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Water Vapor Permeability of Edible Films Prepared from Fish Water Soluble Proteins as Affected by Lipid Type*1

Munehiko Tanaka*2, Shoichiro Ishizaki*2, Toru Suzuki*2 and Rikuo Takai*2

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Abstract: Edible composite films were prepared from fish water soluble proteins (FWSP) and lipid materials. The effect of different types of fatty acids, *i.e.*, lauric acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid together with beeswax and edible oils (peanut oil, corn oil, salad oil, cod liver oil) at 20 or 40% (w/w) of protein on tensile strength, elongation at break, and water vapor permeability (WVP) was investigated. In general, the incorporation of lipid materials resulted in the decrease of tensile strength, the increase of elongation at break, and the reduction of WVP with some exceptions. Overall effect of unsaturated fatty acids was greater than that of saturated fatty acid or edible oils. Especially, WVP of FWSP film was significantly decreased by the incorporation of 40% oleic acid with a reduction of around 4.8 times.

Key words: *Fish water soluble proteins*, *Edible film*, *Water vapor permeability*, *Fatty acids*, *Edible oils*

Introduction

Edible films prepared from many protein sources have recently attracted a lot of attention for the use in food protection and preservation, since they provide a number of advantages over synthetic films, including biodegradable and environmental characteristics.[1–3] Although edible films obtained from such substances as proteins, starch, cellulose, pectin, alginate, and carrageenan tend to have stronger texture, their water vapor barrier characteristics are usually poor because of their hydrophilic nature.[4,5]

Water vapor permeability (WVP) of edible films can be improved by incorporating such lipid materials as neutral lipids, fatty acids or waxes.[6–8] Two types of preparation of protein films with lipid materials have been reported, *i.e.*, bilayer films in which a hydrophobic lipid layer is laminated over a film[6,9] and emulsion films in which the lipid material is uniformly dispersed throughout the film.[10,11] Although emulsion films are not so effective barrier as bilayer films, they possess superior mechanical properties.[3] The incorporation of emulsion droplets in the film increases the distance traveled by water molecules which diffuse through the film, thereby decreasing WVP.[12] About all that can be concluded from all of the aforementioned studies is that lipid materials seem to be the most effective barrier to the movement of water. By taking advantage of the good emulsifying properties of proteins, edible films in which the lipid materials are distributed in particles have been formed.

The main objective of this study was to investigate changes in selected properties of fish water soluble protein (FWSP) films resulting from the incorporation of various lipid materials. The influence of different types and amounts of lipid materials on the mechanical properties and WVP of FWSP-lipid composite films was examined.

Materials and Methods

Materials

Beeswax, lauric acid ($C_{12:0}$, min. 99.0%), palmitic acid ($C_{16:0}$, min. 95.0%), stearic acid ($C_{18:0}$, min. 99.0%), oleic acid ($C_{18:1}$, min. 60.0%), linoleic acid ($C_{18:2}$, min. 88.0%), linolenic acid ($C_{18:3}$, min. 60.0%), glycerol and lecithin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Corn oil and peanut oil were obtained from Hayashi Chemicals, Ltd. (Tokyo). Cod liver oil and salad oil were purchased from Toho Chemicals, Ltd.(Tokyo) and Nisshin Oil, Ltd.(Tokyo), respectively.

Preparation of Fish Water Soluble Proteins

Preparation of fish water soluble proteins (FWSP) from the flesh of blue marlin *Makaira mazara* was carried out as outlined in the preceding paper.[13] Briefly, FWSP was extracted from blue marlin flesh by homogenizing with 5 volumes of 0.1 M Tris-HCl buffer (pH 7.6).

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*2 Department of Food Science and Technology, Tokyo University of Fisheries, 5–7, Konan 4-chome, Minato-ku, Tokyo 108–8477, Japan.

After the homogenates were centrifuged, the supernatants were collected and dialyzed against cold distilled water. Proteins in the dialyzate were used as FWSP in this study.

Preparation of Films

Film-forming solutions were prepared from denatured solution of 3% FWSP (heating at 70°C for 15 min) at pH 10 with glycerol at 50% (w/w) of FWSP as a plasticizer. The lipid materials together with 10% (w/w) of lecithin to lipids were melted in the heated FWSP solution and homogenized with a HG 30 homogenizer (Hitachi, Ltd, Tokyo) at 15,000 rpm for 1 min. The temperature of homogenization was 80°C for beeswax and stearic acid.

