gelatin powder (2.4 gram, w/w) was mixed with 20 mL distilled water and hydrated at room temperature for 20 min. Each Gelatin/CMC/Chitosan solution was prepared separately. The gelatin underwent mechanical stirring at 50°C for 20 min until completely dissolved after which the plasticizer (glycerol/sorbitol) was added and mechanically stirred for another 20 min at 50°C (Cao *et al.*, 2009). CMC (0.8 g) in 30 mL of distilled water was heated and stirred for 30 min at 50°C (Su *et al.*, 2010). The chitosan was then mixed with the Gelatin/CMC/Plasticizer solution and stirred for 30 min at 50°C. This film forming solution was then cast on a flat petri dish (8.5 cm dia) and oven dried at 45°C (Laboratory Dryer FSD-380, Protech®, Malaysia).

Mechanical properties

Tensile strength and Elongation at break (EAB)

Determination of tensile strength was measured according to the method(s) suggested by Nur Hazirah *et al.* (2016) also Calvalho and Grosso (2004) with modifications. Four readings each for three separate samples of each rectangular strip (1cm × 6 cm) per film formulation were averaged. Films were placed on the texture analyzer's pair of grips (5 kg load), and stretched by 15 mm.

The elongation at break (EAB) (%) were determined via ASTM (1997) method (D882-97) with some modifications. Four readings from three separate rectangular strips (1cm × 6 cm) per film formulation were averaged. Films were placed on the texture analyzer's pair of grips (5 kg load) and stretched by 15 mm (Texture analyzer- Stable Microsystem, TA XT Plus, US) (Calvalho and Grosso, 2004; Nur Hazirah *et al.*, 2016).

EAB is calculated as follows:

$$\text{Elongation at break (EAB) (\%)} = \frac{\text{Elongation at rupture} \times 100}{\text{Initial grip length}}$$

Determination of Puncture Force

Puncture force was measured according to the method suggested by Nur Hazirah *et al.* (2016) and Gontard *et al.* (1993), with slight modification. Affixing each film to a 52.6 mm diameter cell, a 3 mm diameter probe moving at 1mm/s determined the puncture force via a texture analyzer (Texture analyzer- Stable Microsystem, TA XT Plus, US), and

texture expert software, V.1.15 (Surrey).

Film thickness

Film thickness was measured to the nearest 0.1 mm with a digital micrometer (Mitutoyo, Model 406-305, Japan). Five measurements per film were taken at random positions (Yakimets *et al.*, 2005).

Determination of water vapor permeability (WVP)

Water vapor permeability (WVP) were determined by using the desiccant method (ASTM, 1997), with some modifications. Each edible film was stretched over the opening of a container holding silica gel and then placed in a desiccator containing distilled water under a controlled atmosphere. Periodic weights helped determined the rate of water vapor movement through the specimen into the desiccant. Each cell was weighed (± 0.001 g) by an analytical balance at 24 h intervals over 7 d (Al-Hassan and Norziah, 2012). Water vapor permeability was calculated using the following formula:

$$\text{Water vapor permeability (WVP) (g. mm/h}^2\text{.Pa)} = \frac{w \cdot x}{tA \Delta P}$$

Where w is weight gain of film, x is average film thickness; (A) is permeation area ($2.25 \times 10^{-4} \text{m}^2$); DP is the partial vapor pressure of the atmosphere with silica gel and pure water (3.159 KPa, at 25°C); and t is time (24 h x 7 d) (Gontard *et al.*, 1993).

Determination of thermal properties

Thermal properties were measured by differential scanning calorimetry (DSC) (TA Instrument, DSC Q2000, USA). Using an empty pan as reference, 5.0 (± 0.5) mg film samples were weighed in an aluminum pan and hermetically sealed for analysis at a heating rate of 10°C/min from 0 to 90°C (Gómez-Estaca *et al.*, 2009). Using thermogram Q series software, peak melting (T_m , °C) and glass transition temperatures (T_g , °C) were obtained (Rivero *et al.*, 2010). The helix-coil transition temperature (T_m) was also determined for each film's endothermic peak. Glass transition temperature (T_g) was calculated as the inflexion point of the base line shift in discontinuity for a sample's specific heat (Sobral *et al.*, 2001).

