ฟิล์มผสมไคโตแซน-โซเดียมเคซีเนตที่เติมสารสกัดใบเตยเพื่อชะลอการเกิดออกซิเดชันของอาหารทอดในระหว่างการเก็บรักษา

Chitosan-Sodium Caseinate Composite Films with Pandan Leaf Extract for Lipid Oxidation Retardation in Fried Foods during Storage

ณัฏฐิกา ศิลาลาย*, ธัญญาภรณ์ ศิริเลิศ, สุกัญญา แสนใจ และ วิรดา คิดเห็น
Nattiga Silalai*, Tunyaporn Sirilert, Sukanya Sanjai and Wirada Kidhen
ภาควิชาเทคโนโลยีการอาหาร คณะวิทยาศาสตร์ มหาวิทยาลัยสยาม
Department of Food Technology, Faculty of Science, Siam University

Received : 25 January 2022
Revised : 5 May 2022
Accepted : 29 August 2022

บทคัดย่อ

วัตถุประสงค์ของงานวิจัยเพื่อพัฒนาฟิล์มห่ออาหารด้วยการเติมสารสกัดใบเตยในฟิล์มเพื่อลดหรือช่วยชะลอการเกิดออกซิเดชันของไขมันในอาหารด้วยการแปรปริมาณสารสกัดใบเตยร้อยละ 5, 10 และ 15 ซึ่งในงานวิจัยพบว่าปริมาณสารสกัดใบเตยร้อยละ 15 และอัตราส่วนที่เหมาะสมของสารผสมระหว่างไคโตแซนและเคซีนที่ใช้ในการผลิตฟิล์มไบโอพอลิเมอร์ คือ 80:20 โดยใช้กลีเซอรอลเป็นพลาสติไซเซอร์ที่แปรความเข้มข้นร้อยละ 1 และ 2 ซึ่งความหนาของฟิล์มที่ได้อยู่ในช่วง 0.098-0.198 เซนติเมตร จากผลการทดลองฟิล์มผสมไคโตแซน-โซเดียมเคซีเนตที่เติมสารสกัดใบเตยและกลีเซอรอลร้อยละ 1 มีค่าอัตราการซึมผ่านไอน้ำ เท่ากับ 100.48 ± 2.17 g/m^2•24 hr, ค่า Tensile strength เท่ากับ 0.41 ± 0.03 N และ ค่า elongation เท่ากับ ร้อยละ 69.3% ในขณะที่ความสามารถในการต้านทานน้ำมันหรือไขมันของฟิล์มนั้นได้นานเกิน 30 วัน นำฟิล์มที่ได้มาใช้ในการเก็บรักษาอาหารทอดที่อุณหภูมิ 30, 40 และ 50 องศาเซลเซียส เพื่อศึกษาความสามารถในการต้านออกซิเดชันและปริมาณสารประกอบฟีนอลของฟิล์ม พบว่า อัตราการเสื่อมสลายของความสามารถในการต้านออกซิเดชันจะเกิดขึ้นอย่างช้าๆเมื่อเพิ่มปริมาณสารสกัดใบเตยซึ่งสอดคล้องกับปริมาณสารประกอบฟีนอลในฟิล์ม โดยที่ฟิล์มเติมสารสกัดใบเตยร้อยละ 5, 10 และ 15 และเก็บรักษา ณ อุณหภูมิ 30 องศาเซลเซียส พบว่า อัตราการเสื่อมสลายของความสามารถในการต้านออกซิเดชันมีค่าเท่ากับ 2.1813, 1.4471 และ 0.8768 ตามลำดับ อย่างไรก็ตามความสามารถในการต้านออกซิเดชันของฟิล์มไบโอพอลิเมอร์ที่เติมสารสกัดใบเตยลดลงเมื่ออุณหภูมิในการเก็บรักษาเพิ่มขึ้น

คำสำคัญ : สารสกัดใบเตย ; ไบโอพอลิเมอร์ฟิล์ม ; ปฏิกิริยาออกซิเดชันของไขมัน ; ความสามารถในการต้านออกซิเดชัน ; ภาชนะบรรจุอาหาร

Abstract

The objective of this research was to develop food wrap films for retardation of lipid oxidation by varying pandan leaves extract concentrations (5, 10 and 15%). The result indicated that pandan leaves extract concentrations at 15% and the suitable ratio of chitosan to casein for film formation was 80:20 with varying 1% and 2% glycerol as a plasticizer. Thickness of the films with 1% and 2% glycerol ranged from 0.098 to 0.198 cm. Its water vapor transmission rate, tensile strength and elongation was 100.48 ± 2.17 g/m^2•24 hr, 0.41±0.03 N and 69.3%, respectively. Grease and oil resistance of all films were more than 30 days. The films, stored at 30, 40 and 50°C, were used to investigate antioxidant capacity and total phenolic compounds. It found that degradation rate of antioxidant capacity decreased slightly in accordance with increasing total phenolic compounds of the films during storage. Degradation rate of antioxidant capacity of the films containing 5, 10 and 15% (w/w) pandan leaf extract during storage at 30°C was 2.1813, 1.4471 and 0.8768, respectively. However, the antioxidant capacity of all biopolymer films containing the pandan extract decreased dramatically with increasing temperature.

Keywords : pandan leave, biopolymer film, lipid oxidation, antioxidant capacity, food packaging

*Corresponding author. E-mail : nattiga.sil@siam.edu

Introduction

Food quality is reduced quickly due to microbial spoilage and several chemical reactions during storage. Lipid oxidation is the most important quality deterioration in food products containing high fat. In the chemical reaction process, unsaturated fatty acids reacting with molecular oxygen via a free radical chain mechanism (Gray 1978). Chemical changes in foods caused by lipid oxidation result in development of off-flavors, loss of color and nutrient values including functionality. Several food products containing high fat or lipid are susceptible to oxidation providing lipid peroxides and the free radicals in the products. The accumulation of these compounds could be detrimental to the health of consumers (Addis, 1986; McClements and Decker, 2000).