The prepared film-forming solutions were cast by pipetting 4 m*l* onto rimmed silicone resin plate (50×50 mm^2) setting on a level surface and dried in a ventilated oven at 25°C for 20 h. After the water was evaporated, resulting FWSP films were manually peeled off.

Film thickness was measured using a micrometer (Dial Pipe Gauge, Peacock Co., Tokyo) to the nearest 0.005 mm at 8 random locations around the film. Precision of the thickness measurements was ±5%.

Mechanical Property Measurements

Prior to the testing of mechanical properties, the films were conditioned for 48 h at relative humidity (RH) of 50±5% and 25±0.5°C (Environmental Chamber Model H110K-30DM; Seiwa Riko Ltd., Tokyo). Tensile strength and percentage of elongation at break were determined using a Texture Analyzer (TA.XT2 Stable Micro Systems, UK), operated according to the American Society for Testing and Materials (ASTM) standard method D 882–22.[14] Two rectangular strips (width 20 mm; length 45 mm) were cut from each FWSP film to measure mechanical properties. Initial grip separation and cross-head speed were set at 30 mm and 0.5/s, respectively. Tensile strength (MPa) was calculated by dividing the maximum load (N) necessary to pull the sample film apart by the cross-sectional area (m^2). Average thickness of the film strip was used to estimate the cross-sectional area of the sample. Percentage of elongation at break was calculated by dividing film elongation at the moment of rupture by the initial grip length of samples multiplied by 100%. A total of 8 samples were tested for each film type.

Water Vapor Permeability Measurements

Water vapor permeability values (WVP) were measured using a modified ASTM method[14] reported by Gontard *et al.*[15] FWSP film was sealed in a glass permeation cup containing silica gel (0% RH) with silicone vacuum grease and an O ring to hold the film in place. Silica gels were heated at 180°C for at least 3 h prior to use for the determination. The cups were placed in a desiccator cabinet with distilled water at 30°C. The cups were weighed at intervals of 1 h over a 12 h period and WVP ($g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) of the film was calculated as follows: $WVP=(w \cdot x)/A \cdot t \cdot (P_2–P_1)$,[16] where w is the weight gain of the cup (g), x is the film thickness (mm), A is the area of exposed films (m^2), t is the time of gain (s), and ($P_2–P_1$) is the vapor pressure differential across the film (Pa). This entire procedure was repeated twice, for a total of 9 tests on each film type.

Results and Discussion

Tensile Strength and Elongation

High tensile strengths are generally necessary for edible films in order to withstand the normal stress encountered during their application, subsequent shipping, and food handling. However, flexibility of edible films, *i.e.* elongation at break should be adjusted according to the intended application of edible films. Tensile strength of control FWSP (3%) film (with glycerol at 50% of FWSP) was 5.15±0.57 MPa. This value is comparable to our previously reported value.[13] Tensile strength of films was changed by the incorporation of lipid materials together with 10% (w/w) lecithin to lipids as shown in Figs. 1–3. Among saturated fatty acids (lauric, palmitic, stearic

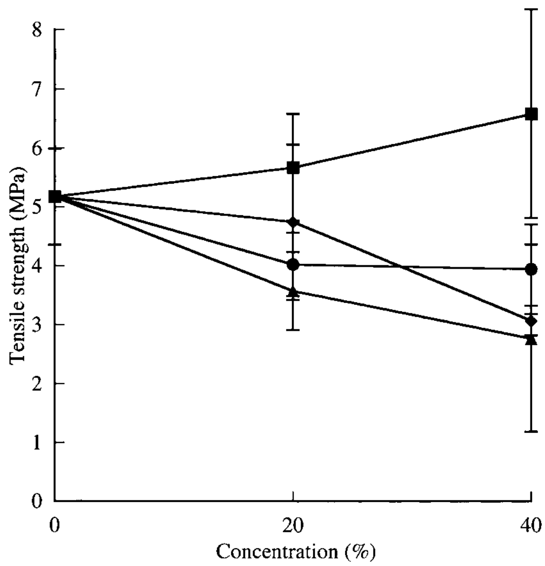

Figure 1. Changes of tensile strength of fish water soluble protein-saturated fatty acid composite films as a function of fatty acid concentration.
■: Lauric acid, ●: Palmitic acid, ▲: Stearic acid, ◆: Beeswax