Light transmittance and transparency

Barrier properties for light transmission (%) and transparency were measured for ultraviolet (UV) and visible light, respectively, at selected wavelengths between 200 and 800 nm by using a UV-Visible recording spectrophotometer (Model Agilent Cary 50 Probe, US). Transparency was determined by following equation:

$$\text{Transparency} = -\log T_{600}/x$$

Table 2. Mechanical and physical tests of Gelatin/CMC/Chitosan composite film at different formulations

Sample	Mechanical tests					Physical tests		
	Tensile strength (MPa)	Elongation at break (%)	Puncture force (N)	Thickness (mm)	WVP 10^{-9} (g.mm/h ² .Pa)	Weight loss (%)		
						3 day	7 day	11 day
A	1.28±0.06 ^d	14.50±1.13 ^b	7.21± 1.04 ^a	0.31 ± 0.03 ^{ab}	5.31±0.30 ^b	6.03±1.99 ^a	20.23±5.66 ^a	30.17±4.90 ^a
B	1.51±0.02 ^c	5.05±0.36 ^a	6.36± 1.42 ^a	0.30 ± 0.02 ^{ab}	3.83±0.18 ^d	5.83±1.17 ^a	17.43±4.78 ^a	30.35±3.52 ^a
C	1.31±0.02 ^b	11.12±0.47 ^c	5.36± 1.06 ^b	0.29 ± 0.02 ^b	3.95±0.03 ^c	4.16±1.19 ^a	11.70±3.33 ^a	20.92±3.73 ^a
D	0.67±0.08 ^e	9.23±0.29 ^d	5.35± 0.30 ^b	0.30 ± 0.02 ^a	4.02±0.10 ^c	6.20±1.70 ^a	19.95±4.943 ^a	30.49±3.54 ^a
E	0.47±0.01 ^f	20.11±0.18 ^a	6.87± 1.59 ^a	0.35 ± 0.05 ^a	4.68±0.36 ^c	4.84±0.65 ^a	16.84±3.20 ^a	28.26±2.84 ^a
F	0.71±0.02 ^e	10.72±0.63 ^c	7.88± 0.97 ^a	0.32 ± 0.02 ^{ab}	6.64±0.21 ^a	5.32±0.83 ^a	18.10±4.38 ^a	28.39±4.16 ^a
G	3.28±0.01 ^a	9.23±0.10 ^d	7.23± 0.22 ^a	0.28 ± 0.02 ^b	4.42±0.08 ^c	4.83±0.53 ^a	11.24±2.92 ^a	23.17±1.33 ^a

Film Formulations: (A) Control, 0% sorbitol; (B) 05% sorbitol; (C) 10% sorbitol; (D) 15% sorbitol; (E) 20% sorbitol; (F) 25% sorbitol; (G) 30% sorbitol. Data expressed as Means (\pm standard deviation). Letters (a - f) indicate significance differences in results ($p < 0.05$) between formulations A–G (post-hoc Tukey HSD)

Where T600 is transmittance at 600nm and x is film thickness (Jongjareonrak, 2006).

Film biodegradability

The measurement of film biodegradability followed the method of Kaur and Gautam (2010), with slight modification. Film samples of 1.5 cm \times 1.5 cm were desiccated until weight became constant and then completely covered with soil. Specimen degradation rate was determined over 11 days by weighing each sample at 3 days intervals. All soil residues were then removed, after which each film was weighed again. Weight loss was calculated as follows:

$$\text{Weight loss (\%)} \text{ (after every 3 days):} \\ [(W_1 - W_2) / W_1] \times 100$$

$$\text{Weight loss (\%)} \text{ (after 11 days):} \\ [(W_1 - W_3) / W_1] \times 100$$

Where,

W₁: Initial weight at the beginning

W₂: Final weight after 3 days

W₃: Final weight after 11 days

X-ray diffraction (X-RD)

Film structure was determined by X-ray diffractometer as described by Rhim *et al.* (2009), with some modification. Each sample was mounted on a 2 \times 2 inch glass slide and placed on the x-ray platform using double-sided tape. Kv (voltage) and mA (current) levels were slowly increased to 40kV and 30mA within a radiation range of $2\theta = 5\text{--}80^\circ$. Each scan required about 30 min.

