Mechanical and physico - chemical properties of biodegradable protein-based films: A comparative study

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Abstract

Biodegradable protein-based films were prepared using the commercial gelatin (bovine hide) (CG), giant catfish skin gelatin (GG), soy protein isolates (SPI) in comparison with commercial wrap film (polyvinyl chloride; PVC). The physical, chemical and mechanical properties of the resulting films were determined and compared. The molecular weight distributions of the GG had high quantities of α-chains and β-components. The GG film had higher mechanical properties [tensile strength: TS 26.42 MPa and elongation at break: EAB 128.25 (%)] but lower thickness (0.0454 mm) than those protein-based films. The PVC film had higher mechanical properties [TS: 44.93 MPa and EAB: 253.71 (%)] but lower water vapor permeability [$3.37x10^{-6}$ $g \cdot mm \cdot h^{-1} \cdot cm^{-2} \cdot Pa^{-1}$], film thickness [0.0103 mm] and film insolubility than protein-based films. The film appearance of protein-based films was similar to commercial wrap film with uniform transparency.

Keywords: biodegradable films, gelatin, giant catfish, polyvinyl chloride, soy protein isolate

1. Introduction

In recent year, biodegradable films have attracted much attention in food and drug packaging (Krochta, 1997). This is because biodegradable films could partly substitute for the commercial packaging due to their made up from non-biodegradable petroleum-based polymeric material, which providing the major source of global warming (Kowalczyk and Baraniak, 2011). Biodegradable films can be used for versatile food product to reduce moisture loss, restrict oxygen absorption and lessen lipid migration to enhance food quality (Falguera et al., 2011). To minimize this problem, biodegradable films can be prepared from various natural resources such as polysaccharide, protein or lipid material. Proteins have been extensively used because of their relative abundance, their film-forming ability, provide desirable mechanical, gas barrier and

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transparency properties, as well as high nutritional value (Cuq et al.,1995; Ou et al., 2004). The mechanical properties of protein-based films are better than polysaccharide and lipid-based films because of proteins have a unique structure (based on 20 different monomers) which provide a wider range of functional properties, especially a high intermolecular binding potential which can form bonds at different positions (Bourtoom, 2009; Kokoszka et al., 2010). In recent years, various kinds of proteins have gained the interested researchers to develop edible films. Generally, the proteins material obtain from two resources; animal and plant proteins. Protein materials that derived from animal sources including collagen, gelatin, fish myofibrillar protein and whey protein have been studied. Proteins from several plant sources including soy, corn, and wheat have also been widely investigated. Gelatin is an important protein widely used for producing edible films/packaging. Gelatins are usually obtained by partial degradation or denaturation from skin, bones and hides of bovine or porcine. Potential source of gelatin that has been utilized include the skin of farmed giant catfish (*Pangasianodon gigas*). Giant catfish are important fishery resource in Southeast asia, particularly in Thailand and Lao (Mekong river). The skin of these fish is a good source of gelatin (Jongjareonrak et al., 2010). It has possible to production of edible films and can be used for some food. Films from different protein types exhibited different properties due to the differences in molecular structure and compositions (Artharn et al., 2008). However, few information in terms of comparison study between commercial wrap films and biodegradable protein-based films have been reported. Therefore, the objectives of this study was to produce the films from different type of proteins (giant catfish skin gelatin, commercial bovine gelatin and soy protein isolate), and the physical, chemical and mechanical properties of each films were also characterized and compared with the commercial wrap film (polyvinyl chloride).

2. Materials and Methods

2.1 Materials

Glycerol and other analytical grade reagents were purchased from Merck (Darmstadt, Germany). Electrophoresis reagents were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

Giant catfish skin gelatin (GG) was prepared according to Rawdkuen et al. (2010). The commercial bovine gelatin type B (240 blooms) (CG) was purchased from Gelita NZ Limited (Woolston Christchurch, New Zealand). Soy protein isolate (SPI) was purchased from Food E.Q.

Co. Ltd. Commercial polyvinyl chloride (PVC) wrap film was purchased from Makro hypermarket in Chiang Rai, Thailand.

2.2 Preparation of protein-based films

CG, GG and SPI powder was mixed with distilled water to obtain the film-forming solution (FFS). The protein concentration of the FFS was fixed at 3% (w/v). Glycerol, used as a plasticizer, was added at 25% (w/v). Only the FFS of SPI was adjusted to pH 10.0±0.1 with 1 *N* NaOH. The FFS was incubated at 60°C for 30 min in a water bath with occasional stirring. De-aerated film forming solution (4 g) was cast onto a rimmed silicone resin plate (50x50 mm) and then evaporated at room temperature for 24 h before dried with a ventilated oven environmental chamber (model H110K-30DM; Seiwa Riko Co., Tokyo, Japan) at 25±0.5°C and 50±5% relative humidity (RH) for another 24 h. The obtained dried films were manually peeled.

