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Review

Characterisation of biodegradable protein based films from gelatin alternative: a review

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Abstract

Protein-based films are thin and flexible films derived from protein sources. They are completely biodegradable and used in food engineering, packaging, drug recovery, and other applications. In food packaging, gelatin is widely used due to properties such as low cost, availability, functional attributes, mechanical (flexibility and tension) and optical (brightness and opacity) strength, barrier against gas flow, and structural resistance to water and microorganisms. Therefore, this paper reviews the characterisation of biodegradable protein-based films from gelatin alternatives, mainly from fish and chicken skin, as food packaging materials. The properties of film packaging derived from gelatin alternatives were compared with films derived from mammalian gelatin. The findings showed that the blended gelatin alternatives with polysaccharide improved physical properties such as water vapour permeability, gas permeability, light transmission and transparency, thermal properties, microstructure, colour, and heat sealability. Moreover, improvements in mechanical properties such as tensile strength and elongation at break were also investigated. This review also comes out with suggestions for future research on the compatibility between gelatin films and food ingredients. This paper provides a comprehensive overview that promotes the development of biodegradable blended films from gelatin alternatives for packaging applications in the food industry and related fields.

Introduction

Packaging products made from natural materials are used to contain, protect, handle, deliver, and present food products. In recent years, there has been increased demand for food packaging that offers improved shelf life for food products (Nazmi and Sarbon, 2020). Packaging takes many forms, such as cardboard cartons, foam, textiles, thermoformed materials, bulk drums, and films. Film packaging has been broadly applied in numerous industries including pharmaceutical, cosmetic, photography, and food packaging. As food packaging, films are generally used to wrap foods for commercial presentation and/or maturation, as well as preservation, without changing the original ingredients (Pascall and Lin, 2013).

Plastic films have been increasingly used for production and packaging, due to their availability and relatively low cost, in addition to superior mechanical performance. The latter includes heat sealability, tensile and tear strength, as well as barriers to carbon dioxide, oxygen, aromatic compounds, and anhydride (Sorrentino *et al.,* 2007). Petroleum-based polymers and plastics have markedly increased in the food packaging marketplace, thanks to their moderately low price and favourable properties. However, those polymers are non-biodegradable and non-renewable (Mohamed *et al.,* 2020). Because of technical and economic difficulties, recycling is limited; in fact, the worldwide waste plastic recycling rate is just less than 3% (HKC, 2016).

Studies have shown that major renewable sources for biodegradable films include polysaccharides, proteins, and lipids. There is great interest in producing biopolymer films based on protein sources due to their environmental-friendly properties (Soo and Sarbon, 2018). Protein-based films can be sourced from plants such as gluten, soy protein, and zein (Chen *et al.,* 2019); and also from animals such as from extracted fish and chicken skin gelatin (Nor *et al.,* 2017; Bakry *et al.,* 2017; Soo and Sarbon, 2018; Staroszczyk *et al.,* 2020).

Therefore, film packaging derived from protein-derived gelatin is of great interest to researchers. Moreover, religion restrictions on pork product consumption in Judaism and Islam, in addition to the bovine spongiform encephalopathy crisis (BSE), have increased demand for Halal and Kosher products

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(Aziz and Chok, 2013). Given the cited issues, an urgent need exists to find alternative sources of gelatin for discerning customers. This increased in demand has increased the research into types of gelatin derived from various sources, including chicken bone (Liu and Xu, 2004) and chicken skin (Sarbon *et al.,* 2013); and fish skin, bone, fins, and scales as alternative raw materials for gelatin production (Cheow *et al.,* 2007; Shakila *et al.,* 2012; Rosli and Sarbon, 2015).

Since there is great interest in blended film manufacturing for biodegradable packaging, this paper reviews the recent research on physical and mechanical properties of gelatin alternative based blended films. This paper has found that biopolymer blending has emerged as an improvement in terms of film properties. Current and future trends in gelatin alternative based films are also discussed.

Biodegradable films

Biodegradable films comprised of biodegradable polymers degrade naturally via composting by microorganisms such as fungi, algae, and bacteria that allow their decomposition to water, carbon dioxide, biomass, and methane (Hanani *et al.,* 2014). The primary biopolymers used to develop biodegradable films include proteins, lipids, polysaccharides, and natural extracts (Suderman *et al.,* 2018; Said and Sarbon, 2020). Biodegradable polymers are considered as the replacement materials for petroleum-based commodity plastics because they are cost-effective and offer competitive mechanical properties (Vieira *et al.,* 2011). These materials have film-forming characteristics, and readily degrade after disposal.

The two major classifications of biodegradable polymers are edible and non-edible, which dominate contemporary research and development efforts. Edible biopolymers with potential for the manufacturing of food packaging applications include: (i) polysaccharides such as carboxymethyl cellulose (CMC), agar, alginate, chitosan, and starch (Nur Hazirah *et al.,* 2016; Nor *et al.,* 2017; Soo and Sarbon, 2018); (ii) proteins such as gelatin, whey, soy, and wheat (Harper *et al.,* 2013; Nor *et al.,* 2017); (iii) lipids such as wax and oil (Chiumarelli and Hubinger, 2014; Janjarasskul *et al.,* 2014), and natural extracts (Hanani *et al.,* 2018; Said and Sarbon, 2020). Non-edible biopolymers are mainly derived from polylactic acid (PLA), starch blended with polyethylene (PE), polyanhydrides, polyvinyl alcohol, polyhydroxyalkanoate (PHA), polyhydroxybutyrate (PHB), and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (Chen *et al.,* 2019).

Protein-based films

Protein-based film can be produced from collagen, gelatin, corn zein, wheat gluten, soy protein, casein and caseinates, mung bean, and others (Said and Sarbon, 2019). Protein-based edible films are generally formed by evaporating solvent/carrier solutions or by dispersing of the proteins. Among biodegradable films, protein-based films are the most impressive gas barriers as compared to films prepared from lipids and polysaccharides (Wittaya, 2012). Protein-based films and coatings also provide good barriers to CO_2 and O_2, but not water, similar to polysaccharide-based films (Cha and Chinnan, 2004). Protein-based films have superior mechanical properties, with greater elongation and tensile strength than lipid and polysaccharide-based films. This is because based on their twenty monomers, proteins have unique structures that deliberate a broader range of functional properties, including a high intermolecular binding potential (Hanani *et al.,* 2013).