Statistical analysis

Minitab 14 software was used for statistical analysis with one-way analysis of variance (ANOVA)

(post-hoc Tukey HSD) to compare average values to a CI of 95%. Each analysis was repeated three times and final data presented as a mean (\pm SD).

Results and Discussion

Mechanical properties for gelatin/CMC/chitosan composite films

Tensile strength and elongation at break (EAB)

Tensile strength (TS) in MPa and percent elongation at break (EAB) results for formulations A-G are shown in Table 2. Tensile strength range from 0.47 to 3.28 MPa. The tensile strength decreases with the combination of both glycerol and increasing sorbitol content in film formulation (B – E). Increased sorbitol concentration across all formulations might have reduced protein-protein interactions, which increased the mobility of polypeptide chains. This findings are aligned with results reported by Thomazine *et al.* (2005) who studied effects from glycerol and sorbitol on gelatin blended films. They found that films plasticized by blends of plasticizers lowered TS compared to films plasticized with a single plasticizer.

Nevertheless, Film-F showed slight increment in TS (0.71 MPa) with increased sorbitol content, accompanied by a significant decrease ($p < 0.05$) in EAB (20.11%). At this state of concentration, Film-F may undergone antiplasticization (Rodríguez-Núñez *et al.*, 2014). Film G (plasticized with sorbitol only), showed the highest TS (3.28 MPa). Film A's TS (plasticized with glycerol only), was lower (1.28 MPa). This was likely due to the larger molecular weight and weaker hygroscopic property of sorbitol compared to glycerol (Sloan and Labuza, 1976).

EAB values ranged from 5.05 to 20.11%. Film-B had the lowest value (5.05%) and film-E

the highest (20.11%). Comparing films plasticized with glycerol (film-A) or sorbitol (film-G), film-A's EAB (14.50%) was significantly higher than film-G's (9.23 %) ($p < 0.05$). Glycerol's smaller molecular weight allowed increased molecular attraction while at the same time it inhibited interactions between biopolymer chains; thus, increasing plasticization (Sonthornvit and Krochta, 2001; Thomazine *et al.*, 2005). These results suggested that increasing sorbitol concentration in Gelatin/CMC/Chitosan composite films with glycerol as plasticizer improves the flexibility of the film by decreases the tensile strength by lowering the polypeptide chains of the film while enhancing EAB for films-B and C.

No trend shown for EAB for films-D, E and F which may had undergone phase segregation as the percentage of sorbitol exceeds 15% (Film-D) – 25% (Film-E) in the film results in the plasticizer concentration exceeds the compatibility limit as suggested by Aulton *et al.* (1981) in the study of the mechanical properties of hydroxypropyl methylcellulose film derived from aqueous systems. However Film-F may undergone antiplasticization as a result from matrix alterations in structure because of carbohydrate chain realignments between CMC and Chitosan (Rodríguez-Núñez *et al.*, 2014).

Puncture Force

Puncture force values ranged from 5.35 N to 7.88 N (Table 2). There were no significant different ($p > 0.05$) between Film-A (7.21 N), Film-B (6.36 N), Film-E (6.87 N), Film-F (7.88 N) and Film-G (7.23 N), while Film-C (5.36 N) and Film-D (5.35 N) are significantly different ($p < 0.05$). Film-A's (glycerol only) higher plasticizing effect was likely due to glycerol's smaller molecular weight and hydrophobicity, which increased relative water and proved a better plasticizer compared to film-G (sorbitol only) (Sornthornvit and Krochta, 2001).

