The Physical Properties and Antioxidant Activity of Sarcoplasmic Protein/Gelatin Film with Marigold Flowers Extract

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***Abstract*—The objectives of this research were to produce sarcoplasmic protein/gelatin films incorporating with marigold flower extract and investigate their physical and chemical properties. The sarcoplasmic protein/gelatin film expressed pale yellow and cloudy appearance. The marigold flowers extract increased redness, yellowness and darkness of films. All sarcoplasmic protein/gelatin films exhibited low moisture content and water vapor permeability. The solubility decreased with the addition of marigold flowers extract components due to their hydrophobic properties. The surface of the film with extract exhibited irregularities in the film structure due to the heterogeneity in the polymer matrix with non-disulfide bonds. Additionally, the cross-linking of the extract in the film contributes to good mechanical properties that enhanced tensile strength and elongation. Moreover, the DPPH scavenging rate of the film with 0.2% marigold flowers extract content increased more than 50%. Therefore, it can be applied in food preservation packaging for extending shelf life.**

***Keywords*—film, gelatin, marigold flowers extract, sarcoplasmic protein**

I. Introduction

In the present, when buying food from department stores, fresh markets, or even small shops, it is often found that cling film or food wrap film is used to wrap food. This film is made from commercial plastic and wildly used in various products, including fresh produce such as vegetables and fruits, various types of meat, chilled food, and prepared food like baked goods, fried fish, and fried snacks. The use of cling film or food wrap film is primarily for packaging and sealing containers to prevent food from coming into contact with or being contaminated by various pollutants, such as bacteria or dust particles present in the environment. It helps reduce exposure to air, preserves the freshness of the food, prevents evaporation or loss of moisture in vegetables or fruits, and also helps prevent the loss of product weight. Food packaging plays a crucial role in maintaining the quality and extending the shelf life of food.

Plastic films take a long time to decompose, leading to increased pollution and severe environmental damage. The plastic used in film production is mostly derived from petroleum. When petroleum-based products are incinerated, they release carbon dioxide gas, which contributes to the greenhouse effect. However, in order to meet the demand for environmentally-friendly food packaging, there has been a development of innovative solutions that incorporate biodegradable materials in packaging production. Biodegradable films, composed of bioactive components, are an alternative option that is environmentally friendly compared to synthetic plastic packaging. Research had focused on enhancing the properties of these films by incorporating essential oils, aromatic compounds, or extracts to develop edible films. Natural biopolymers such as proteins, polysaccharides, and lipids have been utilized to create consumer-friendly films.

Additionally, proteins from fish processing, particularly Sarcoplasmic Proteins (SP), have emerged as an interesting option for film development. SP is a highly water-soluble protein that constitutes approximately 20–40% of the total protein in fish muscle. It consists of myoglobin, myogobulin, and various enzymes. This type of protein is typically removed during seafood processing, especially in the surimi production process, due to its negative impact on the properties of myofibrillar protein gels. However, incorporating this protein as an ingredient in film production could be a promising approach to reduce the environmental impact of fish processing waste and create value-added products. There is extensive research related to biodegradable packaging, and fish-derived protein films offer another widely utilization. Utilizing SP for film production, particularly from industrial by-products, can significantly increase the value of leftovers. Continuous development is being conducted, such as studying gels derived from sarcoplasmic protein [1], investigating antimicrobial properties [2], and exploring their application in edible films [3–5].

The extract from marigold flowers is an interesting option, and the key compounds found in marigold flowers are carotenoids, specifically lutein and zeaxanthin. These compounds exhibit antioxidant activity and perhaps also antimicrobial activity [6].

Therefore, the production of SP films should be formed with gelatin and combined with extracts from marigold flowers. Antioxidant activity of these extract was expected to retard the deterioration of perishable food from lipid oxidation. Therefore, production of gelatin film supplemented with SP was investigated in the present of Marigold Flower Extract (MFE). In addition, their physical and chemical properties were evaluated.

II. Materials and Methods

A. The Production of Sarcoplasmic Protein/Gelatin Film

Protein film production was done following the method of Jirukkakul [7] using 4 g of protein (SP:gelatin 1:1), MFE at 0.2% and 0.5% w/w in 96 mL of distilled water. The mixed solution was blended at 80 ºC for 30 min at a speed of 100 rpm. Then the speed was increased to 700 rpm for 30 min.

The 50 mL of solution was then poured onto the plate (Paisan Superlene, Co., Ltd., Bangkok, Thailand) and was left dry at 37 ºC for 24 h.

