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Properties and characteristics of salmon frame protein isolate films influenced by glycerol and squalene

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Abstract

Characteristics of edible/biodegradable film based on salmon frame protein isolate (SFPI), a processing by-product, containing glycerol at two different concentrations (50 and 65% by weight of protein) with and without 30% squalene (by weight of protein) were investigated. Without squalene, the SFPI film generally had higher hydrophilicity when higher concentration of glycerol was used as indicated by higher ($p < 0.05$) water-vapour permeability (WVP). The addition of squalene augmented yellowness and hydrophobicity of SFPI film as indicated by higher b^* colour coordinate and lower WVP ($p < 0.05$), respectively, in comparison to those without squalene. The squalene also promoted the interactions in SFPI film matrix as revealed by Fourier-transform infrared spectra. These interactions were related to a superior mechanical properties, high barrier properties, and increased thermal resistance of the resulting SFPI film. Therefore, the addition of 30% squalene along with 50% glycerol as plasticiser improved physicochemical, mechanical, barrier, and thermal properties of SFPI film, which could be used as an alternative film for edible/biodegradable food packaging.

Introduction

Edible/biodegradable packaging with bio-based polymers has gained attention as an environmentally friendly approach to replace synthetic polymers. The biopolymers used are mainly polysaccharides, proteins, and lipids. Among biopolymers, proteins are widely used owing to their availability and excellent film-forming capability (Krochta, 2002). Myofibrillar proteins obtained from fish flesh have been utilised as alternative polymers for film formation (Tongnuanchan *et al.,* 2011; Kaewprachu *et al.,* 2017). Currently, the salmon farming and processing industries have grown enormously due to a high market demand associated with good taste, high protein content, and richness in omega-3 polyunsaturated fatty acids. During salmon processing, significant leftovers such as heads, trimmings, mince, frames, and viscera are generated (See *et al.*, 2011). Frames account for 9 - 15% of the whole fish, and are generated by filleting (Liaset *et al.,* 2002). Typically, those leftovers have a low market value, and are generally used for animal feed and fertiliser production. Without appropriate treatment or sustainable waste management, these by-products may cause pollution. Although salmon frames can be used for some dishes in some countries, their popularity is still low due to high bone content. However, salmon frames with a relatively high amount of muscle protein have potential as nutritive protein sources. Moreover, the proteins could serve as biopolymer for making edible or biodegradable films. So far, no edible films prepared from salmon frame protein isolate have been reported.

In order to isolate fish myofibrillar protein, washing is first used to remove haem proteins and lipids. Thereafter, alkaline solubilisation is done to separate meat from bone. Fish protein isolate (FPI) can be used to produce films with good properties and negligible discoloration as compared to those from

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fish meat (Tongnuanchan *et al.*, 2011). Nevertheless, covalent bonds, especially disulphide bonds, lead to high rigidity of the FPI film (Rocha *et al.*, 2013). To tackle this problem, hydrophilic plasticisers, especially glycerol, have been added to FPI films to impart flexibility. However, this inevitably results in poorer water-vapour barrier properties (McHugh *et al.*, 1993). To address this drawback, various hydrophobic compounds have been tested (Limpisophon *et al.*, 2010; Soazo *et al.*, 2011). Regarding the alternative hydrophobic substances, squalene ($C_{30}H_{50}$) is a linear triterpene and highly hydrophobic hydrocarbon which has been used in several industries. It also shows antibacterial and antifungal activities (Desai *et al.*, 1996; Reddy and Couvreur, 2009; Bindu *et al.*, 2015). However, the addition of large hydrophobic squalene molecules in salmon frame protein isolate (SFPI) films has not yet been investigated. The addition of squalene might improve the mechanical performance and the water-vapour barrier properties (WVBP) of the SFPI films. Therefore, the present work aimed to investigate the effects of glycerol concentration in combination with 30% squalene on physicochemical, mechanical, barrier, and thermal properties of SFPI films in order to produce an alternative edible/biodegradable film.