Nowadays, several food packaging that contained some antioxidants, is an alternative way for food spoilage preservation. This can prevent the interaction of humidity or substances, such as oxygen, ethylene, aroma or unusual flavors with the food (Salgado *et al.,* 2011). It leads to long shelf life of food products during storage. However, most food packages are synthetic plastics, which were difficult to decompose becoming waste pollutes in the environment. As a result of this problem, the concept of this research to find biodegradable natural materials to develop food packaging. Biodegradable films are produced mostly from biopolymer substances, such as proteins, polysaccharides, and lipids (Conca & Yang, 1993; Shahidi *et al.,* 1999; Galus & Kadziñska, 2015). Properties of biopolymer films could be modified or improved by employing a combination of these components. Casein was applied to modified films for food packaging since it could provide good strength and low oxygen permeability of films (Bonnaillie *et al.*, 2014). Biopolymer films mainly control the transfer of oxygen and moisture to improve the overall quality and physical properties of foods in order to reduce undesirable characteristics, such as rancidity, the loss of crispness, as well as shelf-life extension. The most commonly method used for controlling the lipid oxidation in the products is the addition of synthetic antioxidants, such as butyl hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary-butyl hydroquinone (TBHQ), and propylgalate (Moore *et al.*, 2003). The variables that affect the susceptibility of lipids to oxidation, include temperature, fatty acid composition, antioxidants, metals, enzyme-catalyzed reactions, and water (*Schaich et al.,* 2013). In recent years, the use of natural antioxidants, such as green tea extract (Giménez *et al.,* 2013a; Wu *et al.,* 2013), brown algae extract (Haddar *et al.*, 2012), essential oil (Tongnuanchan *et al.,* 2013), tocopherols (Barbosa-Pereira *et al.,* 2013), catechin-lysozyme (Rawdkuen *et al.,* 2012) in food packaging application has been famous and growing interest.

Many research indicated that pandan leaves contain some bioactive compounds such as tannin, alkaloids, flavonoids, and polyphenols, showing good antioxidant activity. Therefore, pandan leaf extract was selected as a natural antioxidant substance, which incorporated into food packaging materials. Pandan leaf extract (15% w/w) were able to inhibit and decrease microbiological growth in traditional foods (Aini &

Mardiyaningsih, 2016), and also exhibited an excellent heat-stable antioxidant property (Mohd Nor *et al*., 2008). A wider application of natural antioxidants was placed in the biopolymer films is interesting because of safety and friendly-environmental packaging materials. Therefore, the objectives of the present study were to apply the prepared chitosan-sodium caseinate composite films containing pandan leaf extract on retardation of lipid oxidation in deep-fat frying foods.

Methods

1. Raw materials

Sodium caseinate powder, consisting of 90% of protein, and the rest being lactose, lipids, attached moisture and ashes, was obtained from Vikki Enterprise Co., Ltd., Thailand. Chitosan (deacetylation degree 90%) was supplied by T.C. Union Global Co., Ltd., Thailand. Pandan leaf extract was supplied by Chemipan Corporation Co., Ltd. Other chemical substances were obtained from Sigma-Aldrich, USA and all the chemical substances were analytical grade.

2. Preparation of Chitosan and Sodium Caseinate Solution

In the present study, procedure for chitosan film preparation was according to Sirilert & Kitthaisong (2010) with some modifications. Chitosan solution (2.0%, w/v) were prepared by dispersing chitosan powder (2 g) in 100 mL of formic acid solution (1%, v/v). In the present study, a glycerol was used as a plasticizer with different 2 levels (1.0 and 2.0% glycerol), which were added to each chitosan solution. Sodium caseinate solution 1.0% (w/v) was prepared according to Pereda *et al*., (2008) with some modifications. Sodium caseinate powder (1.0 g) were prepared by dispersing in distilled water 100 mL, and then stirring continuously for 3 h, at room temperature. The pH value of mixed solution between chitosan and sodium caseinate was needed to control carefully for the homogenous solution of chitosan-sodium caseinate (Pereda *et al*., 2008). At pH 6.5, sodium caseinate is remarkably heat-stable, but highly insoluble at the isoelectric point, of which pH between 3.8 and 4.0. Chitosan shows best solubility in the acid condition (pH = 4.4) which is a 1% (v/v) formic acid solution, but remains soluble at pH lower than 6.4. Therefore, the pH of mixed solutions between chitosan and sodium caseinate solution should be careful. In this study, mixed solutions between chitosan and sodium caseinate solution at all ratios had pH around 5.0, and no phase separation or precipitation were observed. Chitosan-Sodium caseinate composite solution was prepared with ratio of chitosan to sodium caseinate solution at 100:0 (Control), 90:10, 80:20, 70:30 and 60:40. These solutions were stirred at 450 rpm for 60 min.

3. Preparation of Chitosan-Sodium Caseinate Composite Film with Pandan Leaf Extract

Pandan leaf extract at different concentrations (5, 10 and 15%, w/w) was added into each chitosan-sodium caseinate solution and stirred continuously until the solution mixed well. The chitosan-sodium caseinate solution containing pandan leaf extract (500 g) was poured into acrylic plastic plate (40 x 60 x 0.5) and dried at 60°C for 18-24 hr. Then, the chitosan-sodium caseinate composite film was removed from the acrylic plastic plate. The chitosan-sodium caseinate composite films were used to investigate physicochemical properties, including thickness by using a micrometer (Fisher Scienific, Pittsburgh, PA) (ASTM D 645-92), tensile strength and elongation by using Instron Universal Testing Machine Model 1000 (ASTM D 882-95a), water vapor transmission rate by using ASTM E96-80 method (ASTM E 96-95, 1996) and grease and oil resistance by using TIS 11-2536 method (TIS 11-2536).

4. Antioxidant Capacity of Pandan Leaf Extract and Lipid Oxidation of Potato Chips

The suitable chitosan-sodium caseinate composite films were selected for the study the antioxidation efficiency of pandan leaf extract in films. The suitable chitosan-sodium caseinate solution was added with concentrated pandan leaf extract with different contents (5, 10 and 15%, w/w) prior to film forming. In this study, the chitosan-sodium caseinate composite film obtained was cut into circular discs about 9 cm in diameter and sealed over a pierced cup lid. The lid with the film containing pandan leaf extract covered the cups, which contained 10 g of potato chips (Figure 1). The prepared samples were stored in incubators at 3 temperatures (30, 40 and 50°C) for 0, 7, 14, 21 and 28 days. Total phenolic compounds and antioxidant capacity (DPPH) of lids made from the films containing pandan leaf extract at each temperature were analyzed, meanwhile, peroxide value (PV) and free fatty acid (FFA) of the potato chips in the cup at each temperature were also investigated throughout 28 days of storage.

