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Utilization of *Bauhinia sirindhorniae* Leaves Extract to Improve the Antioxidant Property of Gelatin/Sarcoplasmic Protein Film

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Abstract

The purpose of developing antioxidant properties of gelatin/sarcoplasmic protein film was to determine the antioxidant properties of *Bauhinia sirindhorniae* leaves extract (BLE). *Bauhinia sirindhorniae* leaves were extracted using the maceration method with ethanol, and it was found that the extract exhibited antioxidant properties (DPPH method) 60.30%, phenolic content 0.13 mg GAE/mL, and flavonoid content 0.85 mg QE/mL. When applied by forming into film sheets with BLE concentrations of 0.2% and 0.5%, the film sheets showed antioxidant properties of 49.22% and 55.10%, respectively. Furthermore, the addition of the extract increased the film's lightness, water vapor permeability, and reduced its hardness. However, the film sheets became water solubility and more flexible. This film is suitable for individual food wrapping, which is secondary packaging such as sachets of oil and ingredients, for the convenience of cooking without needing to tear the pouch, which can dissolve in water. Moreover, BLE can enhance the efficiency of the film in resisting oxidative reactions. Thus, it can be used for food preservation.

Keywords: Antioxidant Activity; *Bauhinia sirindhorniae*; Extract; Edible Film; Physical Properties

1. Introduction

Food deterioration refers to the decline or degradation in the quality of food that affects its nutritional value, rendering it unappealing, unsafe, or unacceptable to consumers. Food deterioration can be categorized into physical, chemical, and microbial aspects, with microorganisms being the primary cause of such deterioration. Physical characteristics affected by food deterioration include changes in color, odor, taste, texture, and appearance. Among chemical factors, the most common cause is oxidative reactions. Oxidation takes place when oxygen reacts with lipids, especially unsaturated fatty acids in triglycerides, resulting in the production of abnormal-smelling and off-tasting compounds, commonly referred to as rancidity. This process involves a chain reaction initiated by free radicals, which in turn trigger additional reactions.

Generally, edible or biodegradable films have low flexibility and tensile strength, along with limitations in water and gas resistance when applied. However, these properties can be enhanced by incorporating essential oils or extracts into the film components to increase mechanical properties and resistance. Additionally, antioxidant properties can be improved, making them suitable for wrapping food products containing fats to reduce oxidation reactions. Preventing or reducing oxidative reactions can be achieved by minimizing exposure to light and oxygen. Additionally, natural and synthetic antioxidants can be utilized. Natural antioxidants are derived from plant sources and have a long history of use in traditional medicine and healthcare. Most research focuses on identifying bioactive compounds in herbal plants by extracting them using suitable solvents. Maceration, a traditional method, involves soaking plant materials like leaves, flowers, fruits, stems, or roots in an appropriate solvent for a minimum of 3 days. After extraction, the mixture is filtered through fine sieves and filter paper. The choice of a suitable solvent is crucial, as it not only separates phytochemicals from the plant material but also allows for the extraction of heat-resistant compounds. Ethanol extraction provides highly pure extracts in significant quantities, making it suitable for research applications. Ethanol is a safe solvent when used appropriately and typically does not introduce unwanted chemical residues into the extracted compounds.

Gelatin films incorporated with antioxidants have been widely studied for their potential to extend the shelf life of food products by reducing oxidative degradation. These films not only act as barriers against oxygen and moisture but also actively inhibit oxidation. For instance, research has explored the incorporation of garlic oil extracted through supercritical methods into a film forming matrix based on cross-linked fish gelatin. This inclusion has shown to positively impact the mechanical, optical, thermal, and barrier properties of the films. Such studies aim to develop materials that exhibit both antioxidant and antibacterial properties, making them suitable for active packaging or food coating to slow bacterial growth and extend product shelf life (Bastos *et al.,* 2024). Other studies have applied chitosan and betanin emulsified Pickering emulsions containing essential oil to enhance the antioxidant ability of bare gelatin films. An accelerated oil oxidation model was employed to assess the effectiveness of these films in suppressing lipid oxidation (Xu and Zhao, 2024). Furthermore, Choi *et al.* (2023) incorporated elderberry extract into high-