Materials and methods

Salmon frames were supplied by Kingfisher Holding Company Ltd., Thailand. The salmon frames were packed in ice, and transported to the laboratory within 3 h. Sodium hydroxide, sulphuric acid, hydrochloride acid, glycerol, potassium hydroxide, and petroleum ether were purchased from Merck (Darmstadt, Germany). Tween-20 was purchased from Fisher BioReagents™ (Waltham, Massachusetts, USA). Copper (II) sulphate, boric acid, potassium (II) sulphate, bromocresol green, and methyl red as well as squalene (≥ 98%) were purchased from Sigma Chemicals (St. Louis, MO, USA). Isopropanol was purchased from RCL Lab-Scan (Bangkok, Thailand).

Isolation of protein from salmon frame

Preparation of salmon frame protein isolate (SFPI) was done as described by Chomnawang and Yongsawatdigul (2013). Salmon frames were firstly ground with the aid of a crushing mill (Model YCM-1.1E; Yor Yong Hah Heng, Bangkok, Thailand) to obtain particle size of less than 3 - 4 mm. Ground samples were mixed with cold distilled water at a ratio of 1:9 (w/v). The mixture was adjusted to pH 12.0 using 2 M NaOH, and held constant for 1 h at 4°C. Thereafter, the bones and connective tissues were removed by filtration through cheese cloth. The filtrate was subjected to centrifugation at 10,000 *g* (20 min, 4°C). The supernatant was then adjusted to pH 4.5 using 2 M HCl for SFPI precipitation. The pellet was collected by centrifugation at 10,000 *g* (20 min, 4°C).

Preparation of defatted SFPI

The defatting of SFPI was performed as described by Tongnuanchan *et al.* (2011). SFPI was mixed in four volumes of isopropanol, and homogenised using a homogeniser (IKA Labortechnik Homogeniser, Selangor, Malaysia) at 10,000 rpm for 3 min. The homogenate was centrifuged at 10,000 *g* (20 min, 4°C). The SFPI was defatted twice in this manner. The defatted SFPI was collected, spread over a stainless tray, and left at room temperature (25 - 28°C) for 12 h for complete isopropanol evaporation. The protein content of SFPI was then determined (AOAC, 2000).

Preparation of film-forming solution (FFS) and film-forming emulsion (FFE) from SFPI

FFS from SFPI was prepared as described by Tongnuanchan *et al.* (2011) with slight modifications. SFPI (1.5% protein content) in distilled water was homogenised at 3,000 rpm for 3 min. The mixture was added with glycerol at 50 and 65% (w/w of protein content), followed by stirring for 5 min. The mixture was adjusted to pH 3.0 using 1 M HCl, and stirred for 30 min to solubilise the protein.

To prepare FFE, the squalene at 30% (w/w of protein content) was mixed with Tween-20 at 25% (w/w of squalene content) (Ali *et al.*, 2019) before addition into the SFPI-FFS prepared as described earlier, followed by homogenisation (13,000 rpm, 3 min).

Both FFS and FFE of SFPI were degassed in a sonicating bath for 5 min before casting. FFS or FFE (20.5 g) were cast onto a plastic circular plate (90 mm diameter) and air-blown at room temperature for 12 h, and then dried in an environmental chamber (WTB Binder, Tuttlingen, Germany) for 48 h (25°C and 50% relative humidity). The dried SFPI films were peeled off and analysed.

Analyses of films

Film thickness and optical properties

The average thickness of film was determined by a digital micrometre (Model GT-313-A; Gotech Testing Machines Inc, Taiwan) from five random locations on each of ten similar film samples.

The colour coordinates (L^*, a^*, and b^*) were determined using a CIE colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) with D_{65} (day light). Total colour difference (ΔE^*) was calculated using Eq. 1 (Gennadios *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \text{(Eq. 1)}$$

where, ΔL^*, Δa^*, and Δb^* = differences of colour coordinates of the sample from those of the white standard (L^* = 92.82, a^* = -1.24, and b^* = 0.46).

The light transmission of SFPI film was determined by a UV-vis spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan) (Shiku *et al.*, 2004) at 600 nm wavelength. The transparency value was determined using Eq. 2 (Han and Floros, 1997):

$$Transparency\ value = (-log\ T_{600}) / x \quad \text{(Eq. 2)}$$

where, T_{600} = fractional light transmission at 600 nm, and x = average thickness (mm) of film sample.

ATR-FTIR analysis

The functional groups and molecular interactions of SFPI films were assessed by using attenuated total reflectance Fourier transformed infrared (ATR-FTIR) spectroscopy as described by Nilsuwan *et al.* (2021). Spectra in the wavenumber range of 650 - 4000 cm^{-1} at 32 scans with resolution of 4 cm^{-1} were collected.