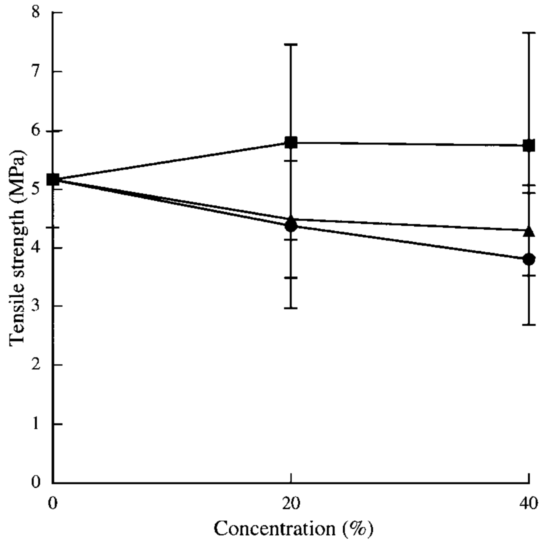

Figure 2. Changes of tensile strength of fish water soluble protein-unsaturated fatty acid composite films as a function of fatty acid concentration.
■: Oleic acid, ●: Linoleic acid, ▲: Linolenic acid

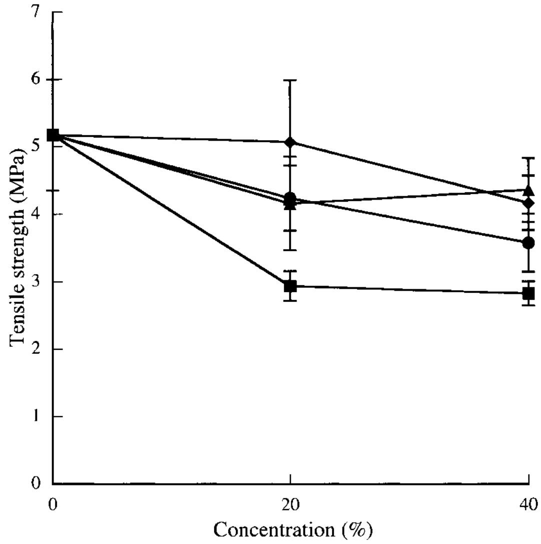

Figure 3. Changes of tensile strength of fish water soluble protein-edible oil composite films as a function of oil concentration.
■: Peanut oil, ●: Corn oil, ▲: Salad oil, ◆: Cod liver oil

acids) and beeswax used in this study, only lauric acid increased tensile strength of composite (Fig. 1) films even though this lipid material is solid at room temperature in films.

Figure 2 presents the effect of unsaturated fatty acids (oleic, linoleic, linolenic acids) on tensile strength of FWSP films. Although the addition of oleic acid led to slight increase of tensile strength, other unsaturated fatty acids reduced tensile strength. From the results given in Fig. 2, it seems that the unsaturation degree of the fatty acids does not influence on tensile strength of FWSP-fatty acid composite films. These findings support those of Hagenmaier and Shaw[17] who studied the formation of hydroxypropylmethyl cellulose-fatty acid film.

In this study, edible oils which are used for ordinary cooking (peanut, corn, salad, and cod liver oils) were also selected as lipid materials from the practical standpoint. The effect of incorporated edible oils on tensile strength of composite films is illustrated in Fig. 3. Among edible oils used, peanut oils gave the smallest tensile strength.

Elongation at break of control FWSP film was 67.8±18.9%.This value agreed well with our previously reported value.[13] Elongation at break of FWSP films was greatly affected by incorporating lipid materials (Figs. 4–6). Figure 4 shows the effect of saturated fatty acids on elongation at break of FWSP films. The incorporation of beeswax remarkably increased the flexibility of films, while that of lauric acid did not cause any changes on elongation at break. On the contrary, it is obvious from