oxygen-barrier gelatin-sodium caseinate biocomposite films. These films were tested on pork shank, demonstrating their ability to delay oxidation by blocking oxygen and providing contact with natural antioxidants. The films' water vapor permeability, oxygen barrier properties, mechanical attributes, and antioxidant capabilities were evaluated to determine their suitability for food applications. Bitencourt *et al.* (2014) explored incorporation of ethanol extracts from turmeric into active films for use in food packaging. Moreover, virgin coconut oil (VCO) was found to enhance physical and mechanical properties of chicken skin gelatin films, making them viable alternatives to those made from other oils or bovine gelatin (Jusoh *et al.*, 2022).

Bauhinia sirindhorniae, locally known as "Sam Sip Song Pradong," was initially discovered in Phutoknoi, Bueng Kan province, and is commonly found in forested areas, along roadsides, rubber plantations, and undeveloped regions of Bueng Kan province. It is considered a native herbal plant of Bueng Kan (Larsen and Larsen, 1997). It possesses therapeutic properties and is known to enhance the immune system. Research has shown that *Bauhinia sirindhorniae* contains over 17 types of bioactive phytochemicals, primarily belonging to the flavonoid group, which are recognized for their excellent antioxidant properties (Sirivan *et al.*, 2005). However, the study of the efficacy of phenolic and flavonoid compounds from *Bauhinia sirindhorniae* extract is still limited (Nithikulworawong, 2012).

Considering the properties of *Bauhinia sirindhorniae* mentioned above, this research aims to assess the effectiveness of *Bauhinia sirindhorniae* leaves extract in resisting oxidative reactions in gelatin/sarcoplasmic protein films, with the goal of employing it to prolonging the shelf life of food products.

2. Materials and Methods

Bauhinia sirindhorniae was obtained from Phutoknoi, Bungkla district, Bueng Kan province. Gelatin and glycerol were obtained from Union Science Co. Ltd., Thailand.

2.1 Preparation of *Bauhinia sirindhorniae* leave extract (BLE)

The *Bauhinia sirindhorniae* leaves to be extracted, which were green and had the longest dimension exceeding 10 cm, were dried using either sun drying or an oven drying at temperatures ranging from 50 to 60°C, until the moisture content was less than 13%. Subsequently, the dried portions were ground or crushed to less than 0.5 cm in size to increase the contact surface area for extraction with 95% ethyl alcohol at a 1:10 ratio. The leaves should be stored in cool, dark conditions (approximately 15-20°C) in tightly closed containers to prevent moisture absorption, exposure to sunlight, and deterioration before use. The extraction process was carried out for 7 days with continuous stirring using a magnetic stirrer. After extraction, the compounds within each type of solvent were either more or less polar, depending on the polarity of the organic solvent used for extraction. The extracted material was then filtered to separate the plant residue from the solvent. The resulting solution was subjected to an evaporator (Dörr *et al.*, 2019).

2.2 Chemical analysis of *Bauhinia sirindhorniae* leave extract (BLE)

2.2.1 Determination of total phenolic content (TPC)

To analyze the extract, 0.2 mL of *Bauhinia sirindhorniae* leaf extract (BLE) was mixed with 5 mL of Folin-ciocalteu reagent and 2% sodium carbonate (Na_2CO_3) solution in a dark room at room temperature for 2 h. The mixture was then centrifuged at 3,000 rpm for 5 s. The absorbance was measured at a wavelength of 760 nm using a UV-Vis spectrophotometer. This experiment was repeated three times. To calculate the total phenolic content of the extract, the results were compared to the standard gallic acid graph. Total phenolic content (TPC) was calculated by the formulation.

TPC (mg gallic acid equivalent/mL) = [(absorbance- x-axis)/ y-axis] × extract content

Where: The x-axis represents absorbance values.
The y-axis represents the concentration of the standard compound (gallic acid).