Mechanical properties

The tensile strength (TS), Young's modulus (YM), and elongation at break (EAB) were measured as described by Tongnuanchan *et al.* (2011). Ten samples (20 mm × 50 mm) were tested using a universal testing machine (Lloyd Instrument, Hampshire, UK) equipped with a 100 N load cell. The initial span was 30 mm between the grips, and the cross-head speed of 30 mm/min was used.

Water vapour permeability (WVP) and oxygen permeability (OP)

The WVP and OP of SFPI films were examined following the standard methods of ASTM-E-96 and ASTM D3985-05, respectively, as described by Nilsuwan *et al.* (2021). The tests were performed at 25°C.

Thermal properties

The degradation temperature (T_d), weight loss (ΔW), and remaining residue of SFPI films were evaluated using a thermo-gravimetric analyser (TGA7; PerkinElmer, Norwalk, CT, USA) from 50 to 800°C at a rate of 10°C/min (Nilsuwan *et al.*, 2016).

Statistical analysis

The experiments followed a completely randomised design (CRD) in triplicate. Analysis of variance (ANOVA) was run, and mean comparisons of data were done with Duncan's multiple range test using the SPSS package (SPSS for Windows, SPSS Inc., Chicago, IL, USA).

Results and discussion

Physicochemical properties

In general, films prepared from salmon frame protein isolate (SFPI) were easy to handle like plastic films. These films did not have fishy or other off-odour. Thicknesses of the SFPI films containing glycerol at 50 and 65% with and without the addition of 30% squalene are shown in Table 1. The thickness was in the range 0.053 - 0.064 mm. No difference ($p > 0.05$) in thickness was observed between films containing 50 and 65% glycerol when they had the same squalene content. The addition of 30% squalene generally increased the thickness of the film, particularly when 65% glycerol was used. Long chained squalene and glycerol molecules could be entangled with protein chains in the film matrix (Ali *et al.*, 2019), leading to an expansion or protrusion of the network in the film. This manifested in the increase in film thickness.

The colour coordinates of the SFPI films prepared with glycerol and squalene at different concentrations are presented in Table 1. Without squalene, SFPI films had L^* (lightness) in the range of 84.31 - 85.12. As squalene was added, a slightly lower L^*-coordinate was observed, indicating that the squalene could decrease lightness of the film. This might be associated with the pale-yellow natural colour of squalene (Ali *et al.*, 2019). All SFPI films exhibited a^* in the range from -2.55 to -1.47. A reduced a^* ($p < 0.05$) was noted when the film was added with squalene, indicating lower redness of the

Table 1. Film thickness, colour parameter, light transmittance at 600 nm (T_{600}), and transparency value of SFPI films with different glycerol contents with and without 30% squalene.

Glycerol content (%)	Squalene content (%)	Thickness (mm)	Colour parameter				T_{600} (%)	Transparency value
			*L**	*a**	*b**	Δ*E**		
50	0	0.053 ± 0.006^{a}	85.12 ± 0.59^{b}	-1.47 ± 0.08^{b}	9.08 ± 0.55^{a}	10.02 ± 0.68^{a}	47.06 ± 7.21^{b}	6.25 ± 1.26^{a}
50	30	0.061 ± 0.003^{ab}	83.42 ± 0.32^{a}	-2.47 ± 0.07^{a}	16.19 ± 0.59^{cd}	17.13 ± 0.63^{de}	21.83 ± 2.66^{a}	10.28 ± 0.80^{b}
65	0	0.056 ± 0.003^{a}	84.31 ± 0.12^{a}	-1.56 ± 0.14^{b}	10.13 ± 0.86^{a}	11.34 ± 0.67^{b}	41.66 ± 5.44^{b}	6.83 ± 0.10^{a}
65	30	0.064 ± 0.003^{b}	83.38 ± 0.91^{a}	-2.55 ± 0.10^{a}	16.86 ± 0.55^{d}	18.21 ± 0.10^{e}	17.96 ± 1.15^{a}	11.73 ± 0.43^{c}