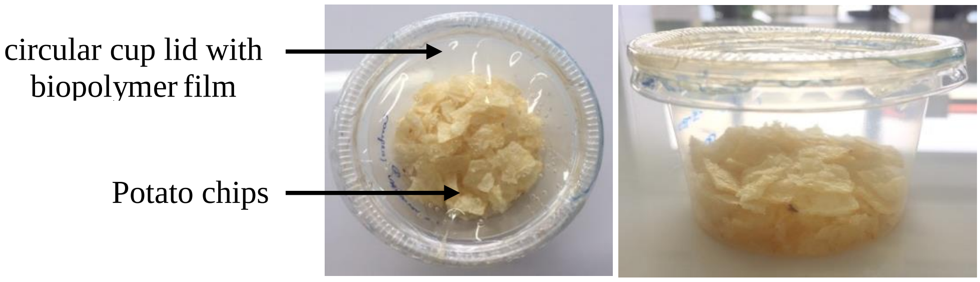

Figure 1 Example of a cup lid made from the chitosan-sodium caseinate composite film containing pandan leaf extract used for determination of antioxidation efficiency of pandan leaf extract in films. Potato chips were representative as food samples for determination of lipid oxidation in fried foods stored in package made from biopolymer films containing pandan leaf extract.

5. Statistical Analysis

All experimental results were analyzed statistically by using Two-way ANOVA with SPSS for Windows (demo version) program at 95% confidence level.

Results

1. Optimal Ratio of Mixed Biopolymers (Chitosan : Caseinate) for Film Forming

In the present study, the effect of glycerol concentration and sodium caseinate on properties of chitosan-sodium caseinate composite film was given Table 1. The physical properties (thickness, water vapor permeability, tensile strength, elongation capacity, and oil resistance) of chitosan-sodium caseinate composite films with different contents of glycerol (1% and 2%, w/w) as a plasticizer were given in Table 1 and Figure 2. The results indicated that thickness of the films with 1% (w/w) glycerol was in range of 0.072 and 0.122 mm, while thickness of biopolymer films with 2% (w/w) glycerol was in the range of 0.098 and 0.198 mm. There was no significant difference ($p>0.05$) in all treatments. In the present study, the thickness of all films was controlled by using the same weight (500 g each biopolymer solution) for film forming.

WVTR value shows the ability of the film to hold water vapor. A low WVTR value means that the water vapor is difficult to pass through. This replied that the shelf life of the product may be longer. According to Table 1, it found that the value of WVTR of the chitosan-sodium caseinate composite films with 1% glycerol was lower than the chitosan-sodium caseinate composite films with 2% glycerol at the same ratio of chitosan-sodium caseinate composite. The value of WVTR of the chitosan-sodium caseinate composite films with 1% glycerol was in range of 100.48 and 110.46 g/m^2•24 hr, while the value of WVTR of the chitosan-sodium caseinate composite films with 2% glycerol was in range of 106.87 and 113.83 g/m^2•24 hr. This was because glycerol had hydrophilic properties, which added polar properties in the film. These properties increased intermolecular distance, which caused moisture to penetrate and increase permeability. Since water vapor was easily diffused by the formation of free space on the film matrix due to reduced molecular density. This caused the presence of hydrophilic groups on glycerol. Therefore, when glycerol concentration was higher, the intermolecular interaction could decrease, then the molecular mobility that facilitate water vapor migration could increase (Yulistiani *et al.*, 2020). On the other hands, it seemed that the WVTR value had tendency to decrease with increasing caseinate in chitosan solution. This was consistent with the studies of Avena-bustillos & Krochta (1993) and Akter *et al.* (2012). The lowest WVTR value (100.93 and 100.48 g/m^2•24 hr) was obtained in the samples with 90:10 and 80:20 (chitosan to caseinate), respectively. This was because the more sodium caseinate in the film could enhance intermolecular interaction between chitosan and sodium caseinate molecules, and water vapor diffusion was inhibited. This was consistent with the result of Pereda *et al.,* (2008), indicating that the complex chitosan-sodium caseinate films showed a strong chain interaction as described by chemical (FTIR), mechanical (tensile and fracture puncture tests) and thermogravimetric (TG) characterization of the complex Chit/NaCas films.

Tensile strength (TS) shows the strength of the biopolymer film that can withstand the load of packaging since the tensile strength value can reflect the mechanical strength of the chitosan-sodium caseinate composite film. It indicated that the increasing glycerol concentration resulting in a reduction of the tensile strength in biopolymer film. All the chitosan-sodium caseinate composite films with 2% glycerol showed the lower TS values, which ranged 0.10 – 0.22 N, while TS values of the chitosan-sodium caseinate composite films with 1% glycerol ranged 0.21-0.41 N. These results were in accordance with the elongation values (%*E*). The elongation values of the chitosan-sodium caseinate composite films with 1% glycerol ranged from 69.31 to 73.72%, while the elongation values of the chitosan-sodium caseinate composite films with 2% glycerol ranged from 71.52 to 81.62%. The results were consistent with the study of Yulistiani *et al.* (2020). The decreasing tensile strength and increasing elongation value of the films adding with glycerol was probably due to disruption of the hydrocolloid interactions in the form of hydrogen bonds, since the energy required for molecular movement and the intermolecular tensile strength of adjacent polymer chains decreased resulting in a decrease in the stiffness of the films and became more elastic.

Table 1 Physical properties of chitosan-sodium caseinate composite films with 1% and 2% (w/w) glycerol

Treatment	Thickness	WVTR	TS	%E
1 (100:0)_1%	0.107 ± 0.019^{D}	110.46 ± 2.17^{ABC}	0.26 ± 0.10^{B}	70.59 ± 4.06^{BC}
2 (90:10)_1%	0.122 ± 0.014^{BDC}	100.93 ± 2.14^{D}	0.26 ± 0.04^{B}	71.82 ± 3.48^{BC}
3 (80:20)_1%	0.108 ± 0.011^{D}	100.48 ± 2.17^{D}	0.41 ± 0.03^{A}	69.31 ± 2.35^{C}
4 (70:30)_1%	0.102 ± 0.016^{CD}	101.20 ± 1.74^{D}	0.21 ± 0.01^{BCD}	73.72 ± 3.95^{BC}
5 (60:40)_1%	0.112 ± 0.016^{BCD}	101.51 ± 2.60^{D}	0.25 ± 0.01^{BC}	71.87 ± 3.03^{BC}
6 (100:0)_2%	0.098 ± 0.016^{CD}	113.83 ± 2.24^{A}	0.14 ± 0.01^{DEF}	81.62 ± 3.05^{A}
7 (90:10)_2%	0.152 ± 0.024^{ABC}	112.29 ± 1.60^{AB}	0.18 ± 0.05^{CDE}	75.57 ± 4.05^{B}
8 (80:20)_2%	0.198 ± 0.038^{A}	108.68 ± 2.74^{BC}	0.22 ± 0.02^{BC}	73.35 ± 1.02^{BC}
9 (70:30)_2%	0.158 ± 0.064^{AB}	107.44 ± 2.13^{C}	0.11 ± 0.01^{EF}	71.52 ± 0.49^{BC}
10 (60:40)_2%	0.144 ± 0.029^{BC}	106.87 ± 2.17^{C}	0.10 ± 0.01^{F}	73.16 ± 4.11^{BC}