2.3 Film properties determinations

2.3.1 Physical and mechanical properties

Film thickness

The film thickness was measured with a hand-held micrometer (Bial Pipe Gauge, Peacock Co., Tokyo, Japan). Nine random locations around each of the ten film samples were used for thickness determination.

Mechanical properties

Prior to testing the mechanical properties, the films were conditioned for 48 h at 50±5% RH at 25°C. The tensile strength (TS) and elongation at break (EAB) were determined by using a Universal Testing Machine (Lloyd Instrument, Hampshire, UK). Ten samples (2x5 cm) with an initial grip length of 3 cm were used for testing. The cross-head speed was set at 30 mm/min with 100N load cell use.

Film appearance

The films appearance were determined by using a Sony Cyber-shot DSC-W510 digital camera (Sony Thail Co.,Ltd., Bangkok, Thailand).

Water vapor permeability

The films' water vapor permeability (WVP) was measured by using a modified ASTM method (1989) as described by Shiku et al. (2004). The films were sealed onto a permeation cup containing silica gel (0% RH) with silicone vacuum grease and an O-ring to hold the film in place.

The cups were then placed in a desiccators saturated with water vapor at 25°C. The cups were weighed at 1 h intervals over a period of 8 h, and the films' WVP was calculated as follows (McHugh et al., 1993)

$$WVP = wxA^{-1}t^{-1}(P_2-P_1)^{-1}$$

w is the weight gain of the cup (g); x is the film thickness (m); A is the area of exposed film (m^2); t is the time of gain (s); and $(P_2-P_1)^{-1}$ is the vapor pressure differential across the film (Pa). The WVP was expressed as $g \cdot mm^{-1} \cdot h^{-1} \cdot cm^{-2} \cdot Pa^{-1}$. A total of three samples were determined for each film.

2.3.2 Chemical properties

Film solubility

The film solubility was determined according to the method of Gennadios et al.(1998). The conditioned film samples were weighed and placed in a 50 ml centrifuge tube containing 10 ml of distilled water. The mixture was shaken at a speed of 250 rpm using a shaker (Heidolth Inkubator 10000, Schwabach, Germany) for 24 h. The un-dissolved debris was then removed by centrifugation at 3,000g for 20 min. The pellet was dried at 105°C for 24 h and weighed. The weight of the solubilized dry matter was calculated by subtracting its difference from the initial weight of the dry matter. It was then expressed as a percentage of the total weight.

Electrophoretic analysis

The protein patterns of protein film (SPI and gelatin from bovine hide and giant catfish skin) were analyzed by SDS–PAGE according to the method of Laemmli (1970). To solubilize the films, the samples were mixed with a solubilizing solution (5% SDS). The mixtures were heated at 85ºC for 1 h in a water bath to dissolve the proteins. The solubilized samples were then mixed with a sample buffer (0.5 M Tris–HCl, pH 6.8 containing 4% (w/v) SDS, and 20% (v/v) glycerol) and 10% (v/v) βME at a ratio of 1:1 (v/v). The samples (20 μg proteins) were loaded into the polyacrylamide gel made of 7.5% running gel and of 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protean II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After electrophoresis, the gel was stained with 0.05% (w/v) Coomassie blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid and then destained with 30% (v/v) methanol and 10% (v/v) acetic acid.

Fourier Transform Infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy was determined according to the method of Limpan et al. (2010). Transmission infrared spectra of the films were recorded at room temperature using a Fourier Transform Infrared Raman spectrometer (PerkinElmer, USA). A total of 64 scans were performed at 4 cm^{-1} resolution. The measurements were recorded between 4000-650 cm^{-1}.

2.4 Statistical analyses

Analysis of variance (ANOVA) was performed. The mean comparison was carried out by Duncan's Multiple Range Tests. Significance of difference was defined at $P < 0.05$. The analysis was performed by using an SPSS package (SPSS 16.0 for window, SPSS Inc, Chicago, IL).