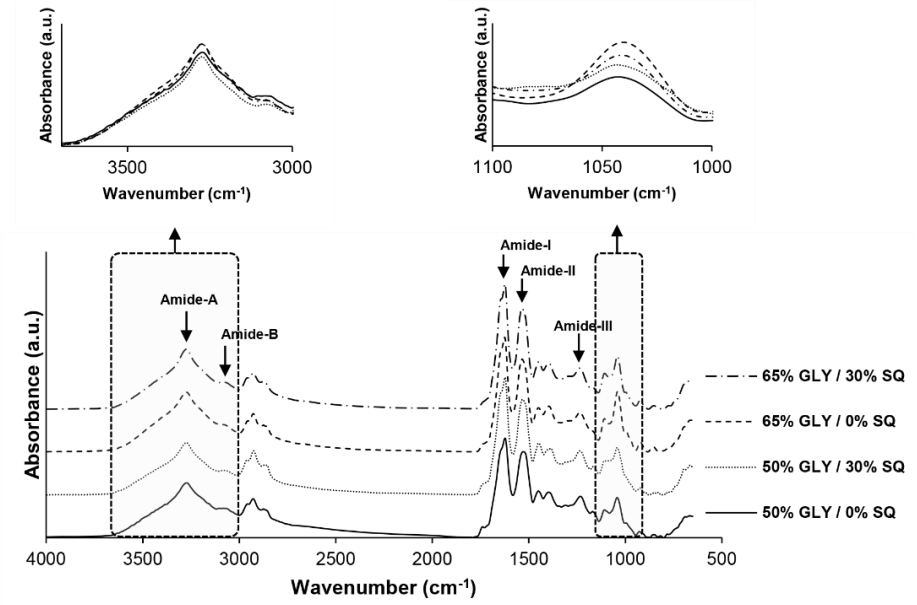
Values are mean ± SD of triplicate (n = 3) determination. Means followed by different lowercase superscripts in the same column are significantly different ($p < 0.05$).

film when compared with film prepared without squalene. Moreover, *b** of all films was in the range of 9.08 - 16.86. The addition of squalene also increased the yellowness and total difference in colour of films as indicated by larger *b** and Δ*E** than films prepared without squalene. This could be related to the yellowness of squalene (*b** = 6.43) (Ali *et al.*, 2019).

The transmission of visible light at 600 nm wavelength and transparency value of the various SFPI films are summarised in Table 1. All SFPI films exhibited high transparency. It was noted that a higher transparency value yielded less transparent or more opaque film. SFPI films with 50 and 65% glycerol had light transmission of 47.06 and 41.66%, respectively, while the transparency value was in the range 6.25 - 6.83. Typically, glycerol as a plasticiser decreases intermolecular interactions of protein chains, thus decreasing compactness of the film matrix. This could facilitate light transmission in the films (Tongnuanchan *et al.*, 2011). However, in the present work, no difference ($p > 0.05$) in light transmission or transparency was observed between films containing glycerol at 50 and 65%. This might be associated with the high levels of glycerol used in the present work (50 and 65%), for which the difference in transmission was negligible. With the addition of 30% squalene, lower ($p < 0.05$) visible light transmission (17.96 - 21.83%) along with higher ($p < 0.05$) transparency value (10.28 - 11.73) of SFPI films were observed (Table 1). The results indicated that films containing squalene became opaque with decreased light transmission. This was plausibly related to the light scattering by squalene in the film (Ali *et al.*, 2019). Thus, both levels of glycerol and squalene directly affected light transmission and transparency of the SFPI films.

ATR-FTIR

FTIR spectra and their major peak wavenumbers for the SFPI films containing 50 and 65% glycerol with and without 30% squalene are depicted in Figure 1.



Region	Peak wavenumber (cm^{-1})			
	50% GLY / 0% SQ	50% GLY / 30% SQ	65% GLY / 0% SQ	65% GLY / 30% SQ
Amide-A	3273	3273	3275	3274
Amide-B	3084	3078	3086	3082
	2928	2925	2927	2928
	2874	2875	2874	2875
Amide-I	1624	1623	1625	1624
Amide-II	1527	1532	1535	1535
Amide-III	1233	1234	1236	1236
	1043	1043	1040	1041

Figure 1. FTIR spectra of SFPI films with different glycerol contents with and without 30% squalene. GLY = glycerol; and SQ = squalene.