Protein-based films are preferable due to superior tensile strength (TS) and water vapour permeability (WVP). However, the TS of protein-based films vary depending on type and protein sources. The number of amino acids (monomers) influences the linkage and bonding within a film, which also affects the properties of each film (Hanani *et al.,* 2014). The TS values for protein-based films derived from gelatin (Fakhreddin *et al.,* 2013; Nor *et al.,* 2017); soy (González and Igarzabal, 2013), and whey (Janjarasskul *et al.,* 2014) protein have been reported to range between 110 - 0.3 MPa. The WVP values for films derived from gelatin (Hanani *et al.,* 2013; Nor *et al.,* 2017); soy proteins isolate (González and Igarzabal, 2013), and whey (Janjarasskul *et al.,* 2014) have been reported to range between 0.5 - 10 g m^{-1} $s^{-1}Pa^{-1}$.

Gelatin and alternative gelatin

Gelatin is a protein product derived from the partial hydrolysis of collagen obtained from the skin (hides), bones, and connective tissues of land animals (Ahmed *et al.,* 2020). It is a high molecular weight polypeptide and an important hydrocolloid, a digestible protein containing all the essential amino acids except tryptophan. The interest in alternative sources of halal gelatin is increasing due to growing concerns among industry and consumers. The growing demand for halal gelatin in halal foods, and the rejection of haram sources of gelatin (mainly porcine gelatin), have encouraged scientists to search for alternative sources (Ahmed *et al.,* 2020).

Alternative gelatins can be extracted from

fish (scale, skin, and bone), poultry (skin and feet), seafood, and even insects. Gelatin sources from fish processing have been investigated by numerous researchers as potential raw materials. These include fish skin (Cheow *et al.,* 2007; Wu *et al.,* 2014; Rosli and Sarbon, 2015), fish bone (Akagündüz *et al.,* 2014), and fish scales (Weng and Zheng, 2015). Additional gelatin sources include chicken processing by-products such as chicken skin (Sarbon *et al.,* 2013) and chicken feet (Lee *et al.,* 2015), insects such as melon bug (*Aspongubus viduatus*) and stink bug (*Agonoscelis pubescens*; Mariod *et al.,* 2011), reptiles such as crocodile and python skins, and also amphibians such as toad skin (Leach, 1957).

Gelatin can be extracted using two methods: (i) acid extraction, and (ii) alkaline extraction. Acid extraction is carried out in an acid medium, and in some cases, an acid pre-treatment before extraction by distilled water is applied. An alkaline extraction refers to a pre-treatment with an alkaline solution, in most cases followed by neutralisation with an acid solution, so the extraction may be carried out in an alkaline, neutral, or acid medium (Mariod and Adam, 2013). An acid extraction process will produce Type A gelatin, while an alkaline extraction process will produce Type B gelatin.

The properties of gelatin are mainly determined by its gel strength or bloom value, including low (< 150), medium (150 - 220), and high (220 - 300); and viscoelastic properties such as gelling and melting temperatures. The quality of gelatin depends on its physicochemical properties, which are influenced by the species, tissue, and processing method(s). Fish gelatin typically has a gel strength range of 0 - 270 g (tested under standard Bloom test conditions), as compared to bovine or porcine gelatin, which have a gel strength range of 200 - 240 g (Rosli and Sarbon, 2015). The gel strengths for various fish skin gelatins have been reported at 98 g for Alaska pollock (Zhou *et al.,* 2006), and 124.9 and 176.9 g for sin croaker and shortfin scad, respectively (Cheow *et al.,* 2007). However, the extracted chicken skin gelatin at 6.67% (w/v) has been reported to have higher gel strength (355 - 1.48 g) than bovine gelatin (259 - 0.71 g) (Sarbon *et al.,* 2013).

Among commercial hydrocolloids, gelatin has been regarded as special and unique, serving multiple functions with a wide range of applications in various industries. The potential uses of gelatin in food, pharmaceutical, photographic, and cosmetic industries are due to its gelling, foaming, and emulsifying properties that contribute to a wide range of applications. The unique properties of gelatin are its solubility in water, and its ability to form thermo-reversible gels with a melting temperature close to human body temperature (Rosli and Sarbon, 2015; Lin *et al.,* 2017). In addition, gelatins have also attracted interest because of their excellent filmogenic properties, good ability to form film, and usefulness as a primary packaging film to protect food from drying and exposure to oxygen and light (Suderman *et al.,* 2018).

Protein-based film from alternative gelatin

Biopolymers based on proteins, including gelatin, have been broadly studied in attempts to develop a single biodegradable film. A single biodegradable film comprises only one biopolymer source. In addition, single gelatin films may be combined and blended with other biomaterials in order to improve the functional properties of the produced film. Studies have shown that the development of blended gelatin films would improve films' physical and mechanical properties (Suderman *et al.,* 2018). However, single biopolymer films derived from proteins or polysaccharides have been reported to suffer from weaknesses such as being poor barriers against water vapour due to strong hydrophilic characteristics. Due to the marked hygroscopic property of gelatin films, which tend to dissolve or swell when in contact with high moisture food products, several approaches have been made to improve gelatin film properties (Hanani *et al.,* 2014).

One of the methods that can improve a film's properties is to blend the gelatin with other biopolymers such as chitosan, starch, or soy proteins (Soo and Sarbon, 2018). Numerous studies have investigated certain properties of biopolymer films by incorporating other polymer components in the film formulation. Blended films are produced by blending two or more complementary polymers with the aim of improving or modifying their barrier and mechanical properties (Bakry *et al.,* 2017). The physical and mechanical properties of single film from gelatin alternative also can be improved by this blending method. In addition, the mechanical properties of gelatin-based films could be improved by modification with plasticisers. Numerous studies have reported the plasticisation effect of sorbitol and glycerol on various sources of starches in developing biodegradable or edible films (Bakry *et al.,* 2017; Nor *et al.,* 2017; Soo and Sarbon, 2018). However, plasticisers may also serve as mechanical anti-plasticisers at low concentrations (2.5%), resulting in stiffer film blends. However, different types of polymer may respond differently to a low concentration of plasticisers. The absence of

anti-plasticising effects may be due to the limited range of diluent concentrations examined, since those plasticisers are usually applied at optimum levels to enhance film flexibility and workability (Suderman *et al.,* 2018).