3. Results and Discussion

3.1 Physical properties

The thickness of different types of protein-based films from the commercial gelatin (bovine hide) (CG), giant catfish skin gelatin (GG), soy protein isolate (SPI) in comparison with commercial wrap film (polyvinyl chloride; PVC) is shown in Table 1. Significant difference in thickness were observed in all films ($p<0.05$). The average thickness of the protein-based films was in range of 0.0454-0.0494 mm, while the thickness of PVC was 0.0103 mm. SPI films showed the highest in thickness (0.0494 mm) followed by CG, GG and PVC films, respectively. The thickness of protein-based films was similar to those values of SPI (47 μm) (Denavi et al., 2009b), cuttlefish (0.041 mm) skin gelatin film (Hoque et al., 2010) and beef (51.75 μm) skin gelatin film (4% protein) (Nur Hanani et al., 2012). Film thickness generally affects film properties such as mechanical properties, water vapor permeability, light transmission, and film transparency. In addition, the further research is required how to reduce the thickness of the protein-based films to be as thin as commercial wrap film or PVC with still provided the same or comparable properties.

Table 1.Thickness, tensile strength and elongation of different protein-based films in comparison with commercial wrap film (PVC).

Sample	Thickness (mm)	Tensile strength (MPa)	Elongation (%)
CG	0.0476 ± 0.0016^{b}	7.88 ± 0.37^{c}	102.05 ± 4.13^{c}
GG	0.0454 ± 0.0027^{c}	26.42 ± 3.39^{b}	128.25 ± 8.02^{b}
SPI	0.0494 ± 0.0010^{a}	4.50 ± 0.27^{d}	79.32 ± 9.61^{d}
PVC	0.0103 ± 0.0160^{d}	44.93 ± 2.65^{a}	253.71 ± 19.57^{a}

Value (n=10) with different superscripts in each column are significantly difference ($p<0.05$).

CG commercial gelatin (bovine hide), *GG* giant catfish skin gelatin, *SPI* soy protein isolate and *polyvinyl chloride; PVC* commercial wrap film.

3.2 Mechanical properties

The mechanical properties of different type of protein-based films in comparison with PVC were expressed in term of tensile strength (TS) (pulling force per film cross-sectional area required to break the film) and elongation at break (EAB) (degree to which film can stretch before breaking) (Table 1). All films had significantly difference in TS and EAB ($p<0.05$). Among all gelatin and SPI films, the GG film showed the highest TS (26.42 MPa) and EAB (128.25%) ($p<0.05$). The result suggested that the difference in film-forming characteristics of gelatin between two sources as well as plant proteins. From the previous studied of Rawdkuen and others (2010) reported that giant catfish skin gelatin films had higher TS and EAB than bovine bone gelatin films. The authors reported that different protein constituents, as well as other components, in different gelatin sources, might govern the film formation (Rawdkuen et al., 2010). Film formation generally takes place by the development of a three-dimensional network of protein molecule by ionic, hydrophobic and hydrogen bonds (Hamaguchi et al., 2007). The different in mechanical properties of the both gelatin films can be attributed to different in their triple helical contents (Avena-Bustillos and others 2011). Chiou et al., (2009) studied the effects of drying temperature on tensile properties of Alaska pollock and pink salmon gelatin films. They found that gelatin films dried below their gelation temperature contained helical structures, whereas films dried above their gelation temperature were amorphous and they said that cold-cast gelatin films (dried below gelation temperature at 4 °C) having higher TS and EAB values than hot-cast gelatin films (dried above gelation temperature at the higher temperatures) (Chiou and others 2009). When compared with PVC film, it showed greater TS over the CG and SPI films by about 6 and 11-times,

respectively. For EAB, the CG and GG films were still less than PVC by around 50%, while SPI films was less than around 70%. Mechanical properties are largely associated with distribution and density of intermolecular and intramolecular interactions in the network (Guerrero and others 2011). According to Chambi and Grosso (2006), these interactions will be dependent of arrangement and orientation of polymers chains. Both gelatin films showed better mechanical properties than SPI film ($p<0.05$). This fact may be attributed to the protein/protein interaction, which is determined by hydrogen bonds or by electrostatic interaction and/or by hydrophobic nature. These interactions are ultimately influenced by both sequence of amino acid residues and by the three-dimensional size of the entire network (Mariniello et al., 2003). Gelatin has a fibrous tertiary structure then forms a triple helical, cross-linked quaternary structure. Among natural polymers, gelatin is probably most analogous to a synthetic polymer because of its linear structure, fairly limited monomer composition and its polydispersity (Simon-Lukasik and Ludescher 2004). On the other hand, SPI is a complex mixture of protein with widely different molecular properties. Most soy proteins are globulins. Thus, SPI has a less organized matrix.