TPC of sample (mg gallic acid equivalent/mL) = TPC × dilution factor (Lasunon *et al.*, 2022)

2.2.2 Determination of total flavonoids content (TFC)

For the determination of the total flavonoid content, 0.3 mL of BLE was mixed with 0.15 mL of 5% sodium nitrate ($NaNO_3$) solution and left for 5 min. Then, 0.15 mL of 10% aluminum nitrate ($Al(NO_3)_3$) solution was added and left for 5 min. Afterward, 1 mL of 1 M sodium hydroxide (NaOH) solution was added. The absorbance was measured at a wavelength of 420 nm using a UV-Vis spectrophotometer with methanol 99% as the blank. This experiment was also repeated three times. The total flavonoid content was determined by comparing the results to the quercetin standard graph. Total phenolic content (TFC) was calculated by the formulation.

TFC (mg Quercetin equivalent/mL) = [(absorbance- x-axis)/ y-axis] × extract content

Where: The x-axis represents absorbance values.
The y-axis represents the concentration of the standard compound (quercetin).

TFC of sample (mg Quercetin equivalent/mL) = TFC × dilution factor (Lasunon *et al.*, 2022)

2.2.3 Determination of antioxidant

The assessment of the antioxidant activity was carried out using the DPPH method. To determine the amount of DPPH required for the preparation, DPPH weight was calculated according to equation, and the volume was adjusted with methanol:

$$g = (m1 * v1 * Mw) / 1{,}000$$

Where:
- g = weight of DPPH (g)
- m1 = DPPH concentration (M)
- v1 = desired volume for preparation (mL)
- Mw = molecular weight (394.4 g/mol)

The control sample was prepared by combining 1.5 mL of ethyl acetate with 1.5 mL of DPPH and incubating it in the dark for 30 min. The UV measurement for the control was then conducted at a wavelength of 515 nm. The absorbance values were employed to calculate the antioxidant activity (AA%) using the following formula:

$$AA\% = ((A\ control - A\ sample) / A\ control) * 100$$

Where:
- AA% = Antioxidant percentage
- A control = The absorbance at 515 nm for the control (without antioxidant).
- A sample = The absorbance at 515 nm for the sample containing the antioxidant.

(Chen *et al*., 2007)

2.3 Formulation of gelatin/sarcoplasmic protein film

The 100 mL of sarcoplasmic protein solution was mixed with 1 g of glycerol, blended for 10 min at room temperature. Meanwhile, 5 g of gelatin and 1 g of glycerol was dissolved in distilled water, mixed for 10 min at 60°C. Next, the two solutions were combined, and 0.2% and 0.5% of BLE was added, mixed for 10 min at room temperature. The resulting solution was then transferred into a plate, each containing 50 mL. Plates were then placed in an incubator at 40°C for 24 h, after which the film was removed from the plate and stored in a desiccator (Jirukkakul and Sodtipinta, 2017).

2.4 Physical analysis of gelatin/sarcoplasmic protein film

2.4.1 Color

The evaluation of the film's properties was conducted using a Hunter Lab Spectrocolorimeter to measure film color. Film samples were positioned at the test point of the instrument and covered with a testing dish. Results were reported using the L* a* b* color space system.

2.4.2 Tensile strength and elongation

The tensile strength of the film was determined according to ASTM D882 standards (ASTM, 1997). It began by cutting samples, each measuring 10 mm in width and 120 mm in length. These samples were conditioned at 23°C and 50% relative humidity for at least 40 h. Subsequently, the test was conducted by securing one end of the sample to the testing grip of a Texture Analyzer (model TA.XT plus, Stable Micro Systems, USA) and applying equal tension on both sides. The test settings

included a pulling speed of 50 mm per min, a load cell of 0.5 kN, and a distance between grips of 100 mm. Tensile strength was calculated as the maximum force divided by the cross-sectional area. Elongation was determined as the increase in film length from its initial length, expressed as a percentage.