Fish skin gelatin has attracted attention as an alternative to bovine and porcine gelatin. The physicochemical and functional properties of fish skin gelatin are sub-optimal as compared to mammalian gelatin. Thus, fish skin gelatin is less favourable in commercial applications (Zilhadia *et al.,* 2018). Fish skin gelatins have a lower concentration of amino acids (62% proline and 50% hydroxyproline) as compared to mammalian gelatins. However, warm-water fish (such as bigeye-tuna and tilapia) gelatins have a higher amino acid content than cold-water fish (such as cod, whiting, and halibut) gelatins (Karim and Bhat, 2009). Since these two sources (mammalian and marine) still have halal issues and health-related concerns, a new potential gelatin source from poultry has been introduced (Sarbon *et al.,* 2013). Many studies have been done on the addition of nanoparticles on different types of gelatin from marine sources, including tilapia skin gelatin/Cloisite Na^+ (Nagarajan *et al.,* 2015), fish skin gelatin/zinc oxide nanoparticle (Arfat *et al.,* 2016), and fish skin gelatin/chitosan nanoparticles (Hosseini *et al.,* 2016). Poultry sources include poultry skin, feet, and bone, and they can replace mammalian sources (Nik Aisyah *et al.,* 2014). There have been many studies related on chicken skin gelatin in gelatin/zinc oxide nanoparticles/tapioca starch (Lee *et al.,* 2019), chicken skin gelatin/tapioca starch (Loo and Sarbon, 2020), and chicken skin gelatin/glycerol (Nor *et al.,* 2017).

Blended alternative gelatin films

The blending of biopolymers has emerged for the purpose of improving film properties. Numerous studies have demonstrated that combining selected biopolymers produces edible films with better properties as compared to the films from a single biopolymer (Suderman *et al.,* 2018). Blended biodegradable films are mixture of polysaccharide with polysaccharide, protein with protein, lipid with lipid, protein with polysaccharide, protein with lipid, or polysaccharide with lipid (Janjarasskul *et al.,* 2014; Bakry *et al.,* 2017; Soo and Sarbon, 2018).

Gelatin alternative film materials can be blended with protein material to improve oxygen permeability because they are less porous and have smoother matrices (Wang *et al.,* 2010). A few studies of blended gelatin alternative films with other protein components have covered fish gelatin-fish protein isolate film blends (Ali *et al.,* 2014), and fish gelatin-egg white film blends (Giménez and Montero, 2012). In addition, polysaccharides have been blended with gelatin alternative films such as fish gelatin-chitosan film blends (Fakhreddin *et al.,* 2013), fish gelatin-lignin film blends (Núñez-Flores *et al.,* 2013), chicken skin gelatin-sorbitol blends (Bakry *et al.,* 2017), chicken skin gelatin-*Centella asiatica* extracts blends (Nazmi *et al.,* 2017), chicken skin gelatin-rice flour blends (Soo and Sarbon, 2018), and chicken skin gelatin-starch-curcumin extract blends (Said and Sarbon, 2020). Overall, these studies have reported the improved barrier and mechanical properties of gelatin-based films blended with polysaccharides, as well as improved water permeability for gelatin films blended with hydrophobic lipids. The following studies on blended gelatin alternative film with lipid components are noteworthy: fish gelatin-corn oil film blends (Hanani *et al.,* 2013), and fish gelatin-sunflower oil film

Table 1. Alternative gelatin (fish) based films blended with different types of materials.

Type of protein blended film	Alternative gelatin	Blended material	Reference
Protein with polysaccharide	Fish skin and fish bone gelatin	Chitosan	Shakila *et al*. (2012); Fakhreddin *et al.* (2013)
	Fish skin gelatin	Pectin	Liu and Zhang (2006)
	Fish skin gelatin	Gellan	Pranoto *et al*. (2007)
	Fish skin gelatin	k-carrageenan	Pranoto *et al*. (2007)
	Commercial type A fish gelatin	Lignin	Núñez-Flores *et al*. (2013)
Protein with protein	Fish skin gelatin	Fish protein isolate	Ali *et al*. (2014)
	Fish skin gelatin	Soy protein isolate	Denavi *et al.* (2009)
Protein with lipid	Fish skin gelatin	Egg white	Giménez and Montero (2012)
	Fish skin gelatin	Sunflower oil	Montero *et al.* (2009)
	Fish skin gelatin	Palm oil	Tongnuanchan *et al.* (2015)

Table 2. Mammalian gelatin (bovine and porcine) based films blended with different types of material.

Type of protein blended film	Protein	Blended material	Reference
Protein with polysaccharide	Bovine gelatin	Chitosan	Rivero *et al.* (2009)
	Bovine gelatin	Pectin	Noemi *et al.* (2011)
	Porcine gelatin	Pectin	
	Porcine gelatin	Gellan	Yeon *et al.* (2004)
	Gelatin	Starch	Al-Hassan and Norziah (2012)
	Commercial gelatin	Lignin	Vengal and Srikumar (2005)
	Porcine gelatin	CMC	Wiwatwongwana and Pattana (2010)
Protein with protein	Commercial gelatin	Whey protein isolate	Wang *et al*. (2010)
	Bovine gelatin	Soy protein isolate	Cao *et al.* (2007)
Protein with lipid	Bovine gelatin	stearic and palmitic acids	Bertan *et al.* (2005)

blends (Pérez-Mateos *et al.,* 2009). Previous findings on gelatin blended films are presented in Table 1, which summarises the alternative fish gelatin films blended with different types of biopolymer; and Table 2, which summarises the bovine and porcine gelatin films blended with different types of biopolymer.

Effect of blending on alternative gelatin film's characteristics

The superiority of gelatin alternative blended films as compared to the single biopolymer films has been determined by investigations into their physical and mechanical properties. These properties include thickness, water vapour permeability (WVP), gas permeability, light transmission and transparency, thermal properties, microstructure, colour, and heat sealability. Previous studies on physical properties such as WVP, light transmission and transparency, thermal properties, morphology, and structure of blended alternative gelatin films are presented in Table 3. Alternative gelatin sources such as fish and chicken skin were blended with proteins (such as soy protein isolate, fish protein, egg white, and mung bean), polysaccharides (such as chitosan, k-carrageenan, lignin, seaweed, CMC, and starch), and lipids (such as palm oil, sunflower oil, and stearic acid). Mechanical properties such as tensile strength and elongation at break of blended alternative gelatin are presented in Table 4.

Physical properties

Thickness

Film thickness is an important parameter since it directly affects the biological properties and shelf life of coated food (Skurtys *et al.,* 2010). An

Table 4. Mechanical properties of blended alternative gelatin film.

