Effects of the Tomato Pomace Oil Extract on the Physical and Antioxidant Properties of Gelatin Films

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***Abstract*—Antioxidants still remain in the tomato pomace which is generated as a by-product from industry and the extraction of tomato pomace oil may useful in edible film production. The edible film solution was prepared by mixing gelatin (2, 4, and 6%). The effects of the tomato pomace oil (TPOE) were evaluated at 0, 0.5, and 1%. The results showed that an increase in gelatin concentration had caused an increase in the tensile strength, moisture content, solubility and swelling power. The edible film with TPOE appeared as a rough film having oil droplets dispersed throughout. The addition of TPOE caused an increase in lightness, redness, and yellowness, while tensile strength, moisture content, and solubility were decreased. The films, made with TPOE at 0.5 and 1%, exhibited antioxidant properties. However, those properties were not significantly different (p<0.05) between film incorporated with TPOE 0.5 and 1%. The suitable conditions for film production in this study were 4% of gelatin and 0.5%.**

***Index Terms*—antioxidant, gelatin films, physical properties, tomato pomace oil extract**

I. Introduction

Tomato pomace is an inexpensive by-product of tomato manufacturing. Effectively, it is what is left over after processing tomatoes for juice, ketchup, and soup, etc. The by-products represent 5-13% of the whole tomato. Pomace is a mixture of tomato peels, crushed seeds and small amounts of pulp that remains after the processing of the tomato for juice, paste, and ketchup [1]. Tomato paste is the primary tomato product produced worldwide, while tomato pomace is the main tomato by-product used for animal feed. An example of using tomato pomace in animal feeds is in sheep feed [2] and in rabbit feed [3] in which tomato pomace (10%) was used instead of rice straw and rice bran. Maize oil, which is used in hamster feed (instead the tomato seeds), can reduce the cholesterol level [4]. Dried tomato pomace has a nutritional profile of about 20% protein, 13-15% fat, 3-5% fat, and 25-57% crude fiber. One study found differences in the contents of minerals like cesium, iron, potassium, molybdenum, and sodium between tomato seeds from conventional or organic systems [5].

The application of oils can be incorporated in gelatin films by coating, by laminating or multi-layering, and by dispersion or emulsion to form composite films. Essential oils are natural volatile complex compounds formed as the secondary metabolites in plants. They have been incorporated to improve the film's properties, such as its antibacterial, antifungal, antiviral and antioxidant properties. Tongnuanchan, et al. [6] studied the structural, morphological, and thermal behavior characterizations of fish gelatin film incorporated with the essential oils of basil and citronella. Films added with both essential oils had lower glass-transition and degradation temperatures than the control film. Moreover, the fish gelatin film with basil leaf essential oil exhibited strong antibacterial activities against food-borne pathogenic and spoilage bacteria [7]. Ahmad, et al. [8] studied the physico-mechanical and antimicrobial properties of gelatin film incorporated with lemongrass oil. Lemongrass oil (5-25%) resulted in decreases in tensile strength, elongation and water vapor permeability. Those films showed an inhibitory effect in a concentration dependent manner against *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, and Salmonella typhimurium*. Sunflower oil in cod gelatin films affected water vapor permeability, the soluble matter content, and the puncture force decrease [9], but when corn oil was used, the gelatin films showed tensile strength and puncture strength increases [10]. Ma, et al. [11] studied the effects of homogenization conditions on the properties of gelatin-olive oil composite films. Gelatin-olive oil films were prepared though emulsification to improve the water barrier ability of gelatin based films. The water barrier capacity and tensile strength showed an enhanced trend as the lipid droplets in the films decreased. The olive oil has a good potential to be incorporated into gelatin in order to make an edible film for some food applications [12]. Tongnuanchan, et al. [13] studied the antioxidant activity of fish skin gelatin incorporated with citrus essential oils. Films, incorporated with lemon essential oil, showed the highest ABTS radical scavenging activity and ferric-reducing anti-oxidant power (FRAP). Film incorporated with plai and turmeric essential oils were shown to have a higher DPPH and ABTS radical scavenging activity than the control film [14]. Gelatin

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film containing *Zataria multiflora* essential oil exhibited excellent anti-oxidant properties and also exhibited excellent antibacterial properties against both Gram-positive and Gram-negative bacteria. Gelatin film with essential oils could be used as an active film to ensure the safety of packaged foods and to extend their shelf-life due to its antioxidant and antimicrobial properties [15].