B. The Analysis of the Physical Properties and Antioxidant of Sarcoplasmic Protein/Gelatin Film

1) The Moisture Content (MC) of the film was determined by heating an aluminum cup in an oven at 105 ºC and then placing it in a desiccator for 24 h as performed previously [8]. Next, the film sample was placed in an aluminum cup and weighed (Wi). After that, the film with the aluminum cup was heated at 105 ºC for 24 h and was then weighed to determine the final weight (Wf). The moisture content was calculated from the Eq. (1).

$$MC(\%) = \frac{(Wi - Wf) \times 100}{Wi} \quad (1)$$

2) Colors were evaluated using a Hunter Lab Spectrocolorimeter. The film was closed with a test cup. Then the measurements were taken by placing the film at the test point of the machine. The results were reported with the Lab system.

3) The Water Vapor Permeability (WVP) of the film was conducted in accordance with ASTM E96 [9], by cutting the sample into a circle of 6 cm in diameter and then applying a thin sealant at the top of the test cup containing distilled water. The sample was then placed onto the cup, and then the top part of the cup was closed. The ring cover was closed by tightly turning the screw. The cup was weighed and placed in a WVP tester. The weight was recorded every 1 h until the weight difference was less than 1%. The WVP value was calculated as follows:

$$WVP = \frac{(G/t) \times thickness}{A \times (P_{A1} - P_{A2})} \quad (2)$$

where:

G / t = change rate of weight per time (g / day);

A = sample surface area (m^2);

$P_{A1} - P_{A2}$ = Internal and external pressure difference of test cup (kPa).

4) The Solubility (S) of film was determined following [10]. The film sample (20 mm × 20 mm) was heated in the hot air oven at 104 ºC for 24 h before weighing (*Wi*). Next, the heated film was put in an Erlenmeyer flask containing 50 mL of distilled water, and then the flask was placed in the shaker (around 2000 rpm) for 24 h at a temperature of 25 ºC. After that, the film sample was filtered through filter paper and heated in the hot air oven again at 104 ºC for 24 h and was then weighed to determine the final weight (*Wf*) Film solubility percentage was determined from the equation.

$$S(\%) = \frac{(Wi - Wf) \times 100}{Wi} \quad (3)$$

5) The surface morphology of the film was examined using Scanning Electron Microscopy (SEM) (JSM-5600, JEOL, Japan). Samples were coated with gold under a vacuum. SEM was carried out to give further insight on the coatings. The examination used an accelerating voltage of 20 kV, and the magnification was 100× for surface of film.

6) The mechanical test of the film was conducted in accordance with ASTM D882 [11]. To begin, a sample 10 mm in width and 150 mm in length was cut and was then stored at 23 ºC at 50% relative humidity for at least 40 h. Then, the sample was tested by firmly fastening the two ends of the sample to the head of the Texture Analyzer (model TA. XT plus, Stable Micro Systems, Ltd., UK). The test was set to have a pulling speed of 50 mm/min, a load cell of 0.5 kN, and a distance between the grips of 100 mm. The Tensile Strength (*TS*) and Elongation (*E*) value were determined from the equation.

$$TS = \frac{Fm}{A} \quad (4)$$

where:

Fm = maximum force (*N*);

A = cross-sectional area (m^2).

$$E(\%) = \frac{(Lf - Li) \times 100}{Li} \quad (5)$$

where:

Li = initial length (m);

Lf = final length (m).

7) Antioxidant activity was determined by DPPH method. The DPPH radical scavenging activity of the extracts were performed according to Jirukkakul [7]. The DPPH sample was prepared by using Eq. (6) to compare the weight of DPPH with the volume to be used. The volume was adjusted using methanol.

$$g = \frac{(m1 \times v1 \times mw)}{1000} \quad (6)$$

where:

g = the weight of DPPH;

m1 = the DPPH concentration;

v1 = the volume to be prepared;

mw = the molecular mass (394.4).

Then, 400 mg of the film sample was dissolved in 3 mL of hexane for 15 h. After that, 0.5 mL sample of the solution was removed and was then mixed with 1.5 mL of ethyl acetate. Then, 1.5 mL was mixed with 1.5 mL of DPPH, and then cured in a dark place for 30 min. After that, the UV was measured at the wavelength of 515 nm and calculated were carried out to determine the antioxidant (AA) by using the following formula:

$$AA(\%) = \frac{(Acontrol - Asample) \times 100}{Acontrol} \quad (7)$$

where:

Acontrol = the UV value of the control sample;

Asample = the UV value of the experimental sample.

The control sample was derived by removing 1.5 mL of ethyl acetate and mixing it with 1.5 mL of DPPH. Then it was incubated in a dark place for 30 min. Next, it was measured for the UV value at a wavelength of 515 nm.