Increased sorbitol concentration appeared to enhance plasticizing effects in the Gelatin/CMC/Chitosan composite films. Puncture force decreased with increased sorbitol concentration at 5% to 15% for films-B, C and D. An increased concentration of sorbitol molecules in the composite film likely enhanced polypeptide chain mobility by weakening the intermolecular forces between the protein-protein interactions (Sobral *et al.*, 2001). Therefore in this study, addition of sorbitol between 5% to 15% in the film contributed to the reduction of forces between adjacent molecules in the gelatin/CMC/chitosan which, in turn, enhanced plasticization while reducing puncture force. Puncture force increased for Films-E and F, but did not reflect a plasticizing

effect with increased sorbitol concentrations of 20% (6.87 N) and 25% (7.88 N), respectively. This was likely due to antiplasticization resulting from matrix alterations in structure because of carbohydrate chain realignments that follow the disruption of carbohydrate-carbohydrate hydrogen bonds between CMC and Chitosan (Rodríguez-Núñez *et al.*, 2014). The findings of the antiplasticization effects just cited also correlated with for TS and EAB results for film-F (25% sorbitol). Thus, the addition of sorbitol concentration contributes to the flexible characteristic as it enhances the plasticizing effect of the Gelatin/CMC/Chitosan composite film by lowering the puncture force.

The trend observed in this study reflected findings by Thomazine *et al.* (2005) on puncture force for gelatin films plasticized with blends of glycerol and sorbitol. They reported that increased sorbitol content and decreased glycerol concentration increased puncture force. This study observed that increasing sorbitol concentration from 5 to 15% allowed sufficient plasticizing, whereas 20 to 25% sorbitol increased the film's resistance.

Physical properties of gelatin/CMC/chitosan composite films

Thickness

Composite film thickness (A–G) ranged from 0.28 to 0.35 mm, respectively (Table 2). However, there were no significant different ($p > 0.05$) between all the films as the films thickness were also controlled in order to ensure the same amount of films forming solution were poured in the petri dish during casting method. Thickness increased with increased sorbitol content, which can be explained in terms of the solid content of the film forming solution. As sorbitol concentrations increased, solid content also increased which may eventually affects the thickness of the film (Han and Krochta, 1999). The characterization of sorbitol may also had contributed by the thickness of the film. Sorbitol that has more hydroxyl group than glycerol lead to higher degree of protein-protein hydrogen bonding thus it can penetrate polymer network quicker thus produce stronger and thicker film (McHugh and Krochta, 1994; Ghasemlou *et al.*, 2011). It can be concluded that the higher concentration of sorbitol across the film lead to higher affinity of the protein network to bind through hydroxyl group, resulting to more compact film with thicker and stronger film properties. The gelatin/CMC/chitosan composite film showed less thicker film compared than the reported result by Suderman *et al.* (2016) at the range of 0.66 mm to 0.75 mm.

However, the difference in the result may due film preparation and the ratio of the materials used.

Water vapor permeability

Water vapor permeability (WVP) also increased as sorbitol concentration increased (Table 2) with no significant differences between film-C, D, E, and G ($p > 0.05$). Film-F (25% sorbitol only) had the highest WVP, followed by film-A; film-C; film-D; film-G; film-E; and film-B. Increased sorbitol content appears to allow a more efficient reorganization of protein networks causing a reduction of intermolecular interaction also increase the mobility of macromolecules. Consequently, the restructuring increases free volume due to a less dense matrix; thus, making the film more permeable to water (Gontard *et al.*, 1993; Cuq *et al.*, 1997; Al-Hassan and Norziah, 2012). This study's results also concurred with previous WVP observations of gelatin films that had been plasticized with glycerol and sorbitol (Thomazine *et al.*, 2005). They reported that films with 55 g of sorbitol per 100 g of gelatin had higher WVP (15.90 g.mm/m² kPa); while those with 25 g of sorbitol per 100 g of gelatin had lower WVP (12.92g.mm/m² kPa).

Film-A, plasticized with glycerol only, had higher WVP (5.31x10⁻⁹g.mm/h².Pa) than film-G (4.42x10⁻⁹g.mm/h².Pa,) ($p < 0.05$), which was plasticized with sorbitol only. The greater plasticizing effect from glycerol is likely due to its lower molecular weight and higher hygroscopic property (Cuq *et al.*, 1997). Thomazine *et al.* (2005) studied gelatin film plasticized with sorbitol and glycerol and suggested that a film's humidity increased with increased glycerol content. The present work found that films plasticized with sorbitol to 20% (films B–E), demonstrated superior WVP compared to formulations plasticized with glycerol only (film-A). Hence, from the WVP analysis the least hygroscopic properties of the sorbitol improves water vapor barrier properties of the Gelatin/CMC/Chitosan composite film compared to the film plasticized by glycerol.