The FTIR spectra of all film samples had similar peaks characteristic of protein at the wavenumbers 3273 - 3275, 3078 - 3086, 1623 - 1625, 1527 – 1535, and 1233 - 1236 cm^{-1}, representing the amide-A (NH-stretching coupled with hydrogen bonding), amide-B (stretching vibrations of CH/CH_2), amide-I (C=O stretching of amide), amide-II (N-H group bending and C-N group stretching), and amide-III (in-plane bending of C-N and N-H of bound amide, or vibrations from CH_2 of glycine backbone and proline side-chains), respectively (Muyonga *et al.*, 2004; Kong and Yu, 2007; Etxabide *et al.*, 2016; Kaewprachu *et al.*, 2017). The addition of squalene to SFPI film resulted in a slight shift of the amide-A and amide-I to lower wavenumbers, thus suggesting interactions between myofibrillar protein chains and squalene mostly through hydrogen bonding. In addition, Tween-20 as an emulsifier was preferably localised on the surface of squalene, and might have interacted by hydrogen bonding with -NH and C=O groups of protein chains, thus contributing to the aforementioned wavenumber shift. Moreover, the shift to lower wavenumbers of amide-B from 3084 - 3086 to 3078 - 3082 cm^{-1} was also observed. This might be related to the interactions between myofibrillar proteins and squalene by hydrophobic-hydrophobic interactions. Nevertheless, when glycerol and squalene were added, amide-II and amide-III peaks shifted to higher wavenumbers (1532 - 1535 and 1234 - 1236 cm^{-1}), which could be related to the decreased interactions in film matrix from dispersion of squalene and glycerol molecules (Ali *et al.*, 2019). Xie *et al.* (2006) documented that the differences in wavenumber of the peaks could correlate with the interactions between functional groups of proteins and the added components. Furthermore, the peak appearing around at 1040 - 1043 cm^{-1} was also detected in all SFPI films, indicating OH groups. This peak indicated the presence of glycerol rich in OH groups as plasticiser in the films (Bergo and Sobral, 2007). As a result, a higher amplitude of this peak was found for the films containing 65% glycerol, as compared to those having 50% glycerol. Additionally, SFPI films also exhibited absorption peaks in the wavenumber ranges 2874 - 2875, 2925 - 2928, and 3078 - 3086 cm^{-1}. Those peaks are typically attributable to symmetric and asymmetric stretching of CH groups along the polymer chain (Ma *et al.*, 2012). When 50% glycerol was added, lower wavenumbers were observed for the aforementioned peaks as compared to those added with 65% glycerol, regardless of squalene addition. This indicated a higher hydrophobic-hydrophobic interaction in the film with the lower amount of plasticiser. Moreover, the addition of squalene also lowered the wavenumbers of these peaks, especially when 50% glycerol was used. Hydrophobic-hydrophobic interactions of protein-protein and protein-squalene type were more pronounced at the lower level of glycerol which acted as a barrier. Therefore, the FTIR spectra confirmed that the addition of squalene influenced the SFPI film network via interactions with proteins.

Mechanical properties

Mechanical properties of SFPI films containing glycerol at 50 and 65% with and without 30% squalene are shown in Table 2. In the absence of squalene, TS and YM of the films with 50 and 65% glycerol were in the ranges of 2.95 - 3.43 and 125.67 - 179.0 MPa, respectively. Glycerol levels showed no marked effect on TS and YM ($p > 0.05$). This was likely because glycerol was used only at a high level (50 and 65%). Squalene significantly enhanced the

Table 2. Tensile strength (TS), Young's modulus (YM), elongation at break (EAB), water vapour permeability (WVP), and oxygen permeability (OP) of SFPI films with different glycerol contents with and without 30% squalene.

Glycerol content (%)	Squalene content (%)	TS (MPa)	YM (MPa)	EAB (%)	WVP (× 10^{-10} g m/m^2 s Pa)	OP (× 10^{-18} mol m/m^2 s Pa)
50	0	3.43 ± 0.71^{a}	179.00 ± 23.26^{ab}	6.25 ± 0.38^{a}	3.74 ± 0.40^{a}	6.74 ± 0.66^{c}
50	30	16.83 ± 0.12^{e}	921.33 ± 73.79^{d}	50.56 ± 6.25^{b}	1.16 ± 0.03^{d}	2.32 ± 0.53^{a}
65	0	2.95 ± 0.88^{a}	125.67 ± 22.30^{a}	5.39 ± 1.11^{a}	4.86 ± 0.40^{b}	4.52 ± 0.47^{b}
65	30	8.29 ± 0.20^{d}	414.33 ± 28.36^{c}	47.77 ± 3.26^{b}	1.97 ± 0.60^{c}	1.76 ± 0.44^{a}