Blended film category	Type of blended film	Property		
		TS (MPa)	EAB (%)	Reference
Alternative gelatin with polysaccharide	Fish gelatin-chitosan	15 - 35	5.0 - 66.6	Shakila *et al.* (2012); Fakhreddin *et al*. (2013)
	Fish skin gelatin-pectin	43.5 - 54.2	2.1 - 3.2	Liu and Zhang (2006)
	Fish gelatin films-gellan	101.76 - 104.39	5.37 - 6.24	Pranoto *et al.* (2007)
	Fish gelatin films-k-carrageenan	103.63 - 104.48	5.04 - 6.81	Pranoto *et al.* (2007)
	Fish gelatin films-lignin	7.5 - 12.13	316.48 - 362.83	Núñez-Flores *et al.* (2013)
	Chicken skin gelatin-CMC	6.81 - 12.43	165.9 - 190.85	Nazmi and Sarbon (2020)
	Chicken skin gelatin-tapioca starch	1.54 - 3.27	50 - 90	Loo and Sarbon (2020)
	Chicken skin gelatin-potato starch	1.78 - 2.76	34.80 - 65.82	Alias and Sarbon (2020)
Alternative gelatin with protein	Fish gelatin-egg white	7.29 - 14.4	37.8 - 426.9	Giménez and Montero (2012)
	Fish gelatin-fish protein	8.14 - 13.98	37.46 - 98.83	Ali *et al.* (2014)
Alternative gelatin with lipids	Fish gelatin-palm oil	12.86 - 21.39	106.61 - 143.30	Tongnuanchan *et al.* (2015)

TS: Tensile strength; EAB: Elongation at break.

Table 3. Physical properties of blended alternative gelatin film.

Blended film category	Type of blended film	Water vapor permeability (WVP) g m^{-1} $s^{-1}Pa^{-1}$	Property					
			Thermal properties (glass transition, T_g)	Light transmission properties (UV)%	Transparency	Microstructure (SEM)	Fourier Transform (FTIR)	Reference
Alternative gelatin with polysaccharide	Fish gelatin-chitosan	883 - 913	45.0 - 55.9	0.008 - 27.6	0.58 - 0.76	Surface: granular and coarser; protrusion of polymerised chains	Amide II and amide III increased	Gómez-Estaca *et al*. (2011); Fakhreddin *et al.* (2013); Wu *et al.* (2014)
	Fish gelatin films with gellan	1.79 - 1.75	-	-	-	Cracks were eliminated with addition of gellan, more dense and compact appearance presents	Amide I: 1652.70 - 1654.63 cm^{-1}	Pranoto *et al.* (2007)
	fish gelatin films with k-carrageenan	2.28 - 2.17	-	-	-	Fish gelatin's internal matrix were lightly modified	Amide A: 3315 cm^{-1}	Pranoto *et al.* (2007)
	fish gelatin films with lignin	2.17 - 4.58	-	-	4.62 - 9.84	Marked disruption surface that inducing a partial laminar-like appearance	-	Núñez-Flores *et al.* (2013)
	Fish gelatin-seaweed	-	57.82 - 59.41	29.68 - 27.01	3.38 - 3.39	The cross-section rougher with discontinuous zones	-	Rattaya *et al*. (2009)
	Chicken skin gelatin-tapioca starch	1.90 - 5.80	-	0.09 - 44	1.9 - 2.9	The composite films had a smoother surface and improved internal structure over pure gelatin films	-	Loo and Sarbon (2020)
	Chicken skin gelatin-potato starch	2.08 - 2.66	-	0.01 - 31.95	0.94 - 1.3	The surface smoothness and internal structure of composite films improved via potato starch incorporation	-	Alias and Sarbon (2020)
Alternative gelatin with protein	soy protein isolate (SPI)-fish gelatin	$2.0 - 2.5 \times 10^{-8}$	-	-	0.95 - 0.99	-	Amide I: 1656	Denavi *et al*. (2009)
	fish gelatin-fish protein	$1.63 - 3.62 \times 10^{-11}$	-	2.57 - 5.84	3.26 - 4.18	Rough Surface	-	Ali *et al.* (2014)
	fish gelatin-egg white	7.29×10^{-8}	-	-	-	Smooth, uniform structure	Amide I: 1634	Giménez and Montero (2012)
	Fish gelatin-mung bean protein	-	30.08	0.37 - 3.83	3.36 - 3.41	Rough surface obtained in CG/MPI blended film and MPI film	Amide I: 1632	Hoque *et al.* (2011)
Alternative gelatin with lipids	Fish gelatin-palm oil	1.11 - 1.65	36.77 - 40.52	-	-	Rougher surface than control film	Amide I: 1641	Tongnuanchan *et al.* (2015)
	Fish gelatin-sunflower oil	3.10 - 3.70	-	-	5.12 - 9.51	-	Amide I: 1690	Montero *et al.* (2009)
	Fish gelatin-fatty acid	-	-	-	-	Existence of crystalline lipid particles within the protein matrix of film, with discrete crystalline particles of stearic acid on the upper surface of the gelatin-stearic blended film	-	Limpisophon *et al.* (2010)

accurate measurement of thickness is important because this can prevent the variation of properties from being erroneously attributed to other parameters (Hong *et al.,* 2011). The thickness of each film normally will be determined using hand-held micrometre (Digital Micrometre 406-350, Mitutoyo Corp., Kanagawa, Japan) with an accuracy of ± 0.01 mm. Generally, film thickness is influenced by the solid content of the film forming solution (Nagarajan *et al.,* 2015). A compact structure formed during film formation might be induced by compounds such as polysaccharides, proteins, and lipids added to the film mixture. For instant, the oil present in film forming solution will react with the molecular chain of polymers by elaborating their network, thus increasing the thickness of films (Sanuja *et al.,* 2015). Moreover, film thickness can also be affected by pH. A study on tilapia skin gelatin/Cloisite Na^+ reported that the highest thickness was obtained at alkaline pH 7 (0.058 mm). This might be due to the coagulation of the protein in film matrix when the pH is close to isoelectric point of gelatin films which is pH 8.38, thus leading to an increase in thickness (Nagarajan *et al.,* 2015).