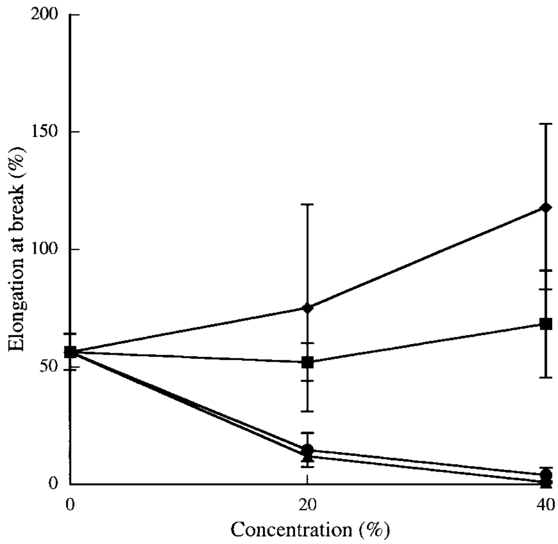

Figure 4. Changes of elongation at break of fish water soluble protein-saturated fatty acid composite films as a function of fatty acid concentration.
■: Lauric acid, ●: Palmitic acid, ▲: Stearic acid, ◆: Beeswax

this figure that the increasing molecular chain length of saturated fatty acids drastically reduced the film flexibility. At higher concentrations of palmitic and stearic acids, the films were no longer flexible and could not be

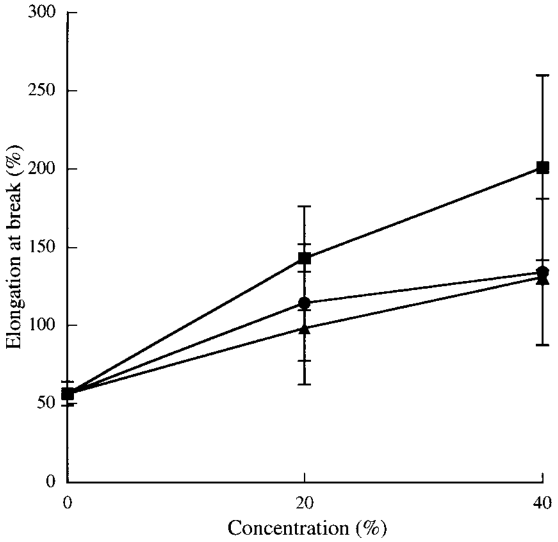

Figure 5. Changes of elongation at break of fish water soluble protein-unsaturated fatty acid composite films as a function of fatty acid concentration.
■: Oleic acid, ●: Linoleic acid, ▲: Linolenic acid

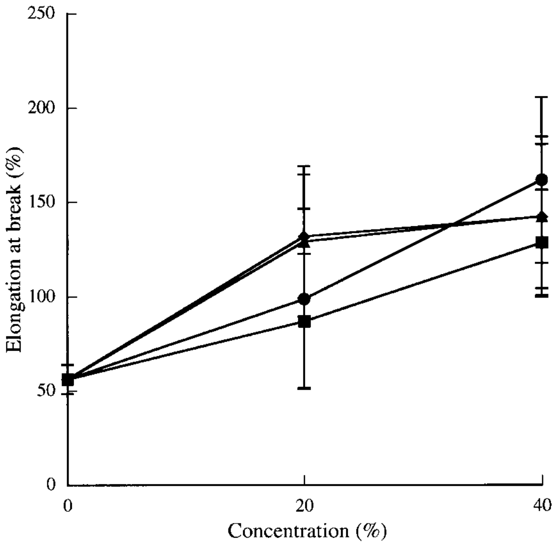

Figure 6. Changes of elongation at break of fish water soluble protein-edible oil composite films as a function of oil concentration.
■: Peanut oil, ●: Corn oil, ▲: Salad oil, ◆: Cod liver oil

handled for measuremens. This could be owing to their high melting points (palmitic acid: 60–63°C, stearic acid: 68–71°C). As with tensile strength data, the lower elongation at break values indicate that composite films of FWSP-saturated fatty acid had less structural integrity than control FWSP films. These results are consisting with the previous finding of Rhim *et al.*[11])

On the other hand, the addition of unsaturated fatty acids emulsified in FWSP casting solutions improved elongation at break, especially the incorporation of oleic acid was quite effective (Fig. 5). Among edible oils, salad oil and cod liver oil brought about the increase of elongation at break at the concentration of 20%, but elongation at break of composite films with 40% oils was quite similar independent of the type of edible oils (Fig. 6). From the results described above, it is plausible that the incorporation of unsaturated fatty acids caused a more profound effect on elongation at break of the FWSP composite films than the saturated fatty acids.