2.4.3 Thickness

The film thickness was measured using a digital micrometer (Mitutoyo, model ID-C112PM, Serial No. 574, Mitutoyo Corp., Kawasaki-shi, Japan). Five random measurements were taken on the film surface.

2.4.4 Moisture content

To determine the film's moisture content, a moisture balance (Mettler-Toledo GmbH, model HE73 Greifensee Switzerland) was used. Aluminum trays were baked at 105°C and then allowed to cool before being filled with the film samples. The instrument automatically calculated the moisture content.

2.4.5 Water vapor permeability

The water vapor permeability (WVP) of the film was tested in accordance with ASTM E96 standards (ASTM, 2000). Circular samples with a diameter of 6 cm were placed over the mouth of a test cup, sealed tightly, and weighed precisely. These cups were then put in a water vapor permeability testing chamber, and weight changes were recorded every hour until the weight difference was less than 1%. WVP was calculated using the formulation.

$$WVTR = (G/t)/A$$
$$WVPN = (WVTR \times thickness)/(P_{A1}-P_{A2})$$

Where: WVTR = Water Transmission Rate ($g/m^2.day$)
G/t = the rate of weight change over time (g/day)
A = surface area (m^2)
$P_{A1}-P_{A2}$ = the pressure difference inside and outside the test cup (kPa).

2.4.6 Solubility

Square film samples measuring 20 mm × 20 mm were subjected to 24 h of drying in an oven at 105°C and weighed (Wi). Following this initial drying, the desiccated films were transferred into Erlenmeyer flask, each containing 50 mL of distilled water. These bottles were then placed inside a shaker operating at 200 revolutions per min, maintaining a temperature of 25°C for 24 h. Subsequently, the films were extracted from the bottles, passed through filter paper to remove excess liquid, and then dried once more in an oven at 105°C for 24 h, with their final weight (Wf) being recorded (Jirukkakul, 2022).

$$S\ (\%) = ((Wi-Wf))/Wi \times 100$$

Where: S (%) = percentage of water solubility
Wi = weight of initial sample (g)
Wf = weight of final sample (g)

2.5 Antioxidant activity

The assessment of the antioxidant activity of gelatin/sarcoplasmic protein film was carried out using the DPPH method. To determine the amount of DPPH required for the preparation, DPPH weight was calculated according to equation, and the volume was adjusted with methanol:

$$g = (m1 * v1 * Mw) / 1{,}000$$

Where:
g = weight of DPPH (g)
m1 = DPPH concentration (M)
v1 = desired volume for preparation (mL)
Mw = molecular weight (394.4 g/mol)

Subsequently, film samples (400 mg) were dissolved in 4 mL of methanol for a duration of 15 h. Following this, 0.5 mL of the sample solution was mixed with 1.5 mL of ethyl acetate, and the resulting solution was further measured at 515 nm using a spectrophotometer. The absorbance values were employed to calculate the antioxidant activity (AA%) using the following formula:

$$AA\%: AA\% = ((A \text{ control} - A \text{ sample}) / A \text{ control}) * 100$$

Where:
AA% = Antioxidant percentage
A control = The absorbance at 515 nm for the control (without antioxidant).
A sample = The absorbance at 515 nm for the sample containing the antioxidant.
(Jirukkakul, 2018).

3. Results and Discussion

3.1 Antioxidant, total phenolic content and total flavonoid content of BLE

The major bioactive compounds found in the BLE were phenolic and flavonoid compounds which were antioxidant. The analysis of the quantities of antioxidants (DPPH) contained 60.30%. The phenolic content was 0.13 mg GAE/mL (mg gallic acid equivalent/mL) and the flavonoid content was mg QE/mL (mg quercetin equivalent/mL). Antioxidants played a crucial role in maintaining health by preventing the formation of reactive oxygen species and protecting cells from oxidative stress reactions (Xia *et al.*, 2023). Meanwhile, the phenolic compounds derived from plants with antioxidant properties, such as those in BLE, were significant in reducing oxidative stress reactions, making them potential antioxidants (Soobrattee *et al.*, 2005). On the other hand, flavonoids belong to a group of biologically active compounds found in various plant-based foods and beverages, including fruits,

vegetables, tea, wine, and beer. They are associated with numerous health benefits, including antioxidant, anti-inflammatory, and anticancer properties (Xia *et al*., 2023). Antioxidant content of BLE was comparable to that of the leaves of *Syzygium cumini L.* (Chanda and Kaneria, 2012) and elephant ginger (Mahmudati *et al*., 2020). This significant antioxidant content can be further applied to combat oxidative reactions in food.