Thermal properties

Figure 1 shows results for all film formulations for water evaporation (zone A); glass transition temperature (zone B); and melting point (zone C). The first peak (29°C) indicates water evaporation for the chitosan-hydrophilic plasticizer (Rodríguez-Núñez *et al.*, 2014), suggesting the initial DSC run endothermic change correlated with the water content of chitosan. The second peak (zone B) reflects the film's glass transition (T_g); films-B and C had the lowest T_g (42.6°C). Other T_g values were as

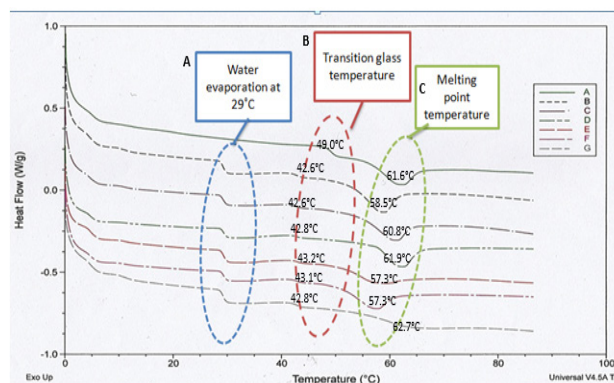


Figure 1. DSC thermograms for formulations A–G. Zone A: water evaporation temperature; Zone B: Glass Transition (T_g) temperature; Zone C: Melting point (T_m) temperature. Film Formulations: (A) Control, 0% sorbitol; (B) 5% sorbitol; (C) 10% sorbitol; (D) 15% sorbitol; (E) 20% sorbitol; (F) 25% sorbitol; (G) 30% sorbitol.

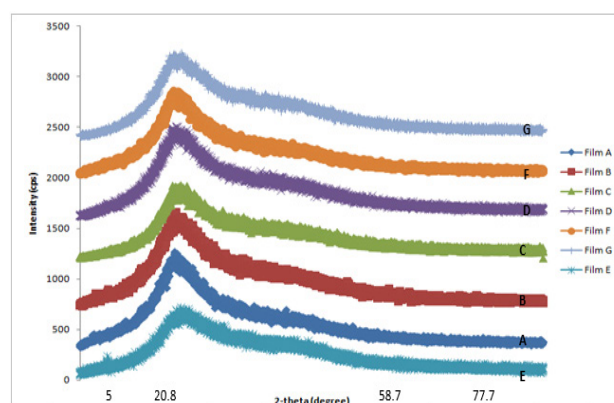


Figure 2. X-ray diffraction patterns (XRD) for formulations A–G. Film Formulations: (A) Control, 0% sorbitol; (B) 5% sorbitol; (C) 10% sorbitol; (D) 15% sorbitol; (E) 20% sorbitol; (F) 25% sorbitol; (G) 30% sorbitol.

follows: films-D and G at 42.8°C; film-F at 43.1°C; film-E at 43.2°C; and film-A at 49.0°C, the highest value, plasticized with glycerol-only compared to film-G (sorbitol-only) at 42.8°C. Film-G's lower T_g was likely due to the higher availability of sorbitol's hydroxyl group (Pouplin *et al.*, 1999), which limits intra- and intermolecular hydrogen bonding between chains, resulting in lower interaction energy between protein-polysaccharides chains. Increased sorbitol content also increased molecular weights causing (i) higher evaporation rates for films-B–G, and (ii) T_g values ranging from 42.6 to 43.2°C. These results were likely caused by limited accessibility to the high junction zone for all film formulations with similar T_g values (Pouplin *et al.*, 1999).

The third peak (zone C) represents melting points for all composite films. Film-G had the highest melting point (T_m) at 62.7°C; followed by film-D (61.9°C), film-A (61.6°C), film-C (60.8°C), film-B (58.5°C), film-E (57.3°C) and film-F (57.1°C). The thermogram (Figure 2) suggested that

Table 3. Gelatin/CMC/chitosan light transmittance (%) and transparency values.