The present research was conducted to study the effects of TPOE on the physical properties of water vapor permeability, tensile properties, color, and solubility, as well as to examine the potential of antioxidant activity by the DPPH radical scavenging activity method including making a determination of the lycopene content in the gelatin film.

II. Materials and Methods

A. Film Preparation

The gelation was purchased from Gelita USA Inc. (USA). The tomato oil was extracted from the tomato pomace which was the by-product of tomato sauce factories located in Nong Khai Province, Thailand. Gelatin powder was mixed with distilled water to obtain the concentrated 2, 4, and 6%. The 0, 0.5, and 1% of TPOE was added into the solution. PEG (1%) was added as the plasticizer. The mixed solution was heated at 40^{o}C for 30 min with stirring. Then 50 ml of film solution was poured in plate (15 cm diameter). The plates were dried at 40 ℃ in the hot air oven for 12 h. Next, the films were peeled off, placed in a plastic bag, and stored in the desiccator before testing.

B. The Physical Characterizations of the Films

1) The Colors of the Films

Gelatin films were determined color by using the Hunter Lab Spectrocolorimeter. Gelatin films were cut for placing over the 1 cm diameter hole test detector. The white plate calibration was used to background. Then they were covered with black cup test for interference protection. The colors were reported in L*a*b* system for lightness, redness and yellowness.

2) The Moisture Content of the Films

The moisture content indicates the shelf-life of film. Generally, film has low moisture content, is considered to dried material. The moisture content of the films were determined using AOAC method [16]. The films were cut in small pieces for 3 g and dried in hot air oven 105^{o}C for 24 h. The loss of weight of the film is calculated as a percentage.

3) The Solubility of the Films

The films were cut in 2x2 cm2, weighed (A) and put in 50 ml of boil distilled water. The flask was shacked for 24 h at 25oC. The residue film was filtrated from the solution and dried at 104 ℃ for 24 h and then weighted (B). The solubility (%) = (A-B) x100/A [15].

4) The Water Vapor Permeability of the Films

The water vapor permeability (WVP) of the film was measured using ASTM E96 method [17]. The films were cut 5 cm diameter, then weighed as the initial weight. The cut film was sealed on the mouth of the test cup which contained 30 ml of distilled water. The test cups were placed in the control chamber containing silica gel (RH=0%). The RH in chamber was 50±5%. Weights were taken every hour at intervals for up to 8 hours. The graph was plotted between the loss of weight and the time to get the slope of each sample. WVP was calculated as follows: WVP (g.mm/m^2.day.kPa)= slope × thickness/(area × dp); dp = partial pressure between 2 sides of film.

5) The Tensile strength of the Films

The mechanical properties of the films were evaluated following the ASTM D882 method [18]. The texture analyzer (Micro Stable Systems, Godalming, UK) was set in tensile mode and was used with a 50 N load cell. The films were prepared in 1x12 cm^2 with initial grip length of 10 cm. The cross head speeds were set at 1 mm/s. The tensile strength was calculated by dividing the maximum force by the cross-section area. Elongation was calculated in the percentage of the extension length.

6) A Scanning Electron Microscope Examination of the Films

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

C. The Antioxidant Activity of the Films

The film, weighing 400 mg, was soaked in 3 ml of hexane for 15 h. The absorbance of the clear solution was measured at 503 nm using a spectrophotometer. The lycopene was calculated using the following formula: Lycopene (mol/l) = A_{503} x 536.9 x 3 x 100/(17.2 x 1000 x 400)

The antioxidant activities of the films were determined by DPPH assay. The 1.5 ml solution was added with 1.5 ml of DPPH. The mixture was mixed using a vortex mixer and allowed to stand at room temperature in the dark for 30 min. The absorbance of the mixed solution was measured at 515 nm using a spectrophotometer. A blank sample was prepared by mixing 1.5% of Ethyl Acetate with 1.5 ml of DPPH in the same manner. The antioxidant activity (AA) was calculated following the formula: AA (%) = (A_{515} of control- A_{515} of sample) x 100/A_{515} of control

III. Results

A. The Color of the Films

The appearance of the gelatin film was transparent, clear, homogenous, and smooth. The 2% gelatin was weaker and thinner than others (Fig. 1). The lightness (L) of the film decreased with gelatin and oil content while the redness (a*) and yellowness (b*) directly increased with TPOE (Table I). It was noted that the orange color of tomato oil extract was correlated with high a* and b* values. The change in color of biopolymer film was mostly affected by the original incorporated oil [14]. This results were in agreement with Arfat, et al. [7] who reported that increasing the amount of basil leaf essential oil markedly increased the b* value with the concomitant decreases in L* value of fish skin gelatin.