Note : different superscripts A, B, C, D, E, F in each column means different mean values ($p \leq 0.05$)

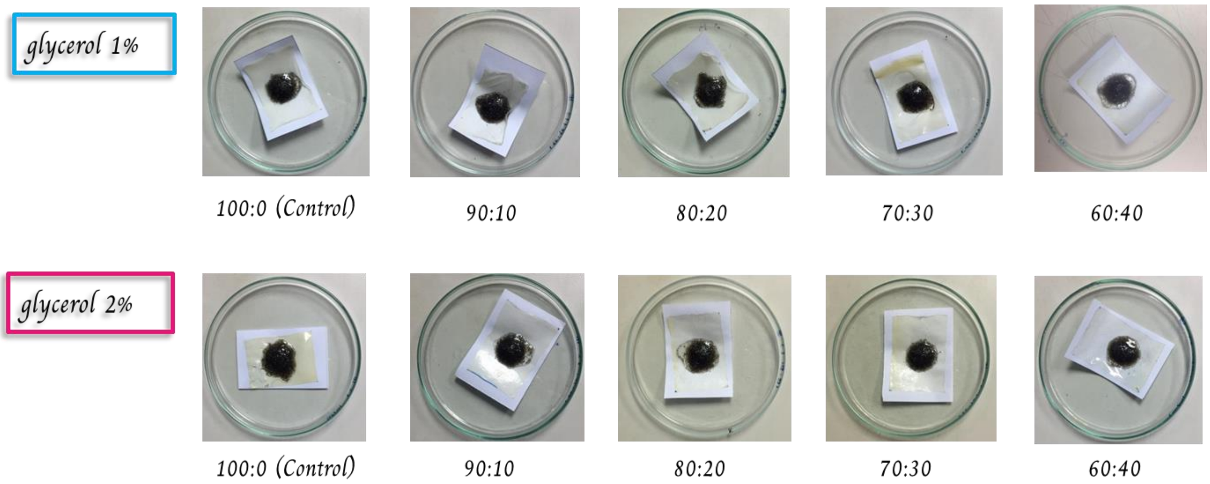

Figure 2 Oil resistance of all chitosan-caseinate composite films stored at room temperature for 30 days

In addition, the oil resistance of the chitosan-caseinate composite films (100:0, 90:10, 80:20, 70:30 and 60:40) indicated that no oil stains on the white papers under the films was found more than 30 days during storage for all ratios of biopolymer films (Figure 2). According to all properties of the films, the chitosan-sodium caseinate composite films with 1% (w/w) glycerol was selected to determine a change rate in water vapor permeability (WVTR) during storage at room temperature (30°C).

Water vapor permeability rate of the biopolymer films without caseinate (100:0) with 1% glycerol showed a steep slope (0.4336), while the slope of WVTR of the rest films added caseinate were slight (range of 0.3353 and 0.3475) as shown in Figure 3. From all physicochemical properties results (thickness, WVTR, tensile, elongation and oil resistance) of the chitosan-caseinate composite films, it was indicated the effect of glycerol and sodium caseinate on its physicochemical properties, especially the WVTR and TS values. The ratio of chitosan to caseinate (80:20) with 1% glycerol had the lowest WVTR and highest TS values. Therefore, the ratio of chitosan to caseinate (80:20) with 1% glycerol was need for film production and study the antioxidant capacity of pandan leaf extract in the chitosan-sodium caseinate composite films.

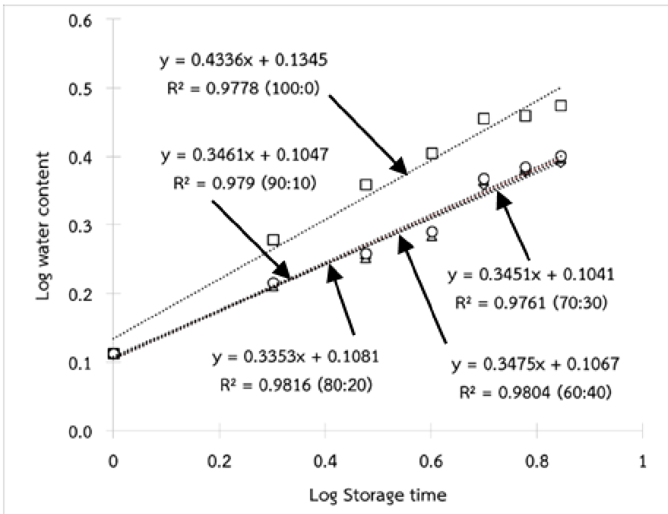

Figure 3 Change rate in water vapor permeability (WVTR) of the chitosan-sodium caseinate composite films with 1% glycerol during storage at room temperature (30 ° C). Ratio of chitosan to caseinate = 100:0 (□), 90:10 (○), 80:20 (◇), 70:30 (△) and 60:40 (▲)

<u>2. Antioxidant Capacity of Pandan Leaf Extract Incorporate into Chitosan-Sodium Caseinate Composite Films</u>

2.1. Antioxidant efficiency and total phenolic compounds content of Pandan leaves extract with various concentrations (5, 10 and 15%, w/w)

Antioxidant capacity of pandan leaf extract with concentration of 5, 10 and 15% (w/w) containing in the chitosan-sodium caseinate composite film was analyzed by using the 2, 2-diphenyl-1-picrylhydrazyl scavenging capacity (DPPH) method. It indicated that the chitosan-sodium caseinate composite films added with 15% (w/w) of pandan leaf extract showed higher efficiency of antioxidant capacity than the films added with 5 and 10% (w/w) of pandan leaf extract throughout storage at 30°C (Figure 4) as considered from the degradation rate of antioxidant activity (slope). The slope of antioxidant degradation of the films with 15% (w/w) of pandan leaf extract was 0.8768, whereas antioxidant degradation rates of the films chitosan-sodium caseinate composite films with 5 and 10% (w/w) of pandan leaf extract were 2.1813 and 1.4471, respectively, as shown in Figure 4a. This result was also consistent with the degradation rate of total phenolic compounds (Figure 4b). Total contents of phenolic compound in the chitosan-sodium caseinate composite films containing pandan leaf extract were observed in Figure 4b. Change rate of total phenolic compounds in the chitosan-sodium caseinate composite films containing 15% (w/w) of pandan leaf extract decreased slightly as compared to the films added with 5 and 10% (w/w) of pandan leaf extract. Degradation rate of a phenolic content in the films containing 15% (w/w) of pandan leaf extract was 1.0293, while the degradation rates of 5 and 10% (w/w) of pandan leaf extract were 1.3223 and 1.3875, respectively, during storage (Figure 4b). Therefore, pandan leaf extract at 15% (w/w) was selected as an antioxidant activity incorporated into the chitosan-sodium caseinate composite films during storage at 30, 40 and 50°C.