3.3 Film appearance

The film appearance of CG, GG, and SPI films was similar to PVC with uniform transparency (Figure 1). When the film sheet covered the white background, the color of the background remained clearly observable, but some film showed more turbidity, especially for the GG film. These results were similar to the light transmission and transparency of the films. Based on the film appearance, the applications of edible protein-based films were interesting, especially in food products. When the consumers see the product packed inside the package, it is easy decide for buying that product.

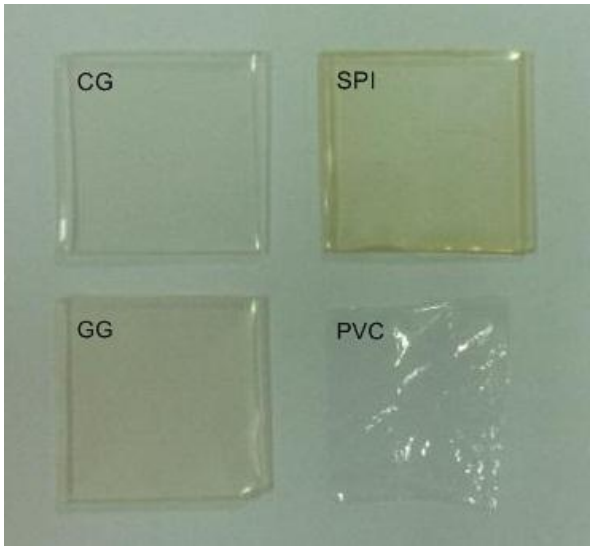

Figure 1. Film appearance of different types of films. *CG* commercial gelatin (bovine hide), *GG* giant catfish skin gelatin, *SPI* soy protein isolate and *polyvinyl chloride; PVC* commercial wrap film.

3.4 Water Vapor Permeability

The WVP of the protein-based films in comparison with PVC is shown in Table 2. The films had significantly different WVP ($p<0.05$). The WVP of protein-based films showed in range of 5.26-10.64 x 10^{-6} g·mm^{-1}·h^{-1}·cm^{-2}·Pa^{-1}, while WVP of PVC was 3.37 x 10^{-6} g·mm^{-1}·h^{-1}·cm^{-2}·Pa^{-1}. In general, protein-based films are poor water vapor barriers due to the inherent high hydrophilicity of the proteins and the substantial amount of hydrophilic plasticizers added to protein-based films. The CG films showed the highest WVP (10.56 x 10^{-6} g·mm^{-1}·h^{-1}·cm^{-2}·Pa^{-1}), while the lowest one was observed in the PVC films. The CG and GG film's WVP were higher than SPI and PVC films, suggesting a decreased value of the film as a barrier to moisture. Denavi et al. (2009b) reported that the WVP of SPI film was lower than gelatin films due to their greater surface hydrophobicity. When compared between CG and GG films found that GG films were low in WVP, this is related to their high hydrophobicity due to they contain lower proline and hydroxyproline compared to mammalian gelatin (Ninan et al., 2010). WVP is dependent on the relative polarity of the polymer used. If the films are cationic and strongly hydrophilic, water interacts with the polymer matrix and the water vapor permeability increase (Rawdkuen et al., 2010). In addition, the further research is required how to reduce the WVP of the protein-based films such as blending with polysaccharide, lipid or cross-linking of films. This have been reported by Chiou et al. (2008) in the case of fish gelatin films from Alaska pollock and Alaska pink salmon skin that showed better barrier properties

when cross-linked with glutaraldehyde (Chiou et al., 2008). Pranoto et at. (2007) reported that the addition of gellan to gelatin films significantly reduced the WVP that nay due to the ionic interaction between gelatin and gellan that formed denser polymeric matrix, thus hindering water molecule transfer through the films (Pranoto et al., 2007).

Table 2. Film solubility and water vapor permeability (WVP) of films.

Sample	Film solubility (%)	WVP (x 10^{-6} g·mm^{-1}·h^{-1}·cm^{-2}·Pa^{-1})
CG	51.83 ± 2.85^{a}	10.56 ± 1.74^{a}
GG	45.06 ± 1.29^{b}	9.81 ± 0.29^{a}
SPI	25.00 ± 0.76^{c}	5.26 ± 0.28^{b}
PVC	ND	3.37 ± 0.27^{c}

Value are given as mean ± SD from triplicate determinations.