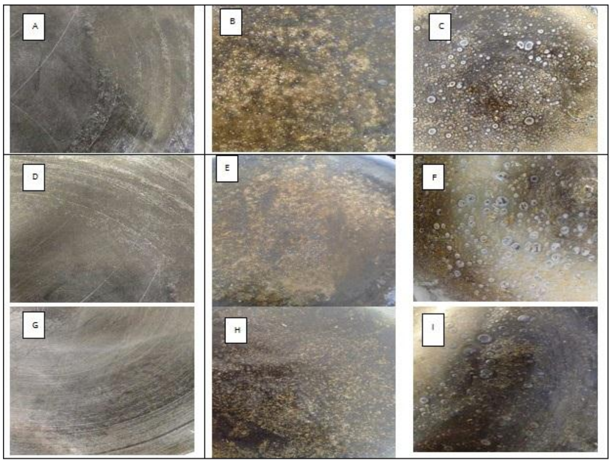

Figure 1. The appearance of gelatin films (Black background); A= gelatin2% oil0%, B= gelatin2% oil0.5%, C= gelatin2% oil1%, D= gelatin4% oil0%, E= gelatin4% oil0.5%, F= gelatin4% oil1%, G= gelatin6% oil0%, H= gelatin6% oil0.5% and I= gelatin6% oil1%.

TABLE I. COLOR OF GELATIN FILMS

Films	*L	*a	*b
gelatin2% oil0%	88.87±0.48^{d}	0.63±0.02^{a}	(-6.22)±0.30^{a}
gelatin2% oil0.5%	88.13±0.52^{d}	0.74±0.04^{a}	2.02±0.81^{c}
gelatin2% oil1%	88.65±0.37^{d}	6.10±0.42^{d}	19.31±1.66^{f}
gelatin4% oil0%	86.39±0.47^{c}	0.62±0.04^{a}	(-3.97)±0.78^{b}
gelatin4% oil0.5%	85.74±0.35^{bc}	1.54±0.18^{b}	4.00±0.36^{c}
gelatin4% oil1%	84.32±0.43^{a}	3.27±0.43^{c}	13.32±2.19^{d}
gelatin6% oil0%	86.42±0.32^{c}	0.59±0.06^{a}	(-3.37)±0.51^{b}
gelatin6% oil0.5%	85.22±0.62^{b}	1.38±0.45^{b}	3.17±0.68^{c}
gelatin6% oil1%	85.41±0.54^{b}	1.49±0.09^{b}	15.46±1.89^{e}

Different letters in the same column indicates the statistically different ($p < 0.05$)

B. The Moisture Content of the Films

The moisture content of the gelatin film was high because gelatin is hydrophilic material while, TPOE is hydrophobic and causes the film to have a low moisture content (Table II). The partial protein-water interactions may account for the moisture content reduction that occurred in the emulsified films [12]. Similar results were observed for gelatin films with *Zataria multiflora* essential oil [15] and gelatin film with olive oil [11]. However, the moisture content of all samples were 5.2-7.5% which is considered to be a low moisture content materials. Thus, it had a longer shelf-life than the high moisture content materials.

C. The Solubility of the Films

The water solubility percentages of films are important for food packaging because its properties have an effect on its applications. Water solubility is an indication of a film's hydrophilicity. The solubility of the films showed a similar trend with moisture content. The gelatin is a water soluble material and can dissolve when it comes in contact with an aqueous medium and loses its fibrous structure [15]. The solubility of 2% gelatin film decreased with TPOE content (Table II). The gelatin films with TPOE were found to maintain, while the gelatin film without TPOE dissolved [12]. These results were similar to Perez-Mateous, et al. [9] who reported that adding oil at >0.6% lowered the solubility ($p<0.05$) because interactions between the protein and the oil in the film contribute to protein insolubilization. The results of the solubility were around 30-60% which is higher than the gelatin film with *Zataria multiflora* oil (27-33%) [15], but is lower than gelatin film with lemongrass oil and gelatin film with sunflower oil (80-87%) [9]. The present results showed that there was no significant difference in the gelatin films with the TPOE content of 4 and 6% ($p<0.05$).

