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Cuttlefish *Sepia pharaonis* skin gelatin-based film: storage stability and its effectiveness for shelf-life extension of chicken meat powder

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Abstract

Stability of cuttlefish **(***Sepia pharaonis***)** ventral skin gelatin film (CG) and film incorporated with Fenton's reagent (H_2O_2 0.02 M + Fe_2SO_4 0.002 M) (FG) was evaluated after 21 days of storage at 50% relative humidity and 25 °C. No changes in mechanical property were observed for CG film but slight increase in tensile strength (TS) was found for FG film after storage ($P < 0.05$). Furthermore, water vapor permeability (WVP) increased for both films ($P < 0.05$), while no marked changes in film solubility and transparency values were found ($P > 0.05$). DSC and TGA study revealed that molecular reorganization with higher thermal stability were formed in the film matrix during storage. When CG and FG film were used to cover chicken meat powder, the samples covered with both films had lower moisture content, peroxide values (PV) and thiobarbituric acid reactive substances (TBARS), compared with control samples (without cover) ($P < 0.05$). Generally, FG film showed more preventive effect than CG film. However, both films were poorer in preventing moisture migration and retarding the color changes of chicken meat powder than low-density polyethylene (LDPE) films. Thus, gelatin-based film, especially modified with Fenton's reagent could be used as a biodegradable packaging material to prevent lipid oxidation in oil enriched foods. Nevertheless, the improvement of its water barrier property is still needed.

Keywords: Cuttlefish, Gelatin film, Fenton's reagent, Storage stability, Shelf-life extension, Food packaging, Chicken meat powder, Lipid oxidation

Introduction

Cuttlefish has become an important fishery product in Thailand as well as other south-east Asian countries. During processing of cuttlefish, skin is generated as a by-product with the low market value. To increase its profitability, cuttlefish skin has recently been used for gelatin extraction (Aewsiri et al. 2009; Hoque et al. 2010). Gelatin has

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been used as a material for preparing biodegradable films with an excellent gas barrier property (Jongiareonrak et al. 2006). Biodegradable films made from renewable biopolymers have become important environmental friendly materials for packaging (Tharanathan 2003; Prodpran and Benjakul 2005; Hoque et al. 2010). Most synthetic films are non-biodegradable and are associated with environmental pollution and serious ecological problems (Tharanathan 2003). As a consequence, biodegradable or edible films from biopolymers have paid increasing attention. Among polymers, proteins from different sources have been used to prepare films due to their abundance and the uniqueness in film-forming ability (Ou et al. 2005; Jongiareonrak et al. 2006; Prodpran et al. 2007). Bondings and degree of interactions involved in the stabilization of a protein film matrix are determined by the amino-acid composition and molecular weight of the proteins (Denavi et al. 2009).

Gelatin has been used as a material for preparing biodegradable films with high transparency and excellent barrier characteristics against gas, organic vapor and oil, compared to synthetic films (Jongiareonrak et al. 2006; Jiang et al. 2007). However, gelatin film has poor water barrier property (Hoque et al. 2011a; 2011b; Jongiareonrak et al. 2006; Jiang et al. 2007; Denavi et al. 2009) and this is the main drawback of gelatin films for their application as a packaging material (McHugh and Krochta 1994; Gómez-Guillén et al. 2009). Recently, Hoque et al. (2011c) reported that Fenton's reagent (H_2O_2 0.02 M + Fe_2SO_4 0.002 M) could increase the mechanical, barrier properties and thermal stability of cuttlefish skin gelatin-based film. This film could be used as an alternative packaging for prevention of lipid oxidation in foods. However, this protein films might undergo changes during extended storage and its function can be altered.

In general, edible film and coatings from proteins can extend the shelf-life of foods by functioning as solute, gas and vapor barriers (Krochta 1997). Artharn et al. (2009) found the lower thiobarbituric acid reactive substances and yellowness of dried fish powder than control (without cover), when round scad muscle protein-based films were used to cover fish powder stored at room temperature. Thus, the aims of this investigation were to study the storage stability of cuttlefish skin gelatin-based films without and with Fenton's reagent, and to investigate the use of the films to extend the shelf-life of dried chicken meat powder.

Materials and methods

Chemicals

Bovine serum albumin and wide range molecular weight protein markers were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Iron (II) sulfate, glycerol, p-dimethylaminobenzaldehyde and tris(hydroxymethyl) aminomethane were obtained from Merck (Darmstadt, Germany). Analytical hydrogen peroxide (30%) was obtained from BDH, VWR International Ltd (Leicestershire, England). Sodium dodecyl sulfate (SDS), Coomassie Blue R-250 and N,N,N',N'- tetramethylethylenediamine (TEMED) were purchased from Bio-Rad Laboratories (Hercules, CA, USA). All chemicals were of analytical grade.

Collection and preparation of cuttlefish skin

Ventral skin of cuttlefish (*Sepia pharaonis*) was obtained from a dock in Songkhla, Thailand. Cuttlefish skin was stored in ice with a skin/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 1 h. Upon arrival, cuttlefish skin was washed with tap water and cut into small pieces (1 × 1 cm^2), placed in polyethylene bags and stored at -20 ºC until use. Storage time was not longer than 2 months. Prior to gelatin extraction, the frozen skin was thawed using running water (25-26 ºC) until the core temperature reached 0 - 2 ºC.

Preparation of gelatin from cuttlefish skin

Gelatin was extracted from cuttlefish skin according to the method of Hoque et al. (2010). Skin was soaked in 0.05 M NaOH with a skin/solution ratio of 1:10 (w/v) with a gentle stirring at room temperature (26–28 ºC). The solution was changed every hour to remove non-collagenous proteins for totally 6 h. Alkali treated skin was then washed with distilled water until the neutral pH of wash water was obtained. The prepared skin was subjected to bleaching in 5% H_2O_2, using a sample/solution ratio of 1:10 (w/v) for 48 h at 4 ºC. The skin treated with H_2O_2 was washed three times with 10 volumes of distilled water. Gelatin was extracted from bleached skin using distilled water at 60 ºC for 12 h, with a sample/water ratio of 1:2 (w/v). During extraction, the mixture was stirred continuously using a paddle stirrer (RW20.n, IKA LABORTECHNIK, Staufen, Germany).