Different superscripts in each column are significantly difference ($p<0.05$). ND = not detected.

CG commercial gelatin (bovine hide), *GG* giant catfish skin gelatin, *SPI* soy protein isolate and *polyvinyl chloride; PVC* commercial wrap film.

3.5 Film solubility

The solubility of different type of protein-based films in comparison with PVC is shown in Table 2. The films had significantly different solubility ($p<0.05$). The water solubility of protein-based films was in range of 25-52%. The CG and GG showed higher solubility than SPI, while PVC films showed insoluble in water ($p<0.05$). The water solubility of GG film was similar to those values of fish skin gelatin films (Tilapia fish) (45%) as found in Nur Hanani et al. (2012). However, in another study using beef skin gelatin films, solubility was found to be as low as 34-40% (Gómez-Estaca et al., 2009). In general, the water solubility of gelatin film is very high (>90%) when compared to others (Ahmad and others 2012; Nur Hanani et al., 2012). According to Denavi et al. (2009b), they reviewed about films solubility of fish gelatin films compared to mammalian gelatin films, the fish gelatin are characterized by a lower content of intra- and interchain covalent cross-links, mainly involving lysine and hydroxylysine residues as well as aldehyde derivatives, whose lower degree of linkage may be responsible for such a higher film solubility (Montero and others 2009). Denavi et al. (2009a) have shown that the solubility of films prepared by casting native SPI with glycerol was significantly affected by drying conditions used. They also reported solubilities higher than 80% when films were dried at low temperature and low relative humidities and attributed this finding to the fact that the proteins are not denatured under these conditions

(Denavi et al., 2009a). In the native state, interactions between their chains (including thiol/disulfide-SH/SS-interchange) would not be favored, thus minimizing the subsequent solubility of the films (Denavi et al., 2009a). Water solubility is an important property of films. Potential application may require water insolubility to enhance product integrity and water resistance. However, in other cases, the water solubility of the film before product consumption might be useful for encapsulating food or additives (Bertuzzi et al., 2007) and to reduced the environmental problem from non-biodegradable films.

3.6 FTIR

FTIR spectra of CG, GG, SPI films in comparison with PVC are showed in Figure 2. The spectra of all protein-based films showed a similar pattern. The band situated at 3272-3283, 1627-1632, 1532-1546 and 1235-1276 cm^{-1} corresponding to amide-A and free water, amide-I, amide-II and amide-III, respectively (Hoque et al., 2010; Nur Hanani et al., 2012). Amide-A represents NH-stretching coupled with hydrogen bonding; amide-I represents C=O stretching/hydrogen bonding coupled with COO; amide-II arises from bending vibration of N–H groups and stretching vibrations of C-N groups; amide-III is related to the vibrations in plane of C-N and N-H groups of bound amide or vibrations of CH_2 groups of glycine (Aewsiri et al., 2009; Muyonga et al., 2004). Guerrero and de la Caba (2010) reported the similar result for SPI films where amide-I, amide-II and amide-III showed peaks at the wavenumbers of 1630 cm^{-1}, 1530 cm^{-1} and 1230 cm^{-1}, respectively. The peak situated around 1031-1040 cm^{-1} might be related to the possible interactions arising between plasticizer (OH group of glycerol) and film structure (Bergo and Sobral 2007). Results showed homogenous mixing and interaction between the proteins and plasticizer. From current spectral data, all films obtained for different protein sources, so they showed in different wave numbers of amide-A and amide-I-III. Yakimets et al. (2005) reported that water content affected FTIR spectra in gelatin film without glycerol addition. The film in this present study contained glycerol as the plasticizer. As a sequence, some water might be bond with the film matrix, through it was equilibrated over the dry silica gel for 2 weeks. This water might have the influence on FTIR spectra to some degree.