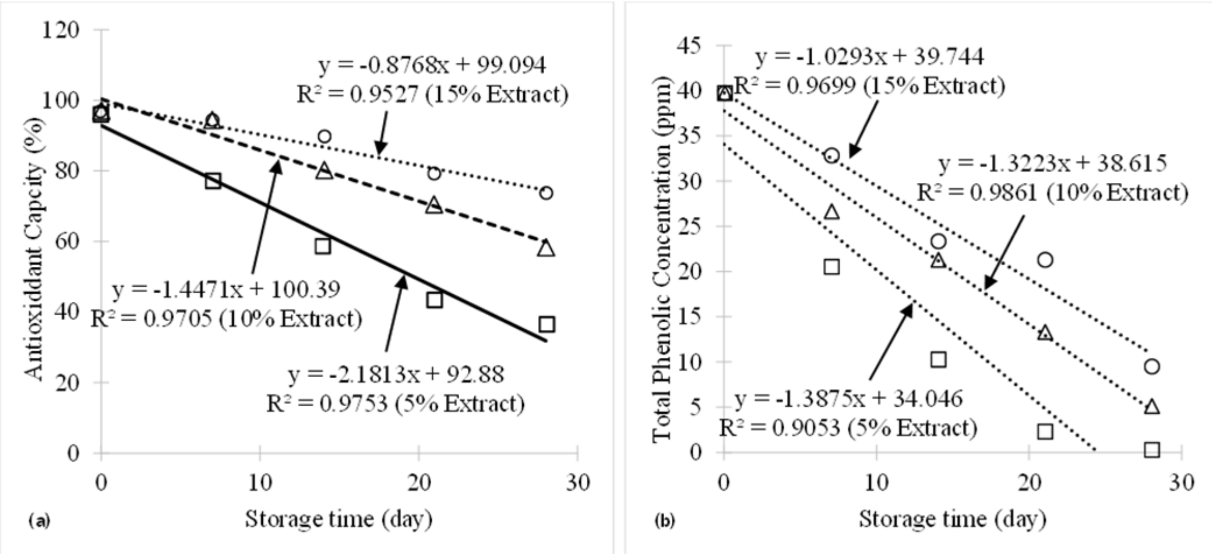

Figure 4 Degradation rate of antioxidant efficiency (a) and total phenolic compounds content (b). Content of pandan leaf extract (5%, □), 10%, △) and 15%, ○) incorporated into the chitosan-sodium caseinate composite films.

2.2. Antioxidant efficiency and total phenolic compounds content of Pandan leaves extract in the films during storage at various temperatures (30, 40 and 50°C)

Effect of temperature (30, 40 and 50°C) on antioxidant capacity and total phenolic compounds in the chitosan-sodium caseinate composite film containing 15% (w/w) of pandan leaf extract was shown in Figure 5. Degradation rates of antioxidant activity of pandan leaf extract in the films stored at different 3 temperatures were temperature dependence. Antioxidant degradation rates of all the films increased with increasing temperature. It indicated that antioxidant capacity decreased dramatically at the highest storage temperature (50°C), followed by 40 and 30°C. This can be observed from the slope of change rates in antioxidant capacity of the films stored at 50, 40 and 30°C, which was 0.8768, 0.8740 and 0.8410, respectively (Figure 5a). This result was consistent with the degradation rate of total phenolic compounds at 50, 40 and 30°C (Figure 5b). Total phenolic compounds of biopolymer films decreased significantly at the highest temperature (50°C), of which change rate was 1.4029, but a slow decrease in degradation rate of phenolic compounds was found in the films stored at 40 and 30°C, of which change rates were 1.3223 and 1.0293, respectively. Therefore, the addition of pandan leaf extract 15% (w/w) showed the highest antioxidant capacity due to the high amount of total phenolic compounds.

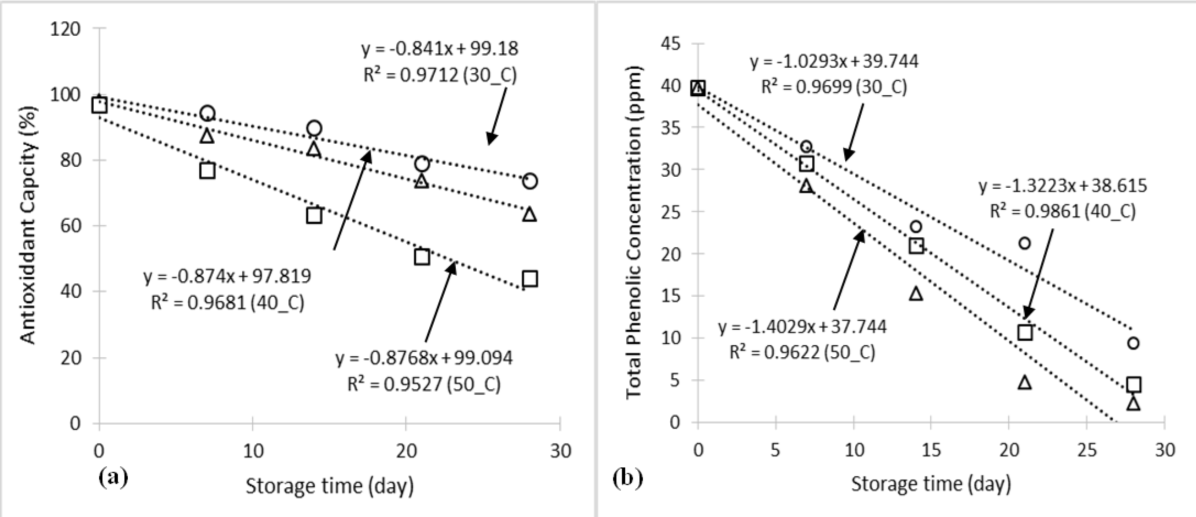

Figure 5 Change rate in the antioxidant efficiency (a) and the total phenolic compounds content (b) of the pandan leaf extract in the chitosan-sodium caseinate composite films during storage at temperature of 30°C (○), 40°C (△) and 50°C (□)

3. Lipid Oxidation of Fat Foods Stored in Packages Covered by the Chitosan-Sodium Caseinate Composite Films Containing Pandan Leaf Extract at Different Temperatures (30, 40 and 50°C)

The most important quality deterioration caused by chemical processes in food systems, especially deep-fat fried foods, was lipid oxidation. In the present research, samples used for the study of lipid oxidation were potato chips, which contained high amount of fat and easily degradable from oxidation reaction and thermal degradation during storage. The potato chips were replaced in the package covered by a lid made from the chitosan-sodium caseinate composite film, consisting of chitosan and caseinate in a ratio of 80:20 with 1% (w/w) glycerol and containing pandan leaf extract 15% (w/w). Lipid oxidation of the potato chips stored the packages as mentioned were stored at 30, 40 and 50°C, and then determined as peroxide value (PV) and free fatty acid (FFA). The results were given in Table 2 and 3.