The extract was centrifuged at 8,000 ×g for 30 min at room temperature using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Beckman Coulter, Inc., Palo Alto, CA, USA) to remove insoluble materials. The supernatant was collected and freeze-dried (Model Duratop™ lP/Dura Dry™ lP, FTS® System, Inc., Stone Ridge, NY, USA). The dry matter was referred to as 'gelatin powder'.

Preparation and storage of film from gelatin incorporated without and with Fenton's reagent

Gelatin powder was dissolved in distilled water and heated at 70 °C for 30 min (Hoque et al. 2010). Gelatin solutions containing 3% protein were prepared. The solution was then added with glycerol at a level of 20% (based on protein content) and mixed thoroughly. The mixtures were stirred at room temperature for 1 h. The mixtures obtained were referred to as 'film-forming solution; FFS'. To prepare the film added with Fenton's reagent, gelatin solution was added with a mixture of H_2O_2 and $FeSO_4$ to yield the final concentration of 0.02 M H_2O_2 and 0.002 M $FeSO_4$, respectively (Hoque et al. 2011c). Thereafter, glycerol was added and stirred as previously described.

FFS incorporated without and with Fenton's reagent were used for film casting. FFS (4 ± 0.01 g) was cast onto a rimmed silicone resin plate (5 × 5 cm^2), air-blown for 12 h at room temperature and dried in an environmental chamber (Binder, KBF 115 # 00-19735, D-78532, Tuttalingen, Germany) at 25 ± 0.5 ºC and 50 ± 5% relative humidity (RH) for 48 h. Dried films were manually peeled-off and subjected to analyses. Films obtained from gelatin (without addition of Fenton's reagent) and films added with Fenton's reagent were referred to as 'CG' and 'FG' films, respectively.

Both CG and FG films were stored in an environmental chamber (Binder, KBF 115 # 00-19735, D-78532, Tuttalingen, Germany) at 25 ± 0.5 ºC and 50 ± 5% relative humidity (RH). Films samples were taken at 0 and 21 days of storage for analyses.

Analyses

Prior to mechanical properties testing, films were conditioned for 48 h at 50 ± 5% relative humidity (RH) at 25 ± 0.5 ºC. For SEM, DSC and TGA studies, films were conditioned in a dessicator containing dried silica gel for 1 week and 2 weeks in dessicator containing P_2O_5 at room temperature (28-30 ºC) to obtain the most dehydrated films.

Film thickness

The thickness of film was measured using a digital micrometer (Mitutoyo, Model ID-C112PM, Serial No. 00320, Mituyoto Corp., Kawasaki-shi, Japan). Ten random locations around each film sample were used for thickness determination.

Mechanical properties

Tensile strength (TS) and elongation at break (EAB) were determined as described by Iwata et al. (2000) using the Universal Testing Machine (Lloyd Instrument, Hampshire, UK). Ten samples (2 × 5 cm^2) with the initial grip length of 3 cm were used for testing. The samples were clamped and deformed under tensile loading using a 100 N load cell with the cross-head speed of 30 mm/min until the samples were broken. The maximum load and the final extension at break were used for calculation of TS and EAB, respectively.

Water vapor permeability (WVP)

WVP was measured using a modified ASTM (American Society for Testing and Materials 1989) method as described by Shiku et al. (2004). The film was sealed on an aluminum permeation cup containing dried silica gel (0% RH) with silicone vacuum grease. The cup was placed at 30 ºC in a desiccator containing the distilled water. It was then weighed at 1 h intervals for up to 8 h. Five films were used for WVP testing. WVP of the film was calculated as follows:

$$WVP\ (g/m/s/Pa) = wl/A/t/(P_2 - P_1).$$

where w is the weight gain of the cup (g); l is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); ($P_2 - P_1$) is the vapor pressure difference across the film (Pa).

Film solubility

Film solubility in water was determined according to the method of Gennadios et al. (1998) with a slight modification. The conditioned film sample (3 × 2 cm^2) was weighed and placed in 50 ml-centrifuge tube containing 10 ml of distilled water with 0.1% (w/v) sodium azide. The mixture was shaken continuously at room temperature

for 24 h using a shaker (Heidolph UNIMAX 1010, Schwabach, Germany). Undissolved debris film matter was determined after centrifugation at 3000 ×g for 10 min at 25 ºC using a centrifuge (Allegra 25R Centrifuge, Beckman Coulter, Krefeld, Germany) and drying them at 105 ºC for 24 h to obtain the dry unsolubilized film matter. The weight of solubilized dry matter was calculated by subtracting the weight of unsolubilized dry matter from the initial weight of dry matter and expressed as the percentage of total weight.

Transparency value of film

The transparency value of the film was calculated by the following equation (Han and Floros 1997):
Transparency value = $(-\log T_{600})/x$.

where T_{600} is the fractional transmittance at 600 nm as measured by UV–Visible spectrophotometer (model UV-160, Shimadzu, Kyoto, Japan) and x is the film thickness (mm). The greater transparency value represents the lower transparency of the films.

Differential scanning calorimetry

Thermal properties of film samples were determined using differential scanning calorimeter (DSC) (Perkin Elmer, Model DSC-7, Norwalk, CT, USA). Temperature calibration was performed using the Indium thermogram. The film samples (2–5 mg) were accurately weighed into aluminum pans, sealed, and scanned over the temperature range of -30 to 120 ºC with a heating rate of 10 ºC/min. The dry ice was used as a cooling medium and the system was equilibrated at -30 ºC for 5 min prior to the scan. The empty aluminum pan was used as a reference. The second scan was also performed in the same manner followed the quench cooling of the sample after completing the first scanning.

Thermo-gravimetric analysis (TGA)

Conditioned films were scanned using a thermogravimetric analyzer (TG A-7, Perkin Elmer, Norwalk, CT, USA) from 50 to 600 ºC at a rate of 10 ºC/min (Nuthong et al. 2009). Nitrogen was used as the purge gas at a flow rate of 20 ml/min.

Microstructure

Microstructure of upper surface and freeze-fractured cross-section of the film samples were visualized using a scanning electron microscope (SEM) (Quanta400, FEI, Tokyo, Japan) at an accelerating voltage of 10 kV. Prior to visualization, the film samples were mounted on brass stub and sputtered with gold in order to make the sample conductive, and photographs were taken at 8000× magnification for surface. For cross-section, freeze-fractured films were mounted around stubs using double sided adhesive tape, coated with gold and observed at the 5000× magnification.