3.2 Physical properties of gelatin/sarcoplasmic protein film with BLE

3.2.1 Color

The color values of gelatin/sarcoplasmic protein films are shown in Table 1. The gelatin/sarcoplasmic protein film with BLE 0.2% exhibits higher L*, a*, and b* values than the gelatin/sarcoplasmic protein film with BLE 0.5% ($p < 0.05$). However, the L*, a*, and b* values of film with BLE 0.2% were not differ significantly from the control film. This can be explained by the intensity of color in the extract, which had a brownish-green hue and was rich in color compared to the catechin film, which was pale yellow-green, resulting in higher L* and b* values and a lower a* value (L* 72.32, a* -3.82, and b* 26.20) (Flórez *et al*., 2023). The intensity of the gelatin/sarcoplasmic protein film with BLE is beneficial in protecting against light, thus serving as an avenue to reduce product deterioration due to exposure to light.

Table 1 Color of gelatin/sarcoplasmic protein film with BLE

BLE concentration	Color		
	L*	a*	b*
0.0%	31.75±1.08^{a}	-0.31±0.04^{b}	9.28±0.09^{a}
0.2%	25.22±3.81^{a}	-0.28±0.31^{b}	9.60±3.16^{a}
0.5%	23.14±0.31^{b}	-0.50±0.04^{a}	3.78±0.15^{b}

$^{a-b}$ the letter exhibited significant difference ($p<0.05$)

3.2.2 Tensile strength, elongation and thickness

The mechanical properties of film are essential for preserving products within containers. Tensile strength refers to the resistance to tearing, where lower tensile strength can make the film more susceptible to damage during handling and storage. The addition of BLE significantly reduced the tensile strength of the film ($p < 0.05$) (Table 2). Generally, the mechanical properties of biopolymer films are controlled by the forces between molecules and the network structure of the film's matrix. Changes in tensile strength result from the discontinuity of the structure due to the interaction between weakened molecules after BLE addition to the film's structure (Liu *et al*., 2023). Wang *et al*. (2021) also observed a similar phenomenon when adding extract from bamboo leaves, which significantly reduced the tensile strength of corn starch-based films.

The elongation at break of the film increased significantly ($p < 0.05$) due to the insertion of the extract into the film matrix, leading to some disruption in the film's polymer chain structure. Consequently, the film became more flexible (Liu *et al*., 2023). The tensile strength (TS) of these films was similar to that of gelatin film, which

measured 6.17-15.57 MPa (Jirukkakul, 2022), and sarcoplasmic protein/chitosan films (19.16 MPa) (Cai *et al*., 2020). Additionally, the thickness of all obtained films had a thickness of 0.11 mm, with no significant difference ($p < 0.05$). The film produced in this experiment exhibited a thickness similar to that of polylactic acid/gelatin film, which measured 0.179 mm (Nilsuwan *et al*., 2020).