Table 2 Peroxide value (PV) of crispy chips stored in packages covered by biopolymer films containing Pandan leaves extract at different storage temperatures

Time (day)	Peroxide value (meq.O_2/Kg)		
	30°C	40°C	50°C
0	8.3 ± 1.4^{Ca}	8.3 ± 1.4^{Da}	8.3 ± 1.4^{Da}
7	8.6 ± 0.8^{Ca}	9.5 ± 0.5^{CDa}	10.5 ± 2.1^{CDa}
14	9.3 ± 0.9^{Cb}	10.9 ± 0.8^{Cab}	11.3 ± 0.8^{Ca}
21	14.1 ± 1.5^{Ba}	15.6 ± 1.0^{Ba}	16.5 ± 1.4^{Ba}
28	26.8 ± 0.6^{Ab}	27.8 ± 0.6^{Aab}	28.8 ± 0.5^{Aa}

Note : The vertically different superscripts (A, B, C, D) refer to statistically different average ($p \leq 0.05$).
The horizontally different superscripts (a, b) refer to statistically different average ($p \leq 0.05$).

At each storage temperature, peroxide value (PV) of crispy chips stored in the packages covered by the chitosan-caseinate composite films (80:20) containing 15% (w/w) of Pandan leaves extract increased with increasing storage time at all temperatures. At each storage time, the peroxide value (PV) of the samples increased slightly with increasing storage temperature (Table 2). The results were in accordance with that obtained in measurement of free fatty acids in chips stored at different temperatures (Table 3). At each storage temperature, free fatty acid (FFA) increased with increasing storage time at all temperatures. At each storage time, the peroxide value (PV) of the samples increased slightly with increasing storage temperature (Table 3). However, both peroxide value and free fatty acids of the chips stored at 50°C were the highest.

Table 3 Free fatty acid (FFA) of crispy chips stored in packages covered by biopolymer films containing Pandan leaves extract at different storage temperatures

Time (day)	Free fatty acid (%)		
	30°C	40°C	50°C
0	0.012 ± 0.001^{Ba}	0.012 ± 0.001^{Ca}	0.012 ± 0.001^{Da}
7	0.013 ± 0.003^{Ba}	0.015 ± 0.001^{BCa}	0.015 ± 0.001^{CDa}
14	0.014 ± 0.003^{ABa}	0.017 ± 0.007^{ABCa}	0.018 ± 0.001^{BCa}
21	0.016 ± 0.003^{Aba}	0.019 ± 0.002^{Aba}	0.020 ± 0.002^{Aba}
28	0.018 ± 0.002^{Aa}	0.022 ± 0.003^{Aa}	0.023 ± 0.003^{Aa}

Note : The vertically different superscripts (A, B, C, D) refer to statistically different average ($p \leq 0.05$).
The horizontally different superscripts (a, b) refer to statistically different average ($p \leq 0.05$).

Moreover, change rates in free fatty acid in the chips were measured by the slope of graph (Figure 6). It indicated that increasing rate of free fatty acid in the chips stored at 50°C was the highest (slope = 0.0004) followed by at 40 and 30°C, which was 0.0003 and 0.0002, respectively.

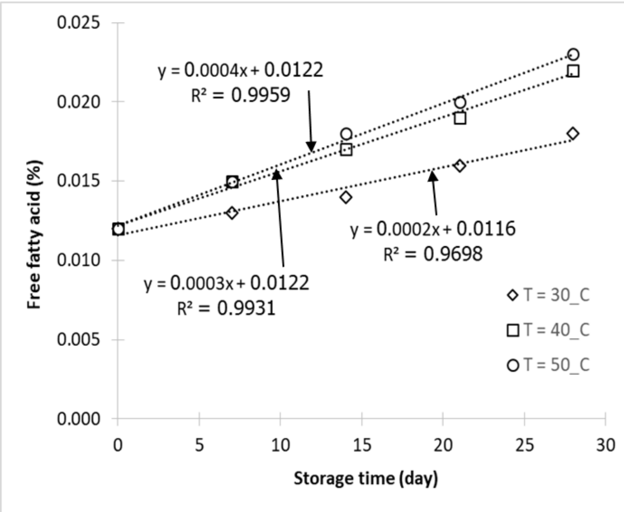

Figure 6 Change rate in free fatty acid of crispy chips during storage at temperature of 30°C (◇), 40°C (□) and 50°C (○)

Discussion

Characteristics of the Chitosan-Sodium Caseinate Composite Film

In each proportion of chitosan to caseinate solution, there was a significant difference of water vapor transmission rate (WVTR) between the films containing 1% and 2% (w/w) glycerol ($p \leq 0.05$). WVTR of biopolymer films containing 1% (w/w) glycerol was higher than that of biopolymer films containing 2% (w/w) glycerol in all ratios. Since glycerol is a hydrophilic plasticizer, it can easily bind to hydrophilic sites on polymer molecules, resulting in reduced internal hydrogen bonding between polymer molecules and increased molecular spacing (Lieberman & Gilbert, 1973). It resulted in an increase in film flexibility and molecular spacing, leading to a bigger hole size in the biopolymer films and a higher water vapor transmission rate. This was consistent with the several studies (Gontard *et al.*, 1993), indicating higher film extensibility with increasing glycerol content. Glycerol-protein interaction reduced self-association of protein molecules causing a decrease in film strength and water vapor permeability barrier properties. However, excessive amounts of glycerol can result in softer and wrinkle films. Tensile strength and Young's modulus had a negative linear correlation with glycerol content (Liu *et al.*, 2017).

An increase in caseinate content decreased WVTR of the chitosan-caseinate composite films. A low WVTR of biopolymer films due to the hydrophobic sites of caseinate molecules resulting in a decrease in water vapor permeability of the films. It also increased film strength due to a reduction of molecular mobility (Lieberman & Gilbert, 1973).

The elongation (*%E*) of the chitosan-casein composite films was opposite the tensile strength. This was consistent with study of Khwaldia *et al.* (2004) showing that the addition of macromolecules, such as proteins, increased film hardness due to strong hydrogen bonds between the protein and chitosan, decreasing the film plasticization (Phadungath, 2005). Therefore, the molecular spaces in the films decreased and the glass transition temperature was higher, resulting in more rigid and lower water vapor permeability rates (Aghazadeh *et al*., 2021).