Effect of cuttlefish skin gelatin film on storage stability of dried chicken meat powder

Preparation of dried chicken meat powder

Fresh chicken meat was purchased from a local market in Hat Yai, Songkhla, Thailand. Meat was washed with cold water. The meat was then steamed for 20 min with an electric steamer (Jixing, CS-032, Guangdong, China). After cooling in air, the steamed chicken was shredded manually. Prepared sample was subjected to drying using a hot-air oven with an air velocity of 1.5 m/s at 60 °C until moisture content was less than 5%. The dried sample was powderized using a blender (Moulinex, Type AY46, Shenzhen, Guangdong, China). The chicken meat powder was screened using a mesh 35 with an aperture size of 500 μm, ASTM E11, serial number 5666533 (FRITSCH GMBH, Laborgerätebau, Industriestrasse 8, D-55743 Idar-Oberstein, Germany).

Quality changes of dried chicken meat powder covered with cuttlefish skin gelatin films during storage

Chicken meat powder (15 g) was transferred to a cylindrical glass cup with a diameter of 25 mm. The cup containing chicken meat powder was covered with gelatin-based films from cuttlefish skin without and with incorporation of Fenton's reagent and sealed with an O-ring. LDPE (permeability of CO_2, N_2 and O_2: 1.7×10^{-10}, 0.1×10^{-10} and 0.4×10^{-10} m^3 mm/cm^2 s cmHg at 25 °C, 1 atm pressure, respectively) films with a thickness of 0.038 ± 0.003 mm were also used to cover the samples. Sample without film covering was used as the control. The samples were stored at 28–30 °C and were taken every 3 days for 21 days for analyses of moisture content (AOAC 1999), peroxide value, TBARS and color.

Peroxide value

Peroxide value (PV) was determined as per the method of Richards and Hultin (2002) with a slight modification. Chicken meat powder (1 g) was homogenized at a speed of 13,500 rpm for 2 min in 11 ml of chloroform/methanol (2:1, v/v) using an IKA homogenizer (Selangor, Malaysia). Homogenate was then filtered using Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England). Two milliliters of 0.5% NaCl were then added to 7 ml of the filtrate. The mixture was vortexed at a moderate speed for 30 s and then centrifuged at 3000xg for 3 min to separate the sample into two phases. Two milliliters of cold chloroform/methanol (2:1) were added to 3 ml of the lower phase. Twenty-five microliters of ammonium thiocyanate and 25 µl of iron (II) chloride were added to the mixture (Shantha and Decker 1994). Reaction mixture was allowed to stand for 20 min at room temperature prior to reading the absorbance at 500 nm. A standard curve was prepared using cumene hydroperoxide at a concentration range of 0.5–2 ppm.

TBARS

Thiobarbituric acid-reactive substances (TBARS) were determined as described by Buege and Aust (1978). Chicken meat powder (0.2 g) was mixed with 2.5 ml of a TBA solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl. The mixture was heated in a boiling water bath (95–100 °C) for 10 min to develop a pink color, cooled with running tap water and then sonicated for 30 min, followed by centrifugation at 5000 g at 25 °C for 10 min. The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane (MDA) at the concentration ranging from 0 to 10 ppm and TBARS were expressed as mg of MDA equivalents/kg of sample.

Color

Color of chicken meat powder was determined using a CIE colorimeter (Hunter associates laboratory, Inc., Reston, VA, USA). Color of the chicken meat powder was expressed as L^*-, a^*- and b^*-values. Total difference in color (ΔE^*) was calculated according to the following equation (Gennadios et al. 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of white standard ($L^* = 93.63$, $a^* = -0.92$ and $b^* = 0.42$).

Statistical analysis

Experiments were run in triplicate. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by Duncan's multiple range test, T-test was used for pair comparison (Steel and Torrie 1980). Analysis was performed using the SPSS package (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

Results and discussion

Stability of cuttlefish skin gelatin films

Thickness

Thickness of CG and FG films at day 0 and 21 of storage is shown in Table 1. The higher thickness was observed for FG film, compared with CG film ($P < 0.05$). The result suggested that Fenton's reagent could affect the film matrix via radical mediated protein modification, in which the pretruded film matrix was developed when Fenton's reagent was added. This result confirmed that reported by Hoque et al. (2011c). However, no differences in thickness were observed for both films after storage for 21 days ($P > 0.05$).

Mechanical properties

Mechanical properties of CG and FG films before and after storage for 21 days are shown in Table 1. FG films showed the higher TS but lower EAB, compared with the CG film (without addition of Fenton's reagent) ($P < 0.05$). TS of FG film was 35.65% higher than that of CG film. 'Fenton-type' reaction is a metal-catalyzed oxidation system, where the $HO^{\bullet}$ radicals are produced when certain transition metals react with H_2O_2 (Kocha et al. 1997). $HO^{\bullet}$ radical involves abstraction of the alpha-hydrogen atom from amino acid residues to form a carbon-centered

radical derivative. Two different carbon-centered amino acid radicals can react with one another to form –C–C– protein cross-linked products (Stadtman 2001). Hoque et al. (2011c) also found the similar results for both gelatin and partially hydrolyzed gelatin films, in which TS increased via radical-mediated protein modification induced by Fenton's reagent.

After 21 days of storage, similar mechanical properties were observed for CG film ($P > 0.05$). However, FG film had increased TS and EAB after storage for 21 days ($P < 0.05$). The increases in TS and EAB of films during the storage were possibly due to the increased radical-mediated aggregation, which still took place to some extent during storage. Bigi et al. (2002) reported that the cumulative release of gelatin from the films was remarkably low at higher degree of cross-linking induced by genipin, after 1 month of storage in physiological solution. However, Pérez-Mateos et al. (2009) found the decreased puncture force for the gelatin film without and with oil during storage for 30 days. Increased TS but decreased EAB were observed for fish muscle protein-based film during storage (Tongnuanchan et al. 2011a, 2011b; Artharn et al. 2009). Thus, modification or alteration of film matrix still occurred when Fenton's reagent was incorporated, especially as the storage time increased. Radicals generated in film might be involved in inducing the covalent cross-linking of gelatin, thereby strengthening film matrix.

Water vapor permeability (WVP)

WVP of films prepared from CG and FG at day 0 and 21 of storage is presented in Table 1. FG film showed the lower WVP, compared with GF film ($P < 0.05$). The result suggested that radical-mediated cross-linking of protein molecules in film matrix might decrease the free volume and mobility of polymeric structure, thereby lowering the diffusion of water as indicated by the lower WVP.