C. Statistical Analysis

Statistical tests were performed using SPSS statistics for

one-way analysis of variance in order to compare means to evaluate the quality of SP/gelatin films.

III. Results and Discussion

A. Moisture Content

It is widely recognized that food packaging films play a crucial role in preserving food by shielding it from external environmental factors and preventing moisture loss. Consequently, these films should possess both low MC and WVP. Experimental findings revealed that all SP/gelatin films exhibited low MC, falling within the range of 5.59 to 6.13%, with no statistically significant differences observed (p>0.05). These films were categorized as having low moisture film since their MC was below 14% [7]. Furthermore, they demonstrated low WVP. Even though the introduction of MFE led to an increase in WVP, no significant differences were observed among the various film samples (p>0.05). The obtained values closely resembled those of polylactic acid and gelatin films, which boasted a MC of 5.4%. This phenomenon may be attributed to interactions between gelatin and other constituents, which limited the availability of hydrophilic sites for interaction with water [12].

B. Solubility

The solubility of food packaging films holds significant importance in practical applications. Films with high solubility, for instance, can dissolve when the packaged product is heated in water, eliminating the need to tear open the packaging or allowing for the consumption of the product with the covering intact. In the case of SP/gelatin film, solubility decreased with the addition of MFE components due to their hydrophobic properties (44.50–47.54%). Nonetheless, the solubility of the films in our experiments closely resembles that of Myofibrillar Proteins sardine film (33–47%) [13] and polylactic acid/fish gelatin film (38%) [12]. However, solubility of our film was higher than that of SP/chitosan film (29.77% and 27.11%) [3, 5]. Solubility depends on the structural integrity of the film, the hydrophobicity of the plasticizer, and the degree of protein denaturation. The extract could enhance the water resistance of the film.

C. Water Vapor Permeability

Water vapor permeability is crucial for food preservation. For instance, fruits and vegetables that require moisture release need packaging materials with good WVP. On the other hand, for the preservation of dry foods, it is essential to prevent moisture from coming into contact with the food. Regarding the WVP of SP/gelatin film, it fell within the range of 0.83–1.17 (g·mm/m^2·day·kPa) with no statistically significant differences (p>0.05). This value was higher than that of myofibrillar protein film [14] because SP was more water-soluble. However, adding a lower concentration of lyophilized myofibrillar protein increased WVP due to their polar amino acids and a high number of hydroxyl (OH) groups, thereby resulting in bioplastics with low moisture barrier [15].

D. Mechanical Properties

Packaging films should possess both strength to protect products during storage and a degree of flexibility for various applications. Nevertheless, edible films often maintain their strength at the cost of reduced flexibility, which can limit their practical utility. This scenario was akin to SP films, which typically exhibited TS and E values ranging from 16.55–20 MPa and 3.54–4.06% respectively. The addition of MFE resulted in higher value than control (p<0.05) but the E properties of all samples did not significantly differ from one another (p<0.05). These TS and E values closely resembled those of fish gelatin films (26 MPa and 105%). Films with TS values exceeding 20 MPa are typically considered resistant [12]. This behavior could be attributed to the notable resistance properties of MFE, which contributed to the film's enhanced mechanical properties. The TS of these films was similar to that of SP/chitosan films (19.16 MPa) [3], while myofibrillar/SP films exhibited TS values ranging from 11.39 to 15.88 MPa. The inclusion of SP resulted in a reduction in TS, as SP, with its lower molecular weight and globular structure, could readily dispersed in the film-forming solution and interposed itself between myofibrillar protein chains, leading to decreased interactions among myofibrillar proteins [4]. However, although TS values of these films were higher, the E values were lower compared to myofibrillar protein films (4.915 MPa and 26.8–199.6%) [15] and (2.05–16.60 MPa and 178.08%) [16] due to the typical inclusion of a plasticizer at 50% in myofibrillar protein films. This plasticizer resulted in reduced TS and increased E by decreasing film fragility, improving its working capacity, and decreasing interactions between polymer chains, thereby enhancing E properties (Table 1).

Table 1. Physical and mechanical properties of SP/gelatin film

MFE	MC (%)	Solubility (%)	WVP (g.mm/m^2.day.kPa)	TS (MPa)	E (%)
0%	5.59±0.41	47.54±1.08^a	0.83±0.20	16.55±1.17^a	3.54±0.82
0.20%	5.90±0.30	44.50±0.32^b	1.17±0.07	18.64±0.73^b	3.56±1.06
0.50%	6.13±0.32	42.73±0.17^c	0.98±0.55	20.77±2.43^b	4.06±1.17

Different letters indicate the statistically different (p < 0.05).