Water vapour permeability (WVP)

An understanding of a film's moisture content and permeability characteristics is necessary before its use as food packaging. Water vapour permeability (WVP) measures a film's potential as a barrier against water, which is important as packaged food products' physical and/or chemical deterioration is related to moisture content equilibrium, which is of great importance for maintaining and/or extending shelf-life (Siracusa *et al.,* 2008). The WVP value is mainly determined by placing film with size 2 × 2 cm, and sealed on an aluminium cup containing silica gel. The changes weight of the cup will be calculated. WVP values for gelatin alternative films blended with polysaccharides range from 1.75 - 4.58 × 10^{-8} (g m^{-1} $s^{-1}Pa^{-1}$). These studies have included fish gelatin-chitosan (Fakhreddin *et al.,* 2013; Wu *et al.,* 2014), fish gelatin-lignin (Núñez-Flores *et al.,* 2013), chicken skin gelatin-CMC (Nur Hazirah *et al.,* 2016), chicken skin gelatin-rice flour (Soo and Sarbon, 2018), and chicken skin gelatin-tapioca starch (Alias and Sarbon, 2019). WVP values for gelatin alternative films blended with proteins range from 1.8 - 28.8 × 10^{-8} (g m^{-1} $s^{-1}Pa^{-1}$). These studies included fish gelatin-fish protein (Ali *et al.,* 2014) and fish gelatin-egg white (Giménez and Montero, 2012). WVP Values for gelatin alternative films blended with lipids range from 3.10 - 83.3 × 10^{-8} (g m^{-1} $s^{-1}Pa^{-1}$). These studies have included fish gelatin-corn oil (Hanani *et al.,* 2013), fish gelatin-palm oil (Tongnuanchan *et al.,* 2015), and fish gelatin-sunflower oil (Pérez-Mateos *et al.,* 2009). Overall, alternative gelatin-polysaccharide film blends have the lowest WVP range as compared to alternative gelatin-protein film and gelatin-lipid film blends. Lower WVP values indicate better water vapour barrier properties.

Gas permeability (oxygen and carbon dioxide)

Protein-based films are the most desirable of all biodegradable films, as they offer great gas barrier properties as compared to those prepared with polysaccharide and lipid (Nazmi *et al.,* 2017). Oxygen is a main factor that can cause oxidation, thereby initiating the degradation of the food products. Film oxygen permeability (OP) is used as an efficacy index for preventing lipid oxidation of packaged fatty foods (Cho *et al.,* 2010). Gelatin films are generally good oxygen barriers but have relatively poor water barriers and mechanical properties, thus restricting their potential applications (Chiou *et al.,* 2008). Nonetheless, fish gelatin films have lower mechanical properties than mammalian gelatin, as well as lower oxygen permeability and lower WVP (Hanani *et al.,* 2014). This is due to the differences in the triple helical contents of the gelatin films (Avena-Bustillos *et al.,* 2011).

In addition, the concentration of carbon dioxide plays a significant role in specific packaging systems for the modified atmosphere (Cerqueira *et al.,* 2012). Measuring the permeability of carbon dioxide by polymeric films is therefore important when intended for use on a food surface (Galus and Kadzińska, 2019). A study by Biscarat *et al.* (2015) found that gelatin films were ineffective because films broke during experiments due to the inability of gelatin films to withstand the drastic and instantaneous pressure variance that occurred during gas permeation tests. Nevertheless, the author claimed that the value for carbon dioxide permeability was higher than OP due to the interactions between carbon dioxide gas and free amine groups of gelatins, following the incorporation of polyethylene glycol into the gelatin film.

Light transmission and transparency

Light transmission and transparency studies measure visible and UV light penetration through the film. These properties affect the appearance of the food product, and are critical when defining the performance of films and coatings for applications as food wrappings and containers. One packaging film

function is acting as a barrier for food product from UV radiation and light effects (Li *et al.,* 2014). Films with lower UV light transmission values are good barriers that prevent UV penetration through the film. Measurements of light transmission and film transparency is done using an UV-Vis spectrophotometer (Merck 1 Spectroquant® Pharo 300; Merck KGaA Co., Darmstadt, Germany) from 200 to 800 nm (Nur Hazirah *et al.,* 2016). UV light transmission values of alternative gelatin-polysaccharide blended films ranged from 0.08 - 2.58% at wavelengths of about 200 nm. UV transmission values for fish gelatin-chitosan films ranged from 3.1 - 43% at 280 nm (Wu *et al.,* 2014), while those of chicken skin gelatin-rice flour films ranged from 0.06 - 5.89% at 280 nm (Soo and Sarbon, 2018). The abundant presence of aromatic amino acids in gelatin molecules plays a crucial role in inhibiting UV light transmission. This is because UV light transmission across films can lead to inappropriate deterioration of food, thus affecting their nutritional content as well as the overall flavour and texture of food (Alias and Sarbon, 2019). For alternative gelatin-protein blended films, UV light transmission values ranged from 0.45 - 7.81% at 280 nm for fish gelatin-mung bean protein (Hoque *et al.,* 2011) and fish gelatin-fish protein isolate (Ali *et al.,* 2014). At 200 - 280 nm, gelatin and polysaccharide blended films offer lower UV light penetration.

Transparency is a critical property for conditioned film applications, since it has a direct impact on the appearance of the coated product (Jridi *et al.,* 2014). A film's transparency is an auxiliary criterion that helps assess its compatibility with packaged components (Liu and Zhang, 2006). Previous studies have reported the transparency values for gelatin alternative films blended with polysaccharide ranging from 1 - 3.5 for gelatin-chitosan (Fakhreddin *et al.,* 2013; Wu *et al.,* 2014; Soo and Sarbon, 2018), fish gelatin-seaweed (Rattaya *et al.,* 2009), and chicken skin gelatin-CMC (Loo and Sarbon, 2020). Transparency values for gelatin alternatives blended with proteins ranged from 3.26 - 3.87 for fish gelatin fish protein isolate and fish gelatin-mung bean protein films (Hoque *et al.,* 2011). Values ranging from 4.52 - 9.51 for gelatin alternative blended with lipids were reported from fish gelatin-sunflower oil film (Pérez-Mateos *et al.,* 2009). A lower transparency value represents higher translucence and less opacity, meaning that the films with a value of 0.5 are clearer as compared to films with a 3.5 transparency value (Ali *et al.,* 2014). The addition of polysaccharide, protein (other than gelatin protein), and oil had increased transparency values of gelatin films, thus causing opacity to the film. Transparency decreased perceptibly when adding fish protein isolate and also with sunflower oil. However, only small changes were detected between films with different levels of oil.