After storage of 21 days, WVP of both films increased ($P < 0.05$). The result suggested that the hydrophilic nature of gelatin favored interaction between gelatin molecules and water during storage. An increased hydrophilicity of film matrix contributed to the decreased water barrier property of film. Increased WVP was also observed for cod skin gelatin film with and without addition of sunflower oil after storage for 30 days (Pérez-Mateos et al. 2009). However, red tilapia muscle protein film had the decrease in WVP after storage for 4 weeks (Tongnuanchan et al. 2011a). Different changes in WVP between films from varying proteins might be governed by the differences in amino acid compositions and molecular weight distribution of materials used for film preparation. Bondings and degree of interactions involved in the stabilization of a protein film matrix are determined by the amino-acid composition and molecular weight of the proteins (Denavi et al. 2009). Increased WVP of both films during storage negatively affected the ability to protect the foods from moisture migration.

Table 1. Thickness, mechanical properties, water vapor permeability, solubility and transparency values of films from cuttlefish skin gelatin without and with Fenton's reagent at day 0 and 21 of storage*

Storage time (days)	Film sample	Thickness (mm)	TS (MPa)	EAB (%)	WVP ($x10^{-10}$ g/s/m/Pa)	Film solubility (%)	Transparency values
0	CG	0.038 ± 0.002 bA	32.45 ± 2.49 bA	5.94 ± 0.49 aA	1.02 ± 0.06 aB	93.36 ± 1.31 aA	3.37 ± 0.03aA
	FG	0.042 ± 0.002 aA	44.02 ± 1.20 aB	5.04 ± 0.20 bB	0.92 ± 0.04 bB	71.59 ± 1.76 bA	3.28 ± 0.02bA
21	CG	0.038 ± 0.002 bA	35.50 ± 2.83 bA	6.18 ± 0.55 aA	1.26 ± 0.07 aA	90.58 ± 1.55 aB	3.36 ± 0.02aA
	FG	0.042 ± 0.002 aA	45.84 ± 1.44 aA	5.60 ± 0.48 aA	1.11 ± 0.04 bA	66.85 ± 1.93 bB	3.29 ± 0.03bA

*Values are given as Mean ± SD (n=3).
Different small letters in the same column under the same storage time indicate significant differences ($P < 0.05$).
Different capital letters in the same column under the same sample indicate significant differences ($P < 0.05$).
CG: control films from gelatin (without addition of Fenton's reagent).
FG: films from gelatin added with Fenton's reagent containing 0.02 M H_2O_2 + 0.002 M $FeSO_4$.

Film solubility

Films solubility of CG and FG films at day 0 and 21 of storage is shown in Table 1. CG film showed the higher solubility than FG film. Gelatin from cuttlefish skin had high hydrophilic amino acids, thus it was soluble with ease in water (Hoque et al. 2010). However, Fenton's type reaction induces the covalent cross-linking of gelatin via radical generated (Stadtman 2001), as evidenced by decreased film solubility. Film solubility can be viewed as a

measure of the water resistance and integrity of a film (Rhim et al. 2000). Cross-linking markedly reduced the degree of swelling of gelatin film added with genipin (Bigi et al. 2002). It was noted that the decreases in film solubility were observed for both films after storage for 21 days ($P < 0.05$). During storage of film, interaction among the proteins molecules still occurred to some degree. This might induce the migration of glycerol to the surface. The intermolecular rearrangement of gelatin to form rigid polymeric structure might cause a decreased solubility. The slight decrease in solubility was in accordance with the slight increases in TS and EAB of film. Thus, interaction of gelatin molecules still proceeded in film matrix to some extent during storage.

Transparency value

Generally, FG film had the lower transparency values, compared with CG film ($P < 0.05$) (Table 1), indicating the higher transparency in the former. However, no differences in transparency values were observed for both films after the storage for 21 days ($P > 0.05$). Transparency of cod gelatin film remained unchanged over the storage period of 30 days (Pérez-Mateos et al. 2009). The result suggested that the light transmission property of films was not affected by the extended storage time.

Differential scanning calorimetry (DSC)

CG and FG films stored for 0 and 21 days were subjected to DSC analysis. DSC thermograms of both films and their transition temperatures are shown in Figure 1(A) and Table 2, respectively. Thermograms of all film samples showed only glass transition at temperature range of 76.8 - 87.1 °C, depending on film types and storage times. At day 0, FG films had the higher glass transition temperature (T_g) (81.5 °C) than CG film (T_g = 76.8 °C). The higher T_g found in films added with Fenton's reagent might be due to the greater interaction of protein molecules induced by radical-mediated protein modification process, which restricted the molecular mobility of gelatin in the film matrix. T_g is generally the temperature causing the onset of molecular segmental motion. The greater interaction among the gelatin strands resulted in higher T_g (Sobral and Habitante 2001; Sobral et al. 2001). Thermal stability of films was possibly affected by the presence of intermolecular interaction of proteins, such as hydrogen bonds, ionic interactions, hydrophobic–hydrophobic interactions and covalent bonds, which stabilized the film network (Barreto et al. 2003). In general, the higher T_g was coincidentally attained in the films with the higher stiffness and TS (Table 1).

After 21 days of storage, the Tg of both films shifted to higher temperatures and the transition was less pronounced, compared to those of films at day 0 (Table 2). The result suggested that intermolecular rearrangements between gelatin molecules or between gelatin and glycerol might take place to some degree during extended storage time. Gelatin molecules more likely underwent intermolecular interaction to a higher extent during storage, thereby decreasing chain mobility and increasing T_g. This new molecular organization might contribute to the changes in mechanical properties of films after storage for 21 days.

For the second DSC scan, no transition was observed for both films stored for 0 and 21 days (data not shown). It was postulated that the bound water acting as plasticizer might be removed during the first heating scan. As a consequence, the interaction between gelatin molecules could be enhanced and the more rigid film network was obtained. Thus, the transition temperature of the film became too high and could not be detected in the temperature range tested.