Values are mean ± SD of triplicate ($n = 3$) determination. Means followed by different lowercase superscripts in the same column are significantly different ($p < 0.05$).

strength and elasticity of the films. When 30% squalene was added, increases ($p < 0.05$) in TS and YM occurred at both glycerol levels. TS and YM at 16.83 and 921.33 MPa were found for the SFPI film containing 50% glycerol, while the values were 8.29 and 414.33 MPa for the film containing 65% glycerol. It is worth noting that the addition of squalene strengthened the film by enhancing mechanical resistance of the SFPI film. This might be related to the entanglement of long chained squalene in the film network (Bavisetty and Narayan, 2015). Additionally, no change in EAB was found in the SFPI film as glycerol concentration was increased from 50 to 65% (Table 2). However, increase ($p < 0.05$) in EAB from 5.39 - 6.25 to 47.77 - 50.56% were obtained when SFPI films were prepared with 30% squalene, irrespective of the glycerol concentration used. This could be caused by the intermolecular interactions between SFPI and squalene, by physical chain entanglement, and by secondary bonding interactions as evidenced in FTIR spectra. This could promote reinforcement during extension before film rupture (Ali *et al.*, 2019). Therefore, the addition of squalene in SFPI could improve mechanical resistance of the film, giving stronger and more elastic films.

Barrier properties

The WVP and OP of the films were analysed in order to estimate the water vapour and oxygen gas barrier abilities of the SFPI films containing glycerol at 50 and 65% with and without 30% squalene, and the results are summarised in Table 2. The WVP of SFPI films with 50 and 65% glycerol were 3.74 and 4.86×10^{-10} g m/m^2 s Pa, respectively. A higher ($p < 0.05$) WVP was attained by the films containing 65% glycerol, as compared to those with 50% glycerol. This was mainly due to the high hydrophilicity of film caused by a large amount of glycerol. Regarding the films made with squalene, lower WVP ($p < 0.05$) was found for the films containing 50 and 65% glycerol as compared to those made without squalene. The lowest WVP (1.16×10^{-10} g m/m^2 s Pa) ($p < 0.05$) was found for SFPI film containing 50% glycerol along with 30% squalene, thus suggesting that the addition of squalene effectively improved water vapour barrier properties of an SFPI film. The distribution of squalene throughout the films could increase hydrophobicity of the SFPI film (Ali *et al.*, 2019). It was apparent that when high glycerol amount (65%) was used, the hydrophilicity could still increase the WVP of the film, despite the squalene component. Thus, hydrophilic glycerol should be used at a lower level.

The OP relates to avoiding lipid oxidation of the packaged product (Cho *et al.*, 2010). Table 2 shows the OP values of the various SFPI films. Generally, the OP of SFPI film varied from 1.76 to 6.74×10^{-18} mol m/m^2 s Pa, depending on the concentration of glycerol and the squalene added. In this regard, the OP of SFPI film made with 50% glycerol was 6.74×10^{-18} mol m/m^2 s Pa, while the film with 65% glycerol had an OP of 4.52×10^{-18} mol m/m^2 s Pa. Increasing glycerol concentration improved the oxygen barrier property of the film. This might be related to glycerol increasing the hydrophilicity of SFPI film, thus reducing the affinity to a hydrophobic gas like oxygen. Additionally, the addition of 30% squalene also decreased ($p < 0.05$) the OP further to 2.32 and 1.76×10^{-18} mol m/m^2 s Pa in the SFPI films containing 50 and 65% glycerol, respectively. Thus, squalene could enhance the oxygen barrier properties of SFPI films. This is in contrast to some prior studies reporting that oil and lipid could disperse throughout the matrix and lead to discontinuous structures in protein films. As a result, the films became uncompact, and allowed oxygen to migrate easily (Nilsuwan *et al.*, 2017). However, in the present work, the long hydrocarbon chains of squalene in film matrix might have hydrophobic-hydrophobic interactions by itself or with the hydrophobic parts of protein chains. This compact network had improved ability to block oxygen gas molecules for penetrating through the film.