Thermal properties

Thermal transitions indicate the changes in a material's physical state due to changes in temperature or pressure. Thermal analysis provides information not only about the thermal properties, but also gives clues about the structure of the materials (Shehap *et al.,* 2015). Thermal properties of gelatin films are determined using differential scanning calorimeter (DSC). A protein film's melting transition point (T_m) indicates the temperature at which a disruption of its ordered or aggregate structure (crystalline phase) occurs, in addition to changes from its stabilised state via various protein interactions during film formation, from its native to denatured state (Tang *et al.,* 2009). T_m values for gelatin alternative films blended with polysaccharides ranged between 18 - 54.77°C for fish gelatin-lignin (Núñez-Flores *et al.,* 2013) and fish gelatin-chitosan (Gómez-Estaca *et al.,* 2011), while the T_m value for chicken skin gelatin-tapioca starch ranged between 44 - 49°C. The increasing T_m values of chicken skin gelatin-potato starch films may be explained in terms of the functionalities of potato starch as crosslinker agent in promoting hydrogen bonding interactions, and reducing the mobility of biopolymer chains in the film matrix, thus producing heat-stable films (Alias and Sarbon, 2019). T_m values for gelatin alternative films blended with various proteins ranged between 72.03 - 118.56°C for fish gelatin-mung bean protein (Hoque *et al.,* 2011). In addition, the T*m* values for gelatin alternative films blended with oil ranged between 122.51 - 125.77°C for fish gelatin-palm oil (Tongnuanchan *et al.,* 2015).

Glass transition for protein films (T_g) is related to the molecular segmental motion of its disordered structure (amorphous phase), which undergoes change into a highly viscous or rubbery state from a brittle glass-like solid state (Tang *et al.,* 2009). T_g values for gelatin alternative films blended with polysaccharides ranged between 171.04 - 178.96°C for fish gelatin-seaweed (Rattaya *et al.,* 2009). T_g values for gelatin alternative films blended with various proteins ranged between 30.08 - 124.46°C, including fish gelatin-mung bean protein (Hoque *et al.,* 2011). T_g values for alternative gelatin films blended with lipids ranged between 35.85 - 40.52°C for fish gelatin-palm oil (Tongnuanchan

et al., 2015). High T_g in a composite film indicates a degree of blending that follows intermolecular interactions between gelatins alternative and polysaccharide polymers. On the other hand, the transition enthalpy associated with this peak reflects relative interactions between polymers, signifying higher renaturation levels that indicate a more robust film network than other films (Rattaya *et al.,* 2009).

Microstructure

Most polysaccharide gelatin alternative blended films include blending with lignin (Núñez-Flores *et al.,* 2013), chitosan (Shakila *et al.,* 2012), tapioca starch (Loo and Sarbon, 2020), and rice flour (Soo and Sarbon, 2018). These films have rough surfaces brought about by strong disruptions of the smoother homogeneous structure of parent gelatins. This coarseness might be the result of increased non-covalent and covalent bonds between protein strands and phenolic compounds (Shakila *et al.,* 2012). The addition of polysaccharide to the gelatin films, such as with tapioca starch, causes a decrease in roughness of the cross section of the films. The change in the components of the blended films, as well as the interactions that happened in the film matrix with the addition of tapioca starch, were probable reasons for the changes in the surface and cross-sectional area of the blended films (Loo and Sarbon, 2020). However, some polysaccharide-gelatin blended films have a compacted dense appearance, such as with gellan, which might initiate linkages and or residue between fish gelatin's fibrillar regions and its poly-electrolyte associations with the gelatin. Microstructural observations of these results allow explanations for improvements in WVP and tensile strength for fish gelatin films blended with gellan, which remarkably altered the film's matrix when compared with k-carrageenan (Pranoto *et al.,* 2007).

In contrast, a rough surface structure was noted for gelatin-protein blended films such as gelatin-mung bean (Hoque *et al.,* 2011) and gelatin-fish protein isolate (Ali *et al.,* 2014) as compared to single protein-based films. This is likely due to alignments of protein molecules during the film's formation, or in association with co-extant but differently ordered junction zones that are formed within the film's matrix (Hoque *et al.,* 2011). However, scanning electron microscopy images showed no difference in separation within the matrix of fish skin gelatin-fish protein isolate blended films, indicating compatibility between fish skin gelatin and fish protein isolate. Such a compatible blend more likely favours molecular interactions within the blended film's matrix with improved mechanical and physical properties (Ali *et al.,* 2014). Some gelatin-protein blended films have shown a smooth, uniform, homogeneous structure, such as in gelatin-soy protein isolate (Weng and Zheng, 2015), and gelatin-egg white films (Giménez and Montero, 2012).

SEM studies have also revealed that gelatin films incorporating different percentages of oil and/or lipid components such as gelatin-palm oil (Tongnuanchan *et al.,* 2015), and fish gelatin-fatty acid (Limpisophon *et al.,* 2010) had rougher surfaces than control films. At higher concentrations (100%) of fatty acids, the top layer of film is concentrated with small oil droplets. Studies have found that large numbers of oil droplets floated towards the surface during the process of drying the film. The matrices of these films demonstrated a limited capacity to retain oil droplets; hence, they were not inserted within the film's network. Consequently, most were expelled or localised at the film's upper surface. Such hydrophobic fat droplets might serve as a barrier for water vapour adsorption, thus hindering water transfer, and granting the film a lower WVP (Tongnuanchan *et al.,* 2015). In terms of surface film structure, smooth and uniform structured are preferred in order to gain an interest to the product itself. Most blended films will have a rougher surface structure as compared to single gelatin-based film. However, improvements in the other physical and mechanical properties of packaging film via blending the film are important, as these will maintain the quality of product.