Thermo-gravimetric analysis (TGA)

Thermal degradation behavior of CG and FG films at 0 and 21 days of storage was studied. TGA traces with their corresponding degradation temperatures (T_d) and weight loss (Δw) are shown in Figure 1(B) and Table 2, respectively. At day 0, three main stages of weight loss were observed for both films. The first stage weight loss (Δw_1= 4.54 and 7.02%) was observed approximately at temperature (Td_1) of 61.37 and 66.27 °C, for CG and FG films, respectively. This was plausibly associated with the loss of free water adsorbed in the film. The similar results were found in porcine plasma protein film added with different cross-linking agents (Nuthong et al. 2009) and in collagen hydrolysate film plasticized with glycerol and polyethylene glycols (Langmaier et al. 2008). The second stage weight loss (Δw_2= 19.06 and 16.39%) appeared at Td_2 of 230.73 and 245.80 °C for CG and FC films, respectively. This was most likely due to the loss of lower molecular weight protein fractions as well as glycerol. For the third stage weight loss (Δw_3= 50.54 and 53.56%), Td_3 of 318.17 and 326.76 °C were observed for CG and FG films, respectively. The results revealed that FG film showed higher heat resistance than CG film. The incorporation of Fenton's reagent yielded the stronger film network, leading to the higher heat resistance of the resulting film. Similar thermal degradation of gelatin films has been previously reported. Bigi et al. (2002) reported

that the genipin induced cross-linking enhanced the thermal stability of gelatin film, as shown by the slight increase in denaturation temperature (T_d). Chiou et al. (2009) reported that gelatin from cold-cast fish began to degrade at 270 – 271 °C. Chongjun et al. (2010) reported the thermal degradation of cast gelatin film appeared at initial temperature of 263 °C with a mass loss of about 58%, and the degradation temperature of the film increased to 300 °C when the gelatin was cross-linked with cerium (III) nitrate hexahydrate. Hoque et al. (2010) showed that T_d of major protein component in films prepared from cuttlefish gelatin hydrolysate (249.5 – 255.8 °C) was lower than those from gelatin without hydrolysis (271.4 – 290.2 °C).

After storage for 21 days, both CG and FG films had the higher Td_1, Td_2 and Td_3 with the coincidentally lower Δw_1 than those of films at day 0. However, slightly higher Δw_2, Δw_3 and lower residue mass (representing char content) were observed for both films after storage. Upon storage, the higher degradation temperature of resulting film was coincidental with the higher T_g. Higher heat stability of both films was more likely attributed to increased intermolecular interaction during storage. Higher T_d was also observed for fish muscle protein-based film after storage for 40 days (Tongnuanchan et al. 2011b). Therefore, the changes in molecular arrangements of film matrix during storage affected thermal property of film, regardless of Fenton's reagent incorporated.

Table 2. Glass transition temperature (T_g) thermal degradation temperature (T_d) and weight loss (Δw) of films from cuttlefish skin gelatin without and with Fenton's reagent at day 0 and 21 of storage

Storage Time (days)	Film sample	T_g (˚C)	Δ_1		Δ_2		Δ_3		Residue (%)
			$Td_{1,onset}$ (˚C)	Δw_1 (%)	$Td_{2,onset}$ (˚C)	Δw_2 (%)	$Td_{3,onset}$ (˚C)	Δw_3 (%)	
0	CG	76.83	61.37	4.54	230.73	19.06	318.17	50.54	25.86
	FG	81.57	66.27	7.02	245.80	16.39	326.76	53.56	23.03
21	CG	81.21	72.01	1.87	238.13	21.18	324.26	53.97	22.98
	FG	87.16	73.65	2.05	244.73	21.05	328.97	52.91	23.99

CG: control films from gelatin (without addition of Fenton's reagent).
FG: films from gelatin added with Fenton's reagent containing 0.02 M H_2O_2 + 0.002 M $FeSO_4$.
Δ_1, Δ_2, and Δ_3 denote the first, second and third stage weight loss, respectively, of film.

Microstructure

SEM micrographs of the surface and freeze-fractured cross-section of CG and FG films at 0 and 21 days of storage are illustrated in Figure 2. At day 0, smooth surface was observed for both films. After 21 days of storage, no obvious changes were found on the surface of both films. For cross-section, the rough cross-sectional structure was observed in CG film, whereas FG film samples showed the compact/coarser structure. After storage for 21 days, the crack was formed throughout the film. The fracture was more pronounced in FG films. Those cracks in the film matrix could allow water vapors to migrate through the fracture, as indicated by increased WVP of both films after 21 days of storage. The significant decrease in moisture content and intensive cross-linking between proteins molecules possibly led to the presence of non-uniform shrinkage of the internal network structure. This resulted in the formation of higher micro-crack in the film matrix. Those cracks exhibited the detrimental effect on the water barrier property of gelatin film during storage. The increase in crack with higher gap in cross-section was also found in red tilapia (*Oreochromis niloticus*) muscle protein isolate (FPI) and unwashed mince (UWM) films during storage of 40 days (Tongnuanchan et al. 2011b).

Effects of cuttlefish skin gelatin films on quality changes of dried chicken meat powder during storage

Moisture content of dried chicken meat powder

Moisture contents of dried chicken meat powder without cover (control) and covered with CG and FG films in comparison with those of samples covered with low density polyethylene (LDPE) films during storage of 21 days at 28-30 ºC are shown in Figure 3A. In general, moisture content of dried chicken meat powder uncovered and covered with CG and FG films increased continuously during 21 days of storage ($P < 0.05$). However, the highest increase in moisture content of dried chicken meat powder was observed from the uncovered samples, especially during the first 12 days of storage ($P < 0.05$). The sample covered with LDPE films had much lower moisture content than other

samples during the storage ($P < 0.05$). Dried chicken meat powder was able to bind water molecules via specific hydrophilic domains, such as carboxylic, amino and hydroxyl residues of proteins (D'Arcy and Watt 1981). The higher moisture diffusion from the environment through the packaging material increases the moisture content of packed sample. Additionally, the micro-cracks formed in CG and FG films (Figure 2) might favor the migration of water into chicken meat powder. The result suggested that gelatin film possessed poor water barrier property, mainly due to high amount of hydrophilic amino acids with negligible or no sulfur containing amino acids (Hoque et al. 2010; Jongiareonrak et al. 2006; Jiang et al. 2007; Denavi et al. 2009). Artharn et al. (2009) also reported that moisture content of dried fish powder packed with round scad protein-based film and chitosan film containing 25 % palm oil was higher than that of those packed with HDPE film ($P < 0.05$) during storage of 21 days. Thus, the gelatin and modified gelatin film able to prevent moisture absorption by the products to some extent but their preventive effect was lower than LDPE films.