Table 2 Tensile strength (TS), elongation at break (EAB) and thickness of gelatin/ sarcoplasmic protein film with BLE

BLE concentration	TS (MPa)	EAB (%)	Thickness (mm)
0.0%	16.55±1.17^{a}	3.54±0.82^{c}	0.10±0.02^{a}
0.2%	13.95±0.09^{b}	50.55±8.07^{b}	0.11±0.01^{a}
0.5%	8.25±0.01^{c}	67.12±18.68^{a}	0.11±0.01^{a}

$^{a-c}$ the letter exhibited significant difference ($p<0.05$)

3.2.3 Water vapor permeability

Water vapor permeability (WVP) indicates how easily water vapor can move in or out of packaging. High WVP can accelerate the drying of packaged foods or absorb moisture from the environment, affecting the quality and texture of the product. The WVP of gelatin/sarcoplasmic protein films with BLE was higher at a 0.2% concentration compared to the 0.5% concentration, and this result was statistically insignificant ($p > 0.05$). This outcome was similar to films containing bamboo leaf extracts, where increasing the extract concentration led to a reduction in water vapor permeability (Flórez *et al*., 2023). This behavior was attributed to the water-absorbing properties of the film's porous matrix structure. The WVP value was higher than that of myofibrillar protein film (Florentino *et al*., 2022) because sarcoplasmic protein is more water-soluble. Edible films with high WVP are suitable for products requiring moisture exchange, such as fresh vegetables and fruits, or as secondary packaging.

3.2.4 Solubility

The solubility (S) of gelatin/sarcoplasmic protein films with BLE is related to changes in hydrogen bonding, ionization of amino acids or carboxyl groups, and the disintegration of the film's structure (Mathew *et al*., 2007). The solubility of these films increased with higher BLE concentrations ($p > 0.05$) due to BLE's hydrophilic nature. Edible films can dissolve and degrade in water, making them suitable for specific applications such as secondary packaging or for separating ingredients for convenience in food preparation.

3.2.5 Moisture content

Moisture content is a critical factor for food product quality and shelf life, and packaging should protect against moisture absorption and WVP to maintain food quality. The moisture content of gelatin/sarcoplasmic protein films with BLE ranged from 6% to 8% ($p > 0.05$). This increase in moisture content is due to BLE's hydrophilic nature and is in line with the increased solubility observed. These films exhibit low

moisture content, making them suitable for use as food packaging materials. They offered good solubility, low water vapor permeability and moisture content, making them suitable for applications such as seasoning powder sachets designed for single-use portions.

3.3 Antioxidant activity

The antioxidant properties of gelatin/sarcoplasmic protein films with BLE significantly increased ($p < 0.05$) with the higher concentration of BLE, as shown in Table 3. Antioxidant properties are attributed to the components of antioxidants, such as polyphenols and flavonoids (Cendrowski *et al*., 2017). The hydroxyl groups within the polyphenolic compounds of extracts often attach to carbon atoms in the aromatic ring, facilitating the dispersion of hydrogen atoms to free radicals and inhibiting the formation of oxidation (Bhowmik *et al*., 2022). The dispersion of hydrogen atoms (H) is beneficial in preventing damage from oxidative reactions and chain reactions. Previous studies have also reported that extracts can enhance the antioxidant properties of biopolymer films (Li *et al*., 2021).

Table 3 Water vapor permeability (WVP), water solubility (WS), moisture content and antioxidant activity of gelatin/sarcoplasmic protein film with BLE

BLE concentration	WVP (g.mm/m^2.day.kPa)	WS (%)	MC (%)	Antioxidant activity (%)
0.0%	0.83±0.20^a	47.54±1.08^b	5.59±1.81^b	-
0.2%	0.97±0.24^a	50.43±1.91^b	5.90±0.36^b	49.22±0.01^b
0.5%	0.24±0.97^b	61.36±0.78^a	7.91±0.18^a	55.10±0.01^a

$^{a\text{-}b}$ the letter exhibited significant difference ($p<0.05$)

4. Conclusion

The study of the antioxidant properties of gelatin/sarcoplasmic protein films with BLE, and the comparison of their physicochemical properties when BLE increased from 0.2% to 0.5%, revealed that gelatin/sarcoplasmic protein films with 0.5% BLE exhibited decreased brightness, water vapor permeability, and tensile strength. Simultaneously, they showed improved solubility, moisture content, and antioxidant properties ($p < 0.05$). Based on this information, gelatin/sarcoplasmic protein films with 0.5% BLE can be used for food packaging to extend the shelf life of perishable foods susceptible to oxidative reactions.

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