Thermal properties

To evaluate the thermal stability of SFPI films containing different levels of glycerol (50 and 65% by weight of protein) with and without 30% squalene, thermo-gravimetric analysis (TGA) was conducted, and the results on weight loss (WL, ΔW), thermal degradation temperature (T_d), and residue are summarised in Table 3. Three main stages of WL were noted for all SFPI films. The first stage WL of 5.02 - 6.12% was obtained at temperature ($T_{d1,onset}$) of 57.32 - 71.11°C. WL in this stage was from the evaporation of moisture (Nilsuwan *et al.*, 2016). The highest $T_{d1,onset}$ (71.11°C) was observed for SFPI films prepared with 30% squalene and 50% glycerol.

Table 3. Thermal degradation temperature (T_d) and weight loss (ΔW) of SFPI films with different glycerol contents with and without 30% squalene.

Glycerol content (%)	Squalene content (%)	Δ_1		Δ_2		Δ_3		Residue (%)
		$T_{d1,onset}$	ΔW_1	$T_{d2,onset}$	ΔW_2	$T_{d3,onset}$	ΔW_3	
50	0	57.42	6.12	216.02	15.32	316.41	59.22	19.34
50	30	71.11	5.07	220.60	16.61	317.63	57.52	20.80
65	0	57.32	5.12	202.35	21.73	319.70	55.43	17.72
65	30	58.78	5.02	199.52	26.15	322.59	51.05	17.78

Values are mean ± SD of triplicate (n = 3) determination. Means followed by different lowercase superscripts in the same column are significantly different ($p < 0.05$).

This might be due to the higher proportion of squalene, which was more concentrated in this sample, thus leading to higher T_{d1}. Films containing squalene had lower ΔW_1, thus indicating less water in the film matrix (Ali *et al.*, 2019). The second stage of WL (ΔW_2 = 15.32 - 26.15%) was found at $T_{d2,onset}$ in the range of 199.52 - 220.60°C. It was noticed that films containing higher level of glycerol (65%) exhibited higher WL (21.73 - 26.15%) along with lower thermal degradation temperature ($T_{d2,onset}$ = 199.52 - 202.35°C), related to the degradation of low molecular weight protein fractions along with the loss of structurally bound water and plasticiser (Wetzel *et al.*, 1987). Furthermore, the third stage of WL (ΔW_3 = 51.05 - 59.22%) was observed at $T_{d3,onset}$ of 316.41 - 322.59°C. WL in this stage was associated with the decomposition of highly compact proteins. Higher $T_{d3,onset}$ and lower ΔW_3 were observed for the films made with squalene, as compared to those without squalene, thus suggesting that the squalene might have inter- and intra-molecular interactions with protein in the film matrix, hence giving a stronger film network. This led to improved heat resistance and reduced weight loss. This was also in line with the higher mechanical resistance seen in Table 2. With a lesser amount of glycerol, proteins had more interactions hence strengthening the film matrix and increasing initial thermal degradation temperature (T_{d2}). Additionally, SFPI films with 50% glycerol with and without squalene had higher residue mass than the other films containing 65% glycerol. This confirmed that films made with squalene, especially those with 50% glycerol, had stronger film network than without squalene. Also, glycerol content influenced thermal properties of the films. This effect was more pronounced at the high thermal degradation temperatures. Glycerol at a high level was still retained to some extent, and helped stabilise the network as shown by high T_d.

Conclusion

The properties of SFPI films could be improved effectively by the addition of squalene in combination with a proper amount of glycerol. As 50% glycerol was used, film made with 30% squalene had a high mechanical resistance, high water vapour-, oxygen-, and visible light-barrier properties, as well as good thermal stability. The addition of squalene at 30% could improve TS, EAB, water vapour, and oxygen gas barrier properties of the SFPI film containing 50% glycerol by approximately 390, 709, 69, and 65%, respectively, as compared to the control (film containing 50% glycerol without squalene). Therefore, SFPI film containing 50% glycerol and 30% squalene could be a promising candidate for edible or biodegradable food packaging with good properties. The edible film prepared from SFPI can potentially be used to develop edible packaging, such as pouches or bags for condiments, or wrapping material for food protection or preservation.

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