E. Color

Color plays a role in the appearance of the product. In the experimental films, the color was a pale yellow and had a cloudy appearance. The values for both redness and yellowness increased in samples containing extracts (p < 0.05) because the extracts had a yellow-brown color, resulting in the films appearing more red and yellow, when the concentration of MFE increased, it led to an increase in yellowness, and additionally, it significantly reduced the brightness of the film (p < 0.05). The redness and yellowness values were similar to those of polylactic acid/fish gelatin film (a* = −0.36, b*= 3.30) [12]. Increasing the presence of epigallocatechin gallate in the film resulted in higher a* and b* values, similar to the films from the experiment where a* and b* values increased with the addition of MFE, as these extract had a reddish-brown color. However, the a* value was higher while the b* value was lower than that of

myofibrillar/SP film. This difference may be due to the presence of myoglobin and hemoglobin in the sarcoplasmic fraction, contributing to the redness of the resulting film [4]. If components with carotenoids, which were orange-red pigments, are added, it can significantly increase the b* value, as seen in fish by-product proteins and passion fruit pectin film with b* value of 30.63 [14] (Table 2).

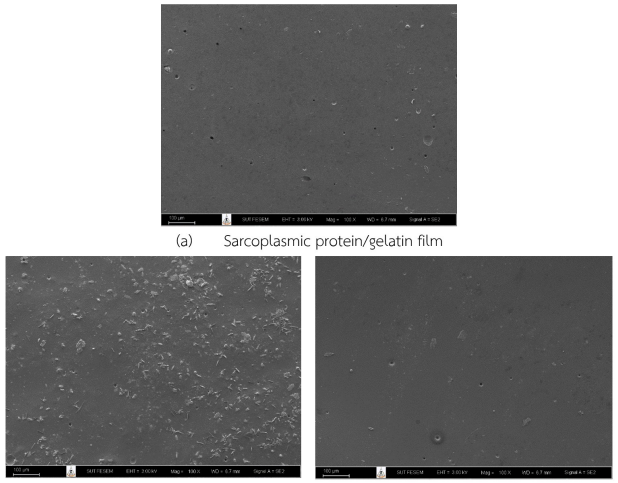
Table 2. Color of SP/gelatin film

MFE	L*	a*	b*
0%	31.75±1.08[a]	(−0.61)±0.04[b]	4.28±0.09[c]
0.2%	27.59±2.16[b]	0.16±0.24[a]	5.87±0.62[b]
0.5%	28.93±1.79[b]	0.13±0.02[a]	9.04±0.48[a]

Different letters indicate the statistically different (p <0.05).

F. Morphology

Morphology of SP/gelatin film without extract had a smooth surface and homogeneous texture due to the compatibility of proteins and gelatin, which acts as a plasticizer, resulting in increased water solubility. The surface of the film with extract exhibited irregularities in the film structure due to the heterogeneity in the polymer matrix with non-disulfide bonds. Additionally, the cross-linking of the extract in the film contributes to good mechanical properties [17]. The SP/gelatin film with MFE showed small particles on the surface, leading to irregularities in the film structure and partial densification of the film, resulting in increased strength but reduced water solubility [18]. This also leaded to an increase in TS, similar to fish protein/montmorillonite films [19]. Furthermore, it increased WVP due to the increased surface area resulting from the irregularities [20] (Fig. 1).



(a) Sarcoplasmic protein/gelatin film

(b) Film with 0.2% marigold flowers extract (c) Film with 0.5% marigold flowers extract

Fig. 1. Morphology of SP/gelatin film (magnification 100×).

G. Antioxidant

The occurrence of oxidation of lipids and proteins is a leading cause of food product deterioration during storage. Therefore, films used to wrap and protect food products must possess antioxidant properties to extend the shelf life of the food. The antioxidant activities of SP films were significantly enhanced (p < 0.05) with increasing extract concentrations. The DPPH scavenging rate of the film with 0.2 and 0.5% MFE content increased by 50.13 and 51.43% (p < 0.05) compared to the control (p < 0.05) because of their flavonoids and phenolic acid compounds regarding to a high antioxidant activity. This value closely resembled that of SP/chitosan film with the addition of 0.5% ginger extract [5].

IV. Conclusion

The SP films incorporating extracts from 0.5% marigold flowers expressed pale yellow and cloudy appearance with low MC, WVP and solubility. The surface of the film with extract exhibited irregularities. The MFE enhanced TS and E. Moreover, the DPPH scavenging rate of the film increased 51.43%. Therefore, it has a potential to apply in food preservation packaging for extending shelf life.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Phoolklang A. conducted the research; Jirukkakul N. analyzed the data, wrote the paper, and approved the final version. All authors had approved the final version.

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