Colour

The colour of packaging cannot be underrated, as it can reflect the appearance of food product. Colour attributes are of prime importance because they explicitly affect acceptability among consumers (Rawdkuen *et al.,* 2012). Regarding the attribute of colours, lightness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*) indicate the values of gelatin-based films. Transparency and lack of colour are two important properties for increasing consumer acceptance in food packaging applications. According to Nagarajan *et al.* (2017), tilapia skin gelatin has been reported to have the highest yellowness (b^* value), and lowest lightness (L^* value) as compared to polylactic acid (PLA) control and gelatin/PLA multilayer film. Karnjanapratuml *et al.* (2019) reported that the higher lightness (L^*) and yellowness (b^*) could be obtained from fish gelatin based-film emulsified with frog skin oil as acompared to palm oil with similar redness (a^*). The

yellowness of a film is related to the incorporation of essential oil. According to Shankar *et al.* (2019), the addition of melanin nanoparticles decreased the lightness (*L**), and increased the redness (*a**), yellowness (*b**) as compared to the neat gelatin film. Hence, the incorporation of melanin nanoparticles into gelatin-based films may help prevent oxidative colour changes in packaged foods. Moreover, in a study by Nafchi *et al.* (2014), the colour characteristic parameter of bovine gelatin had a slightly different value when the nanorod-rich zinc oxide (ZnO–nr) was added. The lightness (*L**) of bovine gelatin was significantly decreased with an increase in nano-ZnO content. The films turned into light yellow and red, which suggests significant increases in *a** and *b**.

Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy is used to determine the intermolecular cross-linking between foods and biomaterials, while also monitoring changes in secondary gelatin structure and functional groups (Al-Saidi *et al.,* 2012). Some researchers have explored FTIR as an instrument to determine biodegradable film properties, and more specifically films produced from gelatin. The functional properties of gelatin films are determined by the attenuated total reflection (ATR) mode of the FTIR spectrophotometer (Thermo Scientific Nicolet iS10 FTIR; Thermo Fisher Scientific, Waltham, USA) (Nur Hazirah *et al.,* 2016). For biodegradable films, the following four major absorption bands represent interactions between functional groups: (i) amide-A, from stretch vibrations of N-H groups; (ii) amide-I, from C-O stretching vibrations coupled with C-N stretching, C-C-N deformation, and in-plane N-H bending; (iii) amide-II, from bending vibrations of N-H groups and stretching vibrations of C-N groups; and (iv) amide-III, from C-N plane vibrations or from N-H groups of bound amides and vibrations from glycine's CH_2 groups (Hashim *et al.,* 2010).

Prior studies have established that absorption bands for functional groups in gelatin-polysaccharide blended films ranged from 3215 to 3321.31 cm^{-1} for amide-A, 1630 to 1680 cm^{-1} for amide-I, 1533 to 1580 cm^{-1} for amide-II, and 1239 to 1242 cm^{-1} for amide-III. These values have been reported for fish gelatin-chitosan (Fakhreddin *et al.,* 2013; Wu *et al.,* 2014), chicken skin gelatin-potato starch and rice starch (Alias and Sarbon, 2019; Cheng and Sarbon, 2020). Alternative gelatin-protein blended films ranged from 3200 to 3500 cm^{-1} for amide-A, 1632 to 1700 cm^{-1} for amide-I, 1530 to 1536 cm^{-1} for amide-II, and 1230 to 1236 cm^{-1} for amide-III. These values have been reported for fish gelatin-mung bean protein blends (Hoque *et al.,* 2011). Values for alternative gelatin-lipid blended films ranged from 3200 to 3500 cm^{-1} for amide-A, 1630 to 1750 cm^{-1} for amide-I, 1550 cm^{-1} for amide-II, and 1236 cm^{-1} for amide-III. These values were reported for fish gelatin-sunflower oil (Pérez-Mateos *et al.,* 2009) and fish gelatin-palm oil blended films (Tongnuanchan *et al.,* 2015).

Absorption band values for gelatin-polysaccharide blended films in the above cited studies varied only slightly from those of the single gelatin film (control). This indicates that no major changes occurred in fish gelatin functional groups due to interactions between gelatin and polysaccharides. Nevertheless, these slight changes in each band did give meaning to these interactions. According to Fakhreddin *et al.* (2013), the increasing in chitosan ratio in chitosan-fish gelatin blended films slightly increased the absorbance of amide-III and amide-II bands. This was related to the carbonyl moieties and amino group, which mainly interact electrostatically, thus allowing for the soluble polyelectrolyte's complex formation (Fakhreddin *et al.,* 2013).

Fish gelatin-protein blended films showed an obvious shift in the amide-A band. A shift towards lower frequencies for fish gelatin-mung bean suggests that interactions between gelatin and mung bean protein isolate have occurred in the film's matrix. Usually, a broadening of OH and NH bands, and a decrease in vibrational wavenumbers indicate molecular hydrogen bond interactions between polymers (Hoque *et al.,* 2011). Generally, FTIR spectra for controls and fish gelatin-lipid blended films have exhibited similar major peaks, although peak amplitudes varied depending on palm oil concentrations. Amide-A peaks for controls gradually shifted to higher wavenumbers as oil concentrations increased, indicating that oil decreased the protein-protein interactions (Tongnuanchan *et al.,* 2015). Furthermore, blending gelatin alternative film with oil possibly induces esterification between functional hydroxyl groups in the gelatin and fatty acids in the oil. This would cause new peaks that are characteristic of fatty acid carbon chains attached to gelatin molecules (Pérez-Mateos *et al.,* 2009).

Heat sealability

Sealing ability is one of the important features for applying films as the starting material for making sachets, pouches, or bags for packaging liquids or dry food ingredients (Tongnuanchan *et al.,*

2016). A seal must be solid enough to keep the product within the package, and not release its contents during handling or storage (Kim and Ustunol, 2001). According to Nilsuwan *et al.* (2017), single fish gelatin film demonstrated the highest seal strength and sealing efficiency as compared to all fish gelatin/emulsified bilayer films. During the lamination, fish gelatin of both films was molten by heat and the interfacial interaction between the two films could be enhanced, favouring the sealing ability of bilayer films. Meanwhile, another study by Nilsuwan *et al.* (2018) reported that the seal strength of tilapia fish gelatin film was lower than PLA film ($p < 0.05$), but higher as compared to other PLA/fish gelatin bilayer films. Nevertheless, these two studies agree that the use of gelatin in heat sealability improves the film. Meanwhile, a study by Rezaei and Motamedzadegan (2015) reported that fish skin gelatin films containing clay nanoparticles and glycerol has high heat sealability since the films did not separate from each other at spots they were heat-sealed but were torn at other places. Thus, it can be concluded that the introduction of nanoparticles into gelatin matrices greatly enhances the overall properties such as the biocompatibility, physical and mechanical properties, and the biodegradability of gelatin-nanocomposite films (Duncan, 2011).