Formulation	Light transmittance (%) at different wavelength			Transparency	
	280	500	800	280	800
A	0.15±0.01 ^a	22.70±0.06 ^a	30.28±0.02 ^a	9.23±0.05 ^a	1.70±0.00 ^f
B	0.034±0.06 ^c	11.80±0.01 ^d	16.18±0.02 ^f	11.47±0.24 ^a	2.64±0.00 ^a
C	0.05±0.00 ^c	10.51±0.09 ^f	17.62±0.05 ^e	11.25±0.16 ^a	2.59±0.01 ^b
D	0.07±0.01 ^b	11.29±0.04 ^e	15.71±0.03 ^g	10.53±0.21 ^c	2.68±0.00 ^a
E	0.06±0.01 ^{bc}	14.93±0.11 ^b	19.06±0.02 ^c	9.36±0.13 ^e	2.07±0.01 ^e
F	0.06±0.00 ^b	13.74±0.01 ^c	18.18±0.03 ^d	9.94±0.12 ^d	2.30±0.00 ^d
G	0.05±0.00 ^c	10.18±0.14 ^g	19.73±0.01 ^b	11.71±0.00 ^b	2.50±0.00 ^c

Film Formulations: (A) Control, 0% sorbitol; (B) 05% sorbitol; (C) 10% sorbitol; (D) 15% sorbitol; (E) 20% sorbitol; (F) 25% sorbitol; (G) 30% sorbitol.

Data expressed as Means (± standard deviation). Letters (a - f) indicate significance differences in results ($p < 0.05$) between formulations A–G (post-hoc Tukey HSD).

increased sorbitol content lowered crystallinity and consequently, T_m also. These findings fit well with the X-ray diffraction analysis, which indicated the amorphous characteristic of Gelatin/CMC/Chitosan composite films.

By comparing both film-A (glycerol only) and film-G (sorbitol only), the T_m for film-G was greater (62.7°C) than T_m of film-A (glycerol only) at 61.6°C. However for the glass transition determination, film-A (glycerol only) showed superior T_g at (49.0°C), compared to film-G's T_g (42.8°C). The peak of Film G's T_m based on the differential scanning calorimetry thermogram (Figure 1) was broad; indicating the blend was not miscible and likely explains the difference when plasticizing with sorbitol. The thermal analysis suggested that the increase in the sorbitol concentration of the Gelatin/CMC/Chitosan composite film lowers the T_m and results in similar T_g throughout the formulations due to higher evaporation rate and molecular weight of film lead to

Light Transmittance and film transparency

Ultra-violet and visible light transmission (%) values for all film formulations at wavelengths 280, 500 and 800 nm are shown in Table 3. Light transmission for all wavelengths increased as sorbitol concentration increased ($p < 0.05$), likely due to the transparent nature of the plasticizer (Paschoalick *et al.*, 2003). Light transmittance (%) ranged from 0.03% to 0.15% for ultra-violet light at 280nm with significant different ($p < 0.05$). 10.18% to 22.70% at visible light 500nm ($p < 0.05$) and 15.71% to 30.28% at 800nm ($p < 0.05$).

Light transparency values ranged from 9.23 to 11.71 for ultraviolet light (280 nm), and from 1.70 to 2.68 for visible light (800 nm). Transparency values decreases for both ultra-violet (UV) and visible light transmission (280 and 800 nm, respectively) with

increasing sorbitol content for films B–E ($p < 0.05$). Rodríguez-Núñez *et al.* (2014) demonstrated similar results (increased film transparency) for chitosan films plasticized by sorbitol and glycerol. This findings suggested that gelatin proteins lowered film transparency and enhanced barrier properties due to the presence of aromatic amino acids that absorb UV light.

Film-F however shows significant ($p < 0.05$) increase in transparency for ultra-violet light (280 nm; 9.94) and visible light (800 nm; 2.30). The increase in the film transparency at sorbitol concentration at 25% in the film may due to the higher molecular weight of sorbitol which exceeds the compatibility limit in the composite film causing phase segregation and physical exclusion of plasticizer and contributes to the opacity of the film as suggested by Aulton *et al.* (1981) and Suppakul *et al.* (2013).