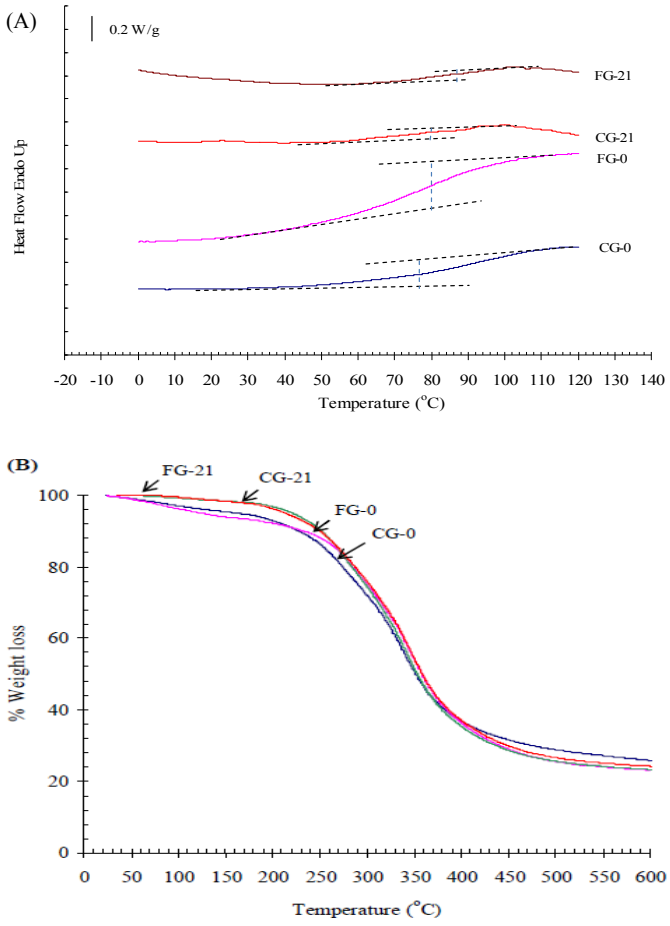

Fig. 1. DSC thermogram (A) and TGA curves (B) of films from cuttlefish skin gelatin (CG) and gelatin film added with Fenton's reagent (FG) at day 0 and 21 of storage.

Lipid oxidation of dried chicken meat powder

Lipid oxidation of dried chicken meat powder uncovered (control) and covered with CG and FG films in comparison with that of samples covered with LDPE films during storage of 21 days was monitored by measuring PV and TBARS (Figure 3B and 3C, respectively). PV value of chicken meat powder samples uncovered and covered with all films increased at day 3 of storage ($P < 0.05$). Thereafter, the decrease was found in all samples at day 6 ($P < 0.05$), except the uncovered sample. The decrease in PV was more likely caused by decomposition of hydroperoxide formed. In general, the highest PV was found in uncovered samples during 6-21 days of storage ($P < 0.05$). No marked changes in PV were observed for sample covered with all films during storage, but the values were slightly different between samples.

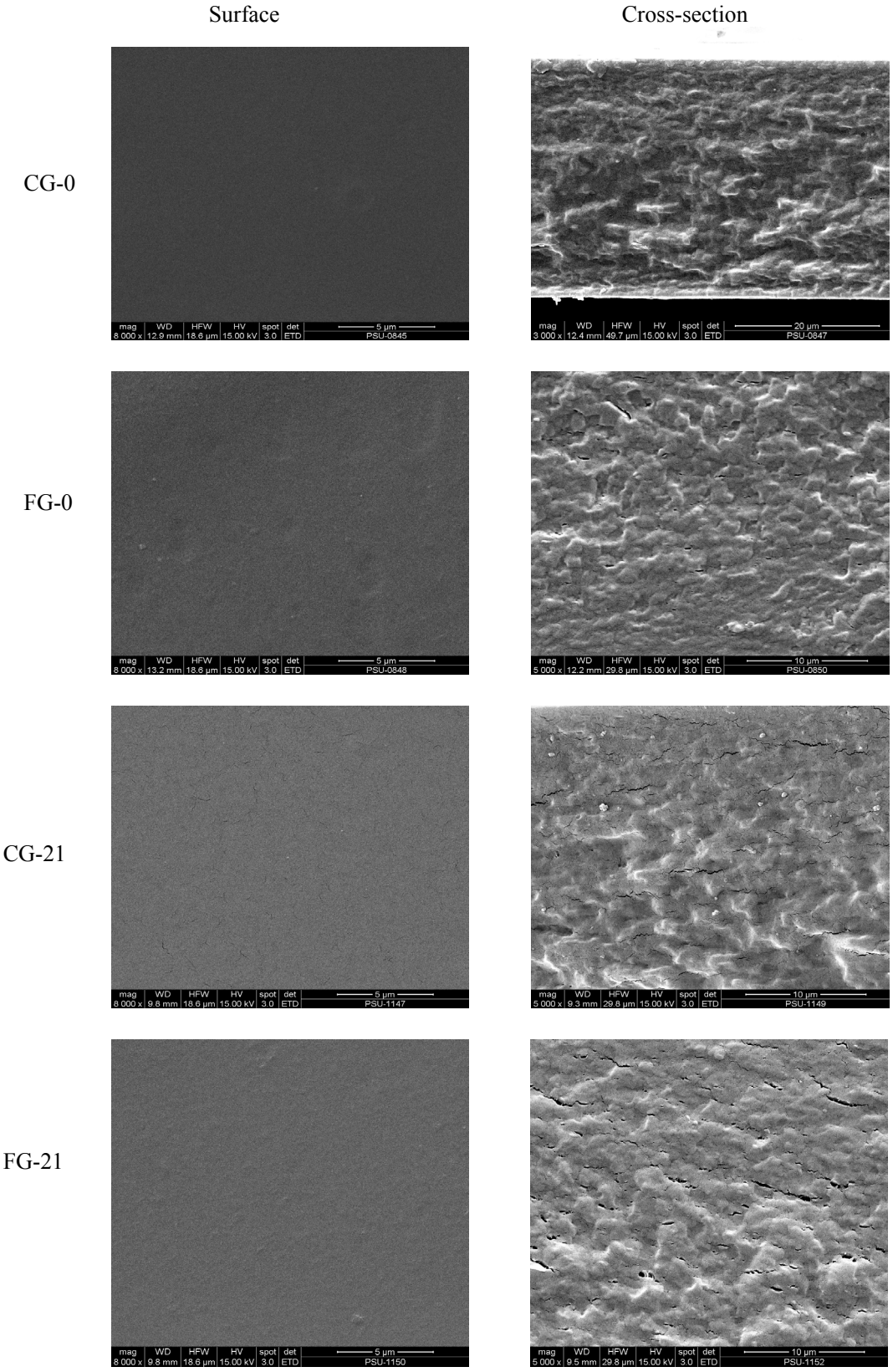

Fig. 2. Morphology of films from cuttlefish skin gelatin (CG) and gelatin film added with Fenton's reagent (FG) at day 0 and 21 of storage. Magnification: 8000x and 5000x for surface and cross-section, respectively.