The addition of protein could reduce the change rate of water vapor permeability (WVTR) in biopolymer films. Water vapor permeability (WVTR) rate of biopolymer films without caseinate (100:0) with 1% glycerol was high, while WVTR rate of biopolymer films decreased with increasing caseinate content. Caseinate is a protein with a relatively hydrophobic groups, which can inhibit water binding in the molecular structures resulting in low water adsorption capacity (Phadungath, 2005).

Antioxidant Capacity of Pandan Leaf Extract

Efficiency of antioxidant capacity of the biopolymer films with 15% (w/w) of Pandan leaf extract decreased slightly, while that of the biopolymer films with 5 and 10% (w/w) of Pandan leaf extract decreased dramatically as observed from the slopes of change rates in antioxidant capacity and total phenolic compounds. This was consistency with the results of their total phenolic compound contents, which play the role of important antioxidants. Antioxidants in Pandan leaves extract includes anthocyanin, carotenoids, tocopherols, tocotrienols, quercetin, alkaloids (Peungvicha *et al*., 1998; Aini & Mardiyaningsih, 2016).

Antioxidant activity rate of Pandan leaves extract in biopolymer films was found to be temperature dependence since antioxidant activity decreased dramatically with increasing temperature. This was probably due to thermal degradation leading to a decrease in its sensitive ingredients (Barreto *et al.*, 2003; Kim *et al.*, 2020). Kim *et al.,* (2020) indicated that total phenolic content and total flavonoid content also decreased with increasing temperature and time. This result was also accordance with the study of Réblová (2006) indicating that antioxidant capacity decreased significantly at higher storage temperature.

Lipid Oxidation of Fat Foods Stored in the Chitosan-Sodium Caseinate Composite Films Containing Pandan Leaf Extract

At each storage temperature, free fatty acid (FFA) and peroxide value (PV) increased with increasing storage time at all temperatures. This was due to thermal and time effect on oxidative stability. High temperature could rapidly accelerate oxidation reaction since the formation of PV and TBARS were dependent on both temperature and storage time (Hoac *et al*., 2006).

Roldan *et al*., (2014), indicating that different combinations of temperature (60, 70 and 80°C) and time (6, 12 and 24 h) affected the oxidative stability of lipids and proteins. Heating induced both lipid and protein oxidation in lamb loins. Higher cooking temperature–time combinations increased conjugated dienes and decreased thiobarbituric reactive substances (TBARS) values.

Conclusions

Physicochemical Properties of Chitosan-Sodium Caseinate Composite Films

Increasing caseinate in chitosan solution decreased WVTR of the chitosan-caseinate composite films. WVTR of biopolymer films containing 1% (w/w) glycerol at a ratio of chitosan to caseinate (80:20) had the lowest water vapor permeability (100.93 g/m^2•24 hr). At the same ratio of chitosan to caseinate (80:20), tensile strength (TS) was higher, but elongation (%*E*) was lower with decreasing glycerol content. TS and elongation (%*E*) of the biopolymers containing 1% glycerol were 0.41 N and 69.31%, respectively. Physicochemical properties of biopolymer films (thickness, WVTR, tensile, elongation and oil resistance), it was indicated that the addition of caseinate in the chitosan solution decreased water vapor permeability rate (WVTR) of biopolymer films (slope = 0.3353 - 0.3475) as compared to biopolymer films without sodium caseinate (slope = 0.4336).

Antioxidant Capacity of Pandan Leaf Extract Incorporated into Chitosan-Sodium Caseinate Composite Films

Antioxidant capacity of chitosan-caseinate composite films increased with increasing pandan leaf extract content. The biopolymer films added with 15% (w/w) pandan leaf extract showed the highest efficiency of antioxidant activity throughout storage. Its change rate of antioxidant capacity of the biopolymer films with 15% (w/w) pandan leaf extract was the lowest (0.8768), whereas change rates of antioxidant capacity of the biopolymer films with 5 and 10% (w/w) pandan leaves extract were 2.1813 and 1.4471, respectively. These were consistent with the results of total phenolic compounds. However, the change rate of antioxidant capacity and total phenolic compounds of all biopolymer films decreased continuously with increasing temperature storage. The antioxidant capacity decreased dramatically at the highest storage temperature (50°C) of which change rate was 1.4029, and followed by 40 and 30°C.

Lipid Oxidation of Potato Chips Stored in the Chitosan-Sodium Caseinate Composite Films Containing Pandan Leaf Extract

According to antioxidant capacity of the biopolymer films, lipid oxidant of full-fat foods (potato chips) kept in the biopolymer films were determined by peroxide value (PV) and free fatty acid (FFA) during storage. PV and FFA of potato chips kept in the chitosan-caseinate composite films decreased with increasing pandan lead extract content. PV an and FFA of potato chips kept in the chitosan-caseinate composite films (80:20) containing 15% (w/w) pandan leaf extract were the lowest; however, these values increased with increasing storage temperatures throughout the time. Both PV and FFA of the foods stored at 50°C were the highest.

Acknowledgements

This project was conducted by the generous financial support of Siam University, Bangkok, Thailand.

References

Addis, P.B. (1986). Occurrence of lipid oxidation products in foods. *Food and Chemical Toxicology, 24* (10-11), 1021-1030.

Aghazadeh, N., Esmaiili, M., Mohtarami, F. (2021). Prediction of equilibrium moisture contents of black grape seeds (*Siah Sardasht cultivar*) at various temperatures and relative humidity: Shelf-life criteria. *Nutrition and Food Sciences Research, 8*(1), 45-52.

Aini, R., Mardiyaningsih, A. (2016). Pandan leaves extract (*Pandanus amaryllifolius Roxb*) as a food preservative. *Indonesian Journal of Medicine and Health, 7*(4), 166-173.

Akter, N., Khan, R.A., Tuhin, M.O., Haque, M.E., Nurnabi, M., Parvin, F., Islam, R. (2012). Thermomechanical, barrier, and morphological properties of chitosan-reinforced starch-based biodegradable composite films. *Journal of Thermoplastic Composite Materials, 27*(7), 1-16.

Avena-bustillos, R.J., Krochta, J.M. (1993). Water vapor permeability of caseinate-based edible films as affected by pH, calcium crosslinking and lipid content. *Journal of Food Science, 58*(4), 904-907.

Barbosa-Pereira, L., Manuel Cruz, J., Sendon, R., Bernaldo de Quirós, A.R., Ares, A., Castro-Lopez, M., Abad, M.J., Maroto, J., Paseiro-Losada, P. (2013). Development of antioxidant active films containing tocopherols to extend the shelf life of fish. *Food Control 31*(1), 236–243.