Mechanical properties

Tensile strength

Adequate elasticity and mechanical strength are commonly necessary for packaging films to resist external stress and maintain barrier properties and integrity during packaging applications (Rao *et al.,* 2010). Tensile strength can be measured using a TA.XT2i Texture analyser (Stable Microsystem, TA.XT Plus; Godalming, Surrey, United Kingdom) (Nazmi and Sarbon, 2020). Tensile strength values for gelatin films blended with polysaccharides ranged from 1.54 to 104.48 MPa. These values were reported for fish gelatin-chitosan which was 11.28 MPa (Fakhreddin *et al.,* 2015), chicken skin gelatin-potato starch which was 2.11 MPa (Alias and Sarbon, 2019), and chicken skin gelatin-tapioca starch which was 3.27 MPa (Loo and Sarbon, 2020). For gelatin films blended with protein, the values of tensile strength ranged from 5.57 to 13.98 MPa. These values were reported for fish gelatin-egg white which was 5.57 MPa (Giménez and Montero, 2012), and fish protein isolate-fish gelatin which was 13.98 MPa (Ali *et al.,* 2014). The values for blended fish gelatin-lipid films ranged from 11.14 to 21.39 MPa. These values have been reported for blended fish gelatin-palm oil film at 21.39 MPa (Tongnuanchan *et al.,* 2015), and blended fish gelatin-fatty acid film at 17.21 MPa (Limpisophon *et al.,* 2010).

Studies have shown that blended alternative gelatin-polysaccharide films have greater tensile strength as compared to gelatin-only films (Fakhreddin *et al.,* 2013). The optimised concentration level for polysaccharide-gelatin interaction where gelatin maintained its dominant phase was < 2% polysaccharide. Thus, it can be concluded that the flexibility and strength of blended films are greatly affected by changing the protein to polysaccharide ratio. To the contrary, the addition of fish protein isolate to alternative gelatin-protein film blends decreases tensile strength. This might be due to the lower reactivity of myofibrillar protein molecules in cross-linking or in interactions between protein molecules (Ali *et al.,* 2014). Similarly, gelatin-lipid film blends also have lower tensile strength as the concentration of palm oil increases. When palm oil droplets are evenly dispersed within a film's network, protein-to-protein interactions are lowered, thus causing lower tensile strength (Tongnuanchan *et al.,* 2015).

Elongation at break

Elongation at break measures the mechanical strain point at which a film break. This is expressed in terms of the percentage of length change from the initial material's length between grips of a test machine. This extendibility is measured as the elongation at break, where a larger value indicates the material absorbs more energy before breakage occurs. The elongation at break is measured by using TA.XT2i Texture analyser (Stable Microsystem, TA.XT Plus; Godalming, Surrey, United Kingdom) (Nazmi and Sarbon, 2020). Reported values for alternative gelatin films blended with polysaccharide ranged from 5.04 to 102.04%. These results were reported for fish gelatin-chitosan (Wu *et al.,* 2014; Fakhreddin *et al.,* 2015), chicken skin gelatin-tapioca starch (Loo and Sarbon, 2020), and chicken skin gelatin-potato starch (Alias and Sarbon, 2019). Values reported for gelatin alternative films blended with protein ranged from 37.8 to 337.6%. These values were reported for fish gelatin-egg white films (Giménez and Montero, 2012) and fish gelatin-fish protein isolate films (Ali *et al.,* 2014). Reported values for gelatin alternative films blended with lipids ranged from 99.31 - 257.17%. These values were reported for fish gelatin-palm oil films (Tongnuanchan *et al.,* 2015) and fish gelatin-fatty acid films (Limpisophon *et al.,* 2010).

The addition of a polysaccharide to a gelatin-based film decreases elongation at break,

while the addition of lipids and proteins increases the gelatin-based film's elongation at break. The addition of protein increases the value of elongation at break due to the lower reactivity of myofibrillar protein molecules in cross-linking or in interactions between protein molecules, thus reducing the stiffness of the film and making the film more elastic (Ali *et al.,* 2014). Lipids and oils function as lubricant. Thus, when oils or lipids are evenly dispersed within a film's network, protein-to-protein interactions are lowered, thus decreasing the stiffness of a film.

Current and future trends for blended biodegradable gelatin films

The use of edible films has found a very important niche of applications through their good performance as carriers for active compounds, including in food packaging, biomedical applications, and others. Recent interest on the development of protein-based films especially from gelatin alternative had widely explored and brought to the introduction of smart and intelligent molecules (in the field of nanotechnology) that may provide information on the properties of the packaged food (quality, shelf life, and microbiological safety) and nutritional values (Siracusa *et al.,* 2008). Various nano-reinforcements currently under development include carbon nanotubes, graphene, nanoclays, 2-D layered materials, and nano whiskers from cellulose. Combining these nanofillers with biopolymers may enhance a large number of physical properties relative to unmodified polymer resin, including barrier, flame resistance, thermal stability, solvent absorption, and rate of biodegradability. Such improvements are typically accomplished at low filler content, and this nano-reinforcement is a very attractive route for generating new functional biomaterials for different applications (Babu *et al.,* 2013). Work in this field has accelerated dramatically in recent years, but some drawbacks still need to be overcome to enable their use in the packaging of consumer products for large applications (Mellinas *et al.,* 2016). However, there are limited information regarding the compatibility between gelatin films and food ingredients. Most materials exhibit different material properties and behaviour at different scales and hierarchical structures. Therefore, the understanding of the relationship among the material structure, properties and process are very important for designing edible packaging films with multi-functionalities (Jeya *et al.,* 2020). Future film packaging should be multifunctional. In order to be used more effectively in food applications, the current and future edible films should offer additional functions such as being heat-proof, water-proof, air-proof, anti-corrosive, oil-resistant, insect-resistant, and disease-resistant.

Conclusion

In conclusion, protein-based film from gelatin alternative blended with polysaccharide has shown a potential application as film packaging for food application. This is due to the fact that gelatin films blended with other materials had improved the physical properties such as water vapour permeability, light transmission and transparency, thermal properties, film structure, microstructure, colour, and heat sealability. Furthermore, the mechanical properties of protein-based film from gelatin alternative such as tensile strength and elongation at break were also improved with the blended system applied to this biopolymer. Although the physical and mechanical properties of film packaging from gelatin alternatives are generally poorer than mammalian gelatin (porcine and bovine), fish and chicken skin, a main by-product of the fish and poultry processing industry, have been gaining popularity as an alternative to bovine and porcine gelatin in recent years. The blending of the gelatin film with different macromolecules results in edible films with better control over their final properties.

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