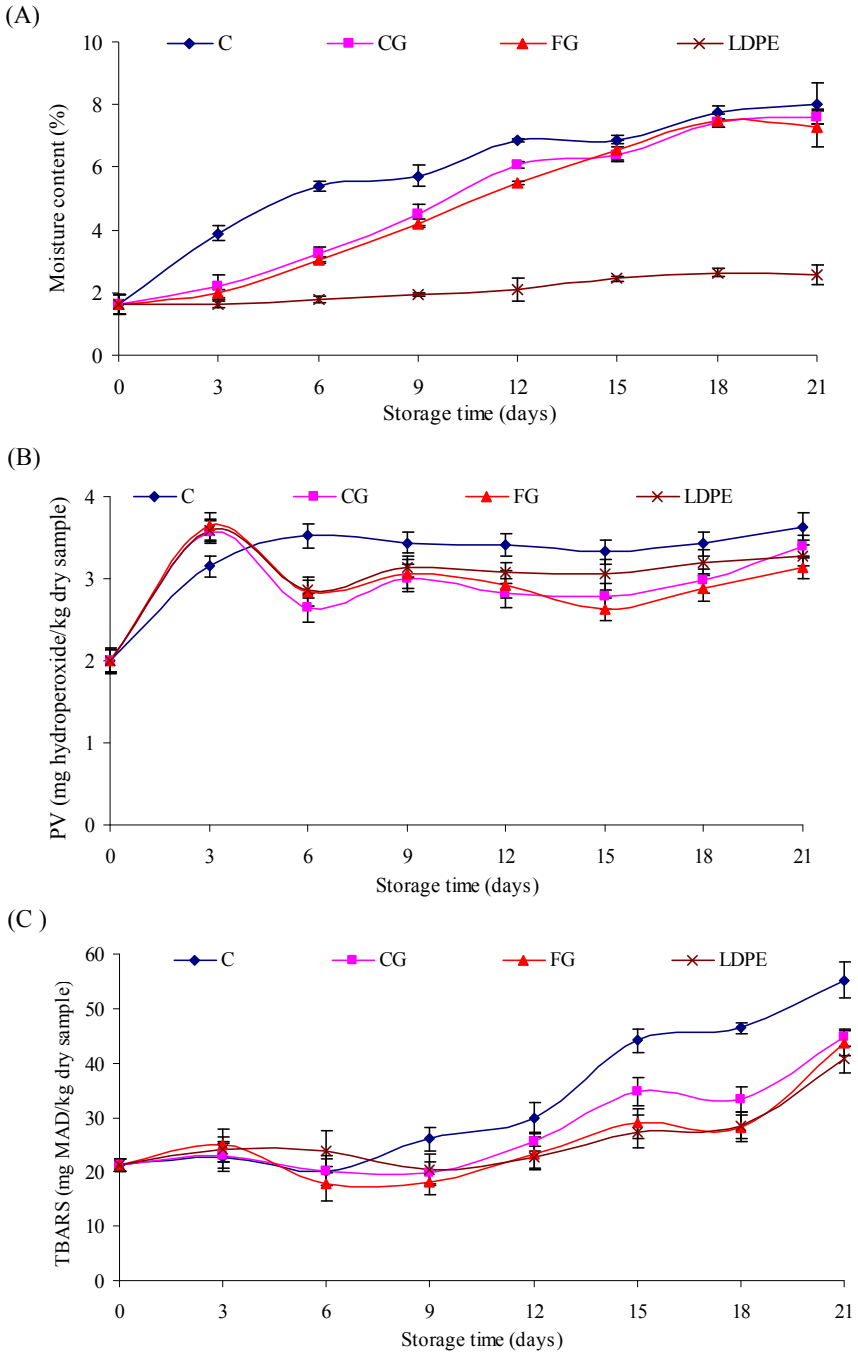

Fig. 3. Changes in moisture content (A), PV (B) and TBARS (C) of dried chicken meat powder uncovered and covered with different films during storage of 21 days. C: Uncovered; CG: cuttlefish skin gelatin film; FG: gelatin film added with Fenton's reagent; LDPE: low density polyethylene. Bars represent the standard deviation (n=3).

Nevertheless, sample covered with FG film tended to have the lowest PV, followed by CG film, suggesting the prevention of oxidation by the FG film. Hydrophilic nature of gelatin can successfully prevent the hydrophobic oxygen gas permeation into the products, thus reducing the oxidation catalytic process. Jongiareonrak et al. (2006) and Jiang et al. (2007) also reported that gelatin film has excellent barrier characteristics against gas, compared to synthetic films. Thus, gelatin film, especially gelatin film incorporated with Fenton's reagent, could retard the lipid oxidation of dried chicken meat powder during extended storage time.