Barreto, P.L.M., Pires, A.T.N., Soldi, V. (2003). Thermal degradation of edible films based on milk proteins and gelatin in inert atmosphere. *Polymer Degradation and Stability, 79*(1), 147-152.

Bonnaillie, L.M., Zhang, H., Akkurt, S., Yam, K.L., Tomasula, P.M. (2014). Casein Films: The Effects of Formulation, Environmental Conditions and the Addition of Citric Pectin on the Structure and Mechanical Properties. *Polymers, 6*, 2018-2036.

Conca, K. R.; Yang, T. C. S. (1993). Edible food barrier coatings. In C.C. Ching, D.L. Kaplan, & E.L. Thomas. (Eds.) *Biodegradable Polymers and Packaging*. (pp. 357-369). Lancaster, PA: Technomic Publishing.

Giménez, B., Moreno, S., López-Caballero, M.E., Montero, P., Gómez-Guillén, M.C. (2013). Antioxidant properties of green tea extract incorporated to fish gelatin films after simulated gastrointestinal enzymatic digestion. *LWT Food Science and Technology 53*(2), 445–451.

Galus, S., Kadziñska, J. (2015). Food applications of emulsion-based edible films and coatings. *Trends in Food Science and Technology*, *45*(2), 273-283.

Gontard, N., Guilbert, S., Cuq, J. (1993). Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science, 58*(1), 206-211.

Gray, J.I. (1978). Measurement of lipid oxidation. A review. *Journal of the American Oil Chemists' Society, 55*, 539–546.

Haddar, A., Sellimi, S., Ghannouchi, R., Martinez-Alvarez, O., Nasri, M., Bougatef, A. (2012). Functional, antioxidant and film-forming properties of tuna-skin gelatin with a brown algae extract. International Journal of Biololgical Macromolecules, *51*(4), 477–483.

Hoac, T., Daun, C., Trafikowska, U., Zackrisson, J., Akesson, B. (2006). Influence of heat treatment on lipid oxidation and glutathione peroxidase activity in chicken and duck meat. *Innovative Food Science and Emerging Technology, 7*(1), 88-93.

Khwaldia, K., Banon, S., Perez, C., Desobry, S. (2004). Properties of sodium caseinate film-forming dispersions and films. *Journal of Dairy Science, 87*(7), 2011-2016.

Kim, J.M., Kang, J.Y., Park, S.K., Han, H.J., Lee, K., Kim, A., Kim, J.C., Choi, S., Heo, H.J. (2020). Effect of storage temperature on the antioxidant activity and catechins stability of Matcha (*Camellia sinensis*). *Food Science and Biotechnology, 29*(9), 1261-1271.

Lieberman, E.R., Gilbert, S.G. (1973). Gas permeability of collagen films as affected by cross-linkage, moisture, and plasticizer content. *Journal of Polymer Science: Polymer Symposia, 41*(1), 33-43.

Liu, F., Chiou, B., Avena-Bustillos, R.J., Zhang, Y., Li, Y., McHugh, T.H., Zhong, F. (2017). Study of combined effects of glycerol and transglutaminase on properties of gelatin films. *Food Hydrocolloids, 65*, 1-9.

McClements, D.J., Decker, E.A. (2000). Lipid oxidation in oil-in-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food system. *Journal of Food Science ,65*(8), 1270–1282.

Mohd Nor, F., Mohamed, S., Ainildris, N., Ismail, A. (2008). Antioxidative properties of Pandanus amaryllifolius leaf extracts in accelerated oxidation and deep-frying studies. *Food Chemistry, 110*(2), 319 - 327.

Moore, M.E., Han, I.Y., Acton, J.C., Ogale, A.A., Barmore, C.R., Dawson, P.L. (2003). Effects of antioxidants in polyethylene film on fresh beef color. *Journal of Food Science ,68*(1), 99–104.

Pereda, M., Aranguren, M.I., Marcovich, N.E. (2008). Characterization of chitosan/caseinate films. *Journal of Applied Polymer Science, 107*(2), 1080-1090.

Peungvicha, P., Temsiririrkkul, R., Prasain, J.K., Tezuka, Y., Kadota, S., Thirawarapan, S.S., Watanabe, H. (1998). 4-Hydroxybenzoic acid: a hypoglycemic constituent of aqueous extract of *Pandanus odorus* root. *Journal of Ethnopharmacology, 62*(1), 79-84.

Phadungath, C. (2005). Casein micelle structure: A concise review. *Songklanakarin Journal of Science and Technology, 27*(1), 201-212.

Rawdkuen, S., Suthiluk, P., Kamhangwong, D., Benjakul, S. (2012). Mechanical, physico-chemical, and antimicrobial properties of gelatin-based film incorporated with catechin-lysozyme. C*hemistry Central Journal, 6*(131), 1-10.

Réblová, Z. (2006). The effect of temperature on the antioxidant activity of tocopherols. *European Journal of Lipid Science and Technology, 108*(10), 858-863.

Salgado, P.R., Fernández, G.B., Drago, S.R., Mauri, A.N. (2011). Addition of bovine plasma hydrolysates improves the antioxidant properties of soybean and sunflower protein-based films. *Food Hydrocoll, 25*(6), 1433–1440.

Schaich, K.M., Shahidi, F., Zhong, Y., Eskin, N.A.M. (2013). Lipid oxidation. In: Eskin, N.A.M, Shahidi, F. (eds) Biochemistry of foods, 3rd edn. Academic, Amsterdam.

Shahidi, F., Arachchi, J.K.V., Jeon, Y.J. (1999). Food applications of chitin and chitosans. *Trends in Food Science and Technology, 10*, 37-51.

Sirilert, T., Kitthaisong, W. (2010). Development of chitosan film laminated with paper material for food packaging forming. *Journal of Food Technology, Siam University, 6*(1), 55-63.

Tongnuanchan, P., Benjakul, S., Prodpran, T. (2013). Physico-chemical properties, morphology and antioxidant activity of film from fish skin gelatin incorporated with root essential oils. *Journal of Food Engingeering ,117*(3), 350–360.

Wu, J., Chen, S., Ge, S., Miao, J., Li, J., Zhang, Q. (2013). Preparation, properties and antioxidant activity of an active film from silver carp (Hypophthalmichthys molitrix) skin gelatin incorporated with green tea extract. *Food Hydrocolloids, 32*(1), 42–51.

Yulistiani F., Khairunisa, N., Fitiana R. (2020). The effect of glycerol concentration and breadfruit flour mass on edible film characteristics. *Journal of Physics: Conference Series, 1450*(012001), 1-6.