Water Vapor Permeability

WVP of the control FWSP film was $1.187\times10^{-10}\pm 0.186\times10^{-10}\,g\cdot m^{-1}\cdot s^{-1}\cdot Pa^{-1}$. This value is in good agreement with previously reported value.[13]) It has been reported that WVP of composite films varies greatly by orientation of molecules within a film,[18,19]) but the effect of film orientation on WVP was not observed with FWSP-lipid material composite films in our study (data not shown).

As shown in Figs. 7–9, it is obvious that the type of lipid materials and their concentration markedly influenced WVP of FWSP-lipid composite films. The incorporation of all lipid materials used up to 20% in this study reduced WVP. Further addition of lipid materials seemed to have less effect on reduction of WSP. It suggests that the configuration of nonpolar components in the structural matrix of proteins is such that the effect of polar groups of proteins and glycerol cannot be limited by incorporating more lipid materials. Fig. 7 presents the effect of saturated fatty acids on WVP of composite films. In general, WVP of composite films is expected to decrease with increasing length of lipid hydrocarbon chain, because the hyrdrophobicity of the fatty acid regulates the water vapor transmission rate through fatty acid composite films[10,11]) and fatty acids with shorter chain lengths tend to have greater chain mobility within the structure of composite films.[20]) However there was no effect of chain length of saturated fatty acids on WVP was observed in this study.

On the contrary, the addition of unsaturated fatty acids in casting film solution significantly reduced WVP of composite films. Generally speaking, low melting point lipids have poor water barrier properties.[4]) Furthermore, the hydrophilic groups of lipid molecules normally promote water molecule sorption, which may bring about easier water vapor migration through the film. The similar phenomenon was also observed in this study. The ef-

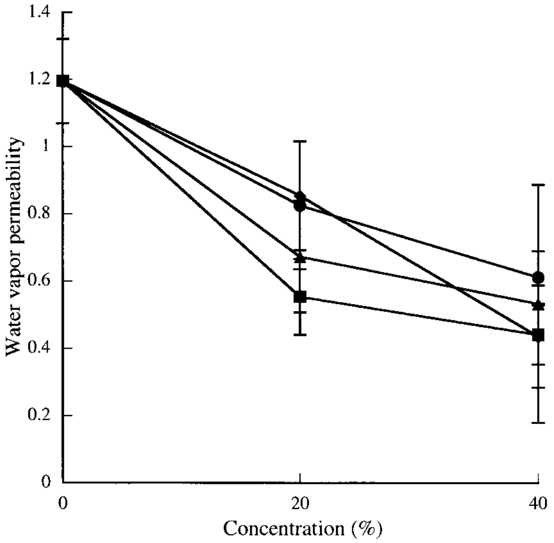

Figure 7. Changes of water vapor permeability ($\times 10^{-10}$ $g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) of fish water soluble protein-saturated fatty acid composite films as a function of fatty acid.
■: Lauric acid, ●: Palmitic acid, ▲: Stearic acid, ◆: Beeswax

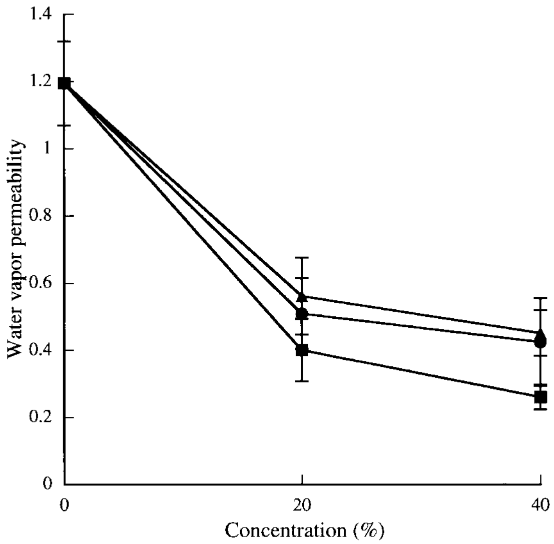

Figure 8. Changes of water vapor permeability ($\times 10^{-10}$ $g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) of fish water soluble protein-unsaturated fatty acid composite films as a function of fatty acid concentration.
■: Oleic acid, ●: Linoleic acid, ▲: Linolenic acid

fect of reducing WVP was dependent upon the degree of unsaturation of C_{18} fatty acids, the smaller the unsaturation degree, the smaller the WVP (Fig. 8). As much as