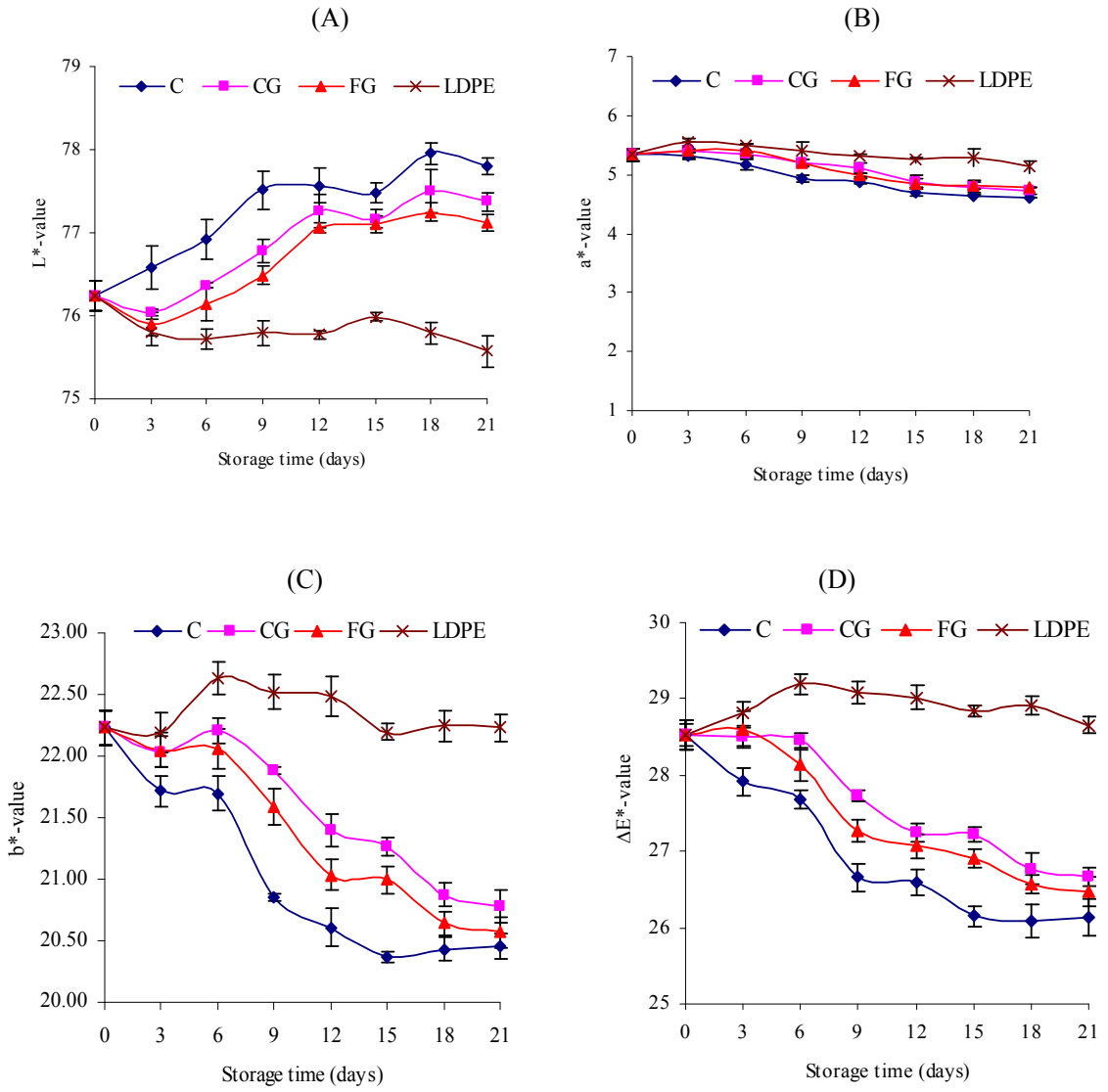

Fig. 4. Changes in L*-value (A), a*-value (B), b*-value (C) and ΔE*-value (D) of dried chicken meat powder uncovered and covered with different films during storage of 21 days. C: Uncovered; CG: cuttlefish skin gelatin film; FG: gelatin film added with Fenton's reagent; LDPE: low density polyethylene. Bars represent the standard deviation (n=3).

TBARS of dried chicken meat powder uncovered (control) and covered with different films during storage of 21 days at 28-30 ºC are presented in Figure 3C. Similar TBARS values of chicken meat powder samples uncovered and covered with all films were observed within the first 6 days of storage ($P > 0.05$). Subsequently, the gradual increase in TBARS was observed for all samples up to 21 days of storage ($P < 0.05$). The sample without cover showed the highest TBARS value than those covered with all films up to 21 days ($P < 0.05$). It was noted that TBARS values of sample covered with FG and LDPE were similar throughout the storage of 21 days. However, sample covered with CG film had the higher TBARS value than others during 15-18 days of storage ($P < 0.05$). The result suggested that FG film, a radical induced modified gelatin film, had higher oxygen barrier properties than CG films. Protein-based films have impressive oxygen and carbon dioxide barrier properties in low relative humidity condition compared to synthetic films (Limpan et al. 2010; Shiku et al. 2003). Therefore, the protein-based film can be used as the packaging material to retard rancidity of foods and also can be served as alternative material for chemically synthesized polymeric films.

Color of dried chicken meat powder

L*, a*, b* and ΔE*-values of dried chicken meat powder uncovered (control) and covered with different films during storage of 21 days are shown in Figure 4. Generally, continuous changes in color values were observed for all samples during storage. The uncovered dried chicken meat powder and powder covered with CG and FG films had the increase in L*-value but decrease in a*-, b*- and ΔE*-values during the extended storage of 21 days ($P < 0.05$). The uncovered sample had the highest L*- value and the lowest a*-, b*- and ΔE*-values during storage time. Highest moisture content in the uncovered sample might contribute to light scattering, leading to increased lightness. When comparing the sample covered with CG and FG films, the former sample had the higher b*- and ΔE*-values than the latter ($P < 0.05$). Generally, chicken meat powder covered with LDPE films had the constant values for L*-, a*-, b*- and ΔE*-values during 21 days of storage. The result suggested that the poorer water barrier property of CG and FG films was more likely associated with the induced changes in color of dried chicken meat powder. Higher moisture content might favor the movement of reactants for discoloration reaction, especially the decrease in a*-values (redness) and b*- values (yellowness). Pigment oxidation may catalyze lipid oxidation, and free radicals produced during oxidation may oxidize the iron atoms or denature the myoglobin molecules, negatively changing the color of the products (Selani et al. 2011). Thus, lipid oxidation products, especially in the control, in the presence of high moisture content, might destruct heme pigments. This resulted in decreased a*- and b*-values during the storage. Seydim et al. (2006) reported that the decreased redness in ground ostrich meat was due to myoglobin oxidation. Furthermore, Artharn et al. (2009) reported the changes in color of dried fish powder during storage due to Maillard reaction effect. Although CG and FG films could retard lipid oxidation of chicken meat powder to some degree, they were not able to maintain the color of the chicken powder during storage.

Conclusions

Both CG and FG films underwent the molecular changes during storage of 21 days. This was associated with the increased mechanical properties but lowered water vapor barrier property. This change directly determined the protective role of films in dried chicken meat powder. FG film could prevent lipid oxidation of chicken meat powder comparably to LDPE film, but had the poorer water barrier property. Thus, the improvement of water barrier property is still needed to maximize the use of cuttlefish skin gelatin films.

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