Biodegradation

Table 2 shows carefully measured biodegradation results as weight loss (%) for films A–G, respectively, on days 3, 7 and 11. No significant differences were noted ($p > 0.05$). Film-D had the greatest weight loss (30.49%) by day 11, and film-C showed the least (20.92%). The addition of sorbitol to films-D and C did not enhance biodegradation as both had similar T_g values of approximately 43°C. Biodegradation rates can be explained in terms of a plasticizer's molecular weight where increased plasticizer concentration lowers the rate of hydrolysis and slows biodegradation.

Table 2 shows that on day three and seven, similar weight loss values were recorded for all Films (A -G) ($p > 0.05$). The percentage of weight loss on day 11 however lower (23.17% - 30.35%) than the previous study by Suderman *et al.* (2016) which recorded the percentage weight loss were in the range of 40.09% to 85.50% in 5 days of observation for CMC based

film blended with chitosan. The difference may be due to the incorporation of sorbitol in this study as rate of biodegradability depends on the degree of cross linking. The dependence may be related to the hindering effect of chemical network on enzymatic degradation (Martucci and Ruseckaite, 2009). In this study, the sorbitol concentration added into composite film across formulation film-B-G affects weight loss (%) of film as the higher molecular weight of sorbitol leads to lesser water solubility in the polymer chain of the film compared to the film plasticized with glycerol only (film-A). Thus increasing sorbitol concentration across the composite film may produce lesser extent of microbial attack, proteolytic enzyme reaction and hydrolysis reaction hence reducing the degree of biodegradation of film (González *et al.*, 2011).

X-ray diffraction patterns (X-RD)

Figure 2 shows X-RD position and intensity patterns (diffraction peak near $2\theta = 20.8^\circ$) obtained by X-ray diffraction studies of all composite films. Films A and B had the highest peak, followed by films D; E; F; G and C. As sorbitol content decreased, characteristic peak intensity identified films plasticized by sorbitol and reflected a semi-amorphous state. Regardless of plasticizer concentration, these amorphous characteristics indicated the absence of recrystallization during film fabrication, likely due to higher stability or increasing moisture, both of which limit tendencies towards semi-crystalline formations (Maria *et al.*, 2008). This study's findings concurred with the diffractograms obtained by Bergo and Sobral (2007) of gelatin films containing glycerol (0%, 15%, 30%, and 45%) at $2\theta = 20^\circ$, all of which demonstrated amorphous characteristics.

Film-G (sorbitol only) had lower peak intensity than film-A (glycerol only, Figure 2), suggesting greater amorphous consistency. A similar study by Cervera *et al.* (2004) on chitosan-amylose starch films, plasticized with sorbitol, demonstrated a greater amorphous structure than chitosan films plasticized with glycerol. According to Sothornvit and Krochta (2001) the molecular differences between the two plasticizers; glycerol and sorbitol are responsible for the different sorption rate of film plasticized with them. Sorbitol has more hydroxyl groups (six hydroxyl groups) to interact with water by hydrogen bonding compared to glycerol (three hydroxyl groups). Gelatin/CMC/Chitosan composite films in the present study were less amorphous than the gelatin films plasticized by glycerol and reported by Bergo and Sobral (2007) (peak intensity approximately 500 u.a.). This result was likely due to chitosan's high crystallinity, which contributes to increased peak

intensity.

Conclusion

In conclusion, the increasing sorbitol concentration in the Gelatin/CMC/Chitosan composite film improves the flexibility of the film by decreasing tensile strength and puncture force while increasing EAB, reduces the less amorphous characteristic in the composite film. The addition of sorbitol across the film formulation also influences the film thickness, water vapor permeability, amorphous consistency, light transmittance (%) and film transparency. Film C (10% sorbitol) demonstrated sufficient packaging properties as influenced by its mechanical properties in terms of flexibility of tensile strength, EAB and puncture force, second lowest WVP and second highest value of transparency to UV-light and high transparency against visible light.

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