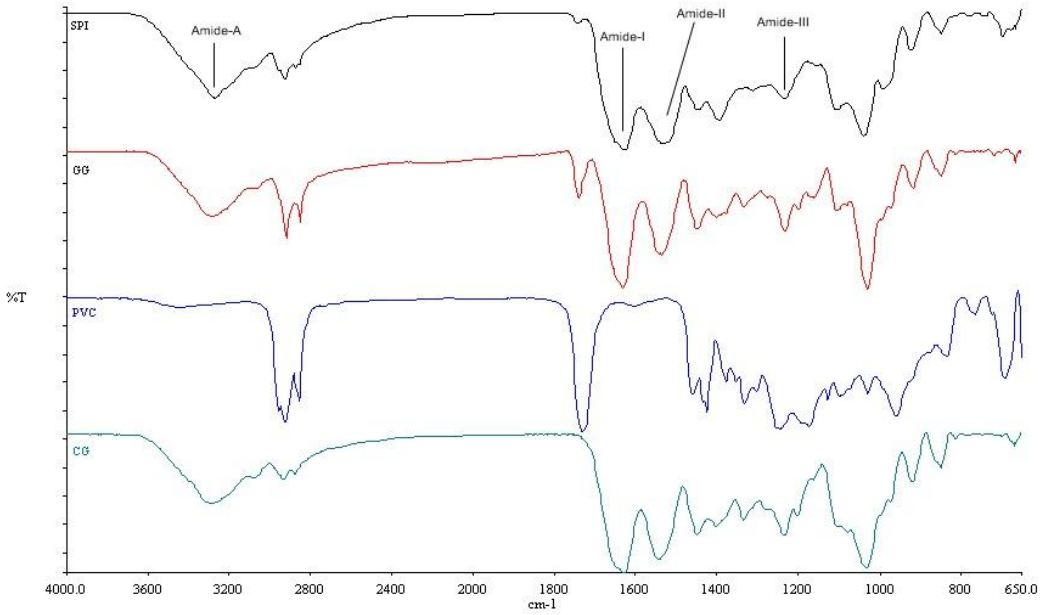

Figure 2. FTIR spectra of films. *CG* commercial gelatin (bovine hide), *GG* giant catfish skin gelatin, *SPI* soy protein isolate and *polyvinyl chloride; PVC* commercial wrap film.

The wave numbers for amide-A for SPI films was lower than that observed from CG and GG film. The amide-I band in range of 1600-1700 cm^{-1} is the most useful peak for infrared analysis of secondary protein structure (Muyouga et al., 2004). For the spectra of PVC, the major absorption band was observed in range of 2850-3000 cm^{-1} (CH stretch) and 850-550 cm^{-1} (CCl stretch). Apart from amino acid composition, distribution and polarity, protein film-forming ability can also be influenced by ionic cross-links between amino and carboxyl groups, hydrogen bonding, intramolecular and intermolecular disulfide bonds and these might be associated with the mechanical properties of the resulting films (Hoque et al., 2010).

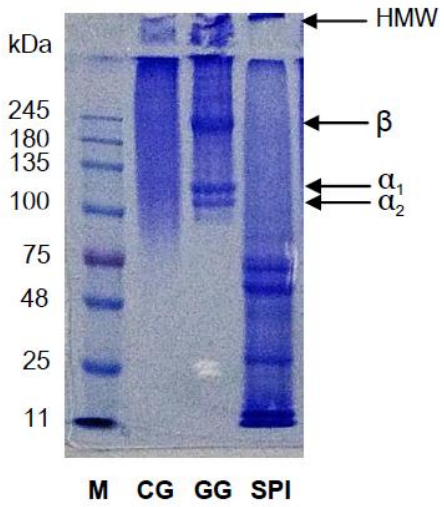

Figure 3. SDS-PAGE patterns of protein-based films. *M protein markers, CG* commercial gelatin (bovine hide), *GG* giant catfish skin gelatin and *SPI* soy protein isolate.

Protein patterns of CG, GG and SPI films are shown in Fig 3. The predominant bands including α-1, α-2 chains and their dimers (β-components) were observed. Marked differences in protein patterns were observed in all of protein-based films. The major components of gelatin (α-1, α-2 and β) were clearly observed in GG films, while these components were not found in CG. Gómez-Estaca et al. (2009) reported that α-1 and α-2 chain in the mammalian gelatin was possible degradation during the extraction process and the amount of β-components was also higher in the fish gelatin. From the results, gelatin from two sources showed difference in protein patterns when compared with SPI films. The protein band of SPI films are shown between 11-75 kDa (β-conglycinin including α, α' and β-subunit). The lower content of high MW cross-links and α-chains might result in the lowered mechanical properties of the CG films, unlike the GG films that have higher mechanical properties.

4. Conclusion

The results demonstrated that films from different proteins exhibited significant different in physical, chemical and mechanical properties. Protein-based films had higher thickness, WVP and film solubility, but they were lower in mechanical properties compared to the PVC film. Further work in this area is required to enhance the properties of protein-based films to closer the commercial wrap film by plasticization, cross-linking of protein film (thermal, chemical, enzymatic)

or nano-composite, especially physical and mechanical properties for benefit to food packaging applications (fresh produce, meat and fishery products).

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