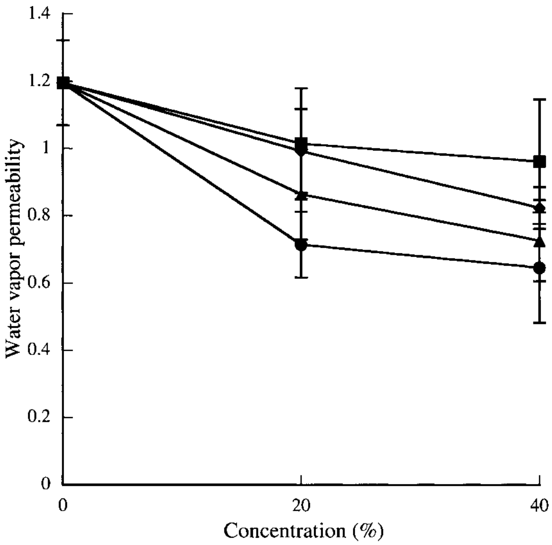

Figure 9. Changes of water vapor permeability ($\times 10^{-10}$ $g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) of fish water soluble protein-edible oil composite films as a function of oil concentration.
■: Peanut oil, ●: Corn oil, ▲: Salad oil, ◆: Cod liver oil

Table 1. Tensile strength, elongation at break, and water vapor permeability of protein films

Films	Tensile Strength (MPa)	Elongation at break (%)	WVP*1
FWSP	5.2	67.8	1.2×10^{-10}
FWSP+oleic acid	5.8	205	2.5×10^{-11}
Myofibrillar protein[22]	17.1	22.7	6.3×10^{-11}
Soybean[23]	5.9	107	1.7×10^{-10}
Wheat gluten[24]	2.7	197	4.3×10^{-9}
Rice bran[2]	16.4	—	9.2×10^{-9}
Corn zein[25]	0.4	—	1.0×10^{-7}
HDpolyethylene*2,26)	25.9	300	2.0×10^{-13}
LDpolyethylene*3,26)	12.9	500	7.7×10^{-13}

*1 Water vapor permeability: $g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-}$
*2 HD: High density
*3 LD: Low density

around 4.8 times reduction in WVP was observed for films with 40% oleic acid. Unsaturated fatty acids used in this study are liquid at room temperature, so they have a certain degree of mobility due to their double bonds which can reduce the density of lipid molecules in the structure of composite films.[21]

Figure 9 illustrates the influence of incorporating edible oils on WVP of FWSP-edible oil composite films, suggesting that corn oil was most effective to reduce

WVP among edible oils used. However, WVP of composite films with edible oils was larger than that with unsaturated fatty acids.

Conclusion

Among lipid materials tested in this study for preparing FWSP composite films, the incorporation of oleic acid was most effective from the standpoint of overall properties including tensile strength, elongation at break, and WVP. As a conclusion, those properties of FWSP-oleic acid composite film are compared with other protein films in Table 1.

Acknowledgement

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魚肉水溶性タンパク質から調製した可食性フィルムの水蒸気透過性におよぼす脂質添加の影響

田中宗彦・石崎松一郎・鈴木　徹・高井陸雄
（東京水産大学食品生産学科）

魚肉水溶性タンパク質 (FWSP) と各種脂質とを混合して可食性フィルムを調製した。ラウリン酸，パルミチン酸，ステアリン酸，オレイン酸，リノール酸，リノレン酸，蜜ロウ，各種食用油を FWSP に対して 20 あるいは 40% 添加した可食性フィルムの引っ張り強度，引っ張り伸び率，水蒸気透過性について測定した。脂質の添加により，いくつかの例外を除いて，引っ張り強度は減少，引っ張り伸び率は増大，水蒸気透過性は減少した。不飽和脂肪酸の添加は，飽和脂肪酸あるいは食用油の添加より効果的であり，特に 40% オレイン酸の添加は水蒸気透過性を約 5 分の 1 まで減少させることができた。

キーワード：魚肉水溶性タンパク質，可食性フィルム，水蒸気透過性，脂肪酸，食用油脂