D. The Water Vapor Permeability of the Film

The Water vapor permeability (WVP) of gelatin films incorporated with TPOE is presented in Table II. WVP of gelatin film incorporated TPOE was slightly increased. The TPOE in the gelatin film might have been distributed irregularly leading to the heterogeneous film structure [8]. The film had the space in film structure and had high surface area which may have affected the WVP in the film. With respect to the 2% gelatin film, incorporated with 1% TPOE, the WVP of film could not be measured because film was thin and small holes were distributed around the film. Following oil content, the 6% gelatin film expressed a high WVP because the film had a greater thickness than the 2% and 4% gelatin films. The hydrophilic domains of gelatin can essentially interact with oil through hydrophobic interaction and thereby enhance interfacial interaction between the matrix and the oil. The phenomenon caused the increase in WVP [15]. The resulting expressed WVP was lower than gelatin film with corn oil [10] and *Zataria multiflora* oil [15] but higher than gelatin with basil leaf oil [7], bergamot oil [8], citrus oil [13], olive oil [12], and ginger, turmeric and plai oil [14]. Similar results of WVP were observed for basil leaf essential oil incorporated in gelatin film which increased with increasing level of oil. It was due to the protruded structures mediated by interaction between chemical components in basil leaf essential oil and the protein matrix. Thus, the protein chains could not form the compact and ordered film network in the presence of oil as indicated by the increased thickness [7].

E. The Tensile Strength of the Films

The mechanical properties of the film were expressed through the applicable usage to maintain its integrity and to withstand external stress [12]. Generally, bioplastic films are weaker than synertic plastic. They could be improved though the use of plasticziers or additives. The mechanical properties of films, incorporated with TPOE, are shown in Table II. The tensile strength increased with the gelatin content because gelatin films were mainly stabilized by weak bonds including hydrogen bonds and hydrophobic interaction. Cross-linking was carried out with glutaraldehyde by inserting a covalent bond between the gelatin strands [15]. The 6% gelatin film without TPOE showed the highest tensile strength. The addition of TPOE in increasing amounts decreased the tensile strength of protein-based film because it interrupted the protein network and the protein-protein interaction, as well as showed the lack of cohesive structural integrity of the oil. The interaction of oil between peptide chains could not be developed [14]. With respect to tensile strength, similar results have been reported that are in agreement with the findings of Tongnuanchan, et al. [14] and Tongnuanchan, et al. [13] who all reported that gelatin films incorporated with root, oregano, and lime essential oils had shown a lower tensile strength than the gelatin films. The lowered elongation was obtained with the addition of TPOE at levels of 1% and 4% in the gelatin films, while 2% and 6% gelatin did not show significant differences in the elongation between the films added with TPOE. The elongation values of film were similarly reported and were in agreement with Ahmad, et al. [8] who had studied gelatin film incorporated with bergamot essential oil.

TABLE II. PHYSICAL PROPERTIES OF GELATIN FILMS

Films	Moisture content (%)	Solubility (%)	Water vapor permeability (g.mm/m^2. day.kpa)	Tensile strength (Mpa)	Elongation (%)
gelatin2% oil0%	6.21±0.53^{b}	60.09±17.86^{c}	0.18±0.03^{a}	26.21±1.11^{bc}	1.49±0.12^{bcd}
gelatin2% oil0.5%	7.64±0.11^{e}	51.81±0.67^{bc}	0.21±0.02^{a}	17.63±9.45^{ab}	1.13±0.16^{ab}
gelatin2% oil1%	5.40±0.09^{a}	34.18±6.21^{a}	na	14.03±4.24^{a}	1.43±0.21^{abc}
gelatin4% oil0%	6.90±0.10^{cd}	55.85±6.73^{bc}	0.29±0.10^{ab}	45.56±7.80^{d}	1.98±0.15^{e}
gelatin4% oil0.5%	6.45±0.24^{bc}	54.81±9.90^{bc}	0.35±0.05^{b}	28.83±5.61^{c}	1.87±0.21^{de}
gelatin4% oil1%	6.22±0.13^{b}	43.96±0.95^{ab}	0.40±0.03^{b}	15.61±1.11^{a}	1.21±0.17^{ab}
gelatin6% oil0%	7.58±0.48^{e}	62.71±8.20^{c}	0.21±0.04^{a}	61.94±4.00^{e}	1.73±0.15^{cde}
gelatin6% oil0.5%	7.26±0.33^{de}	65.57±4.00^{c}	0.40±0.12^{b}	15.70±1.36^{a}	1.50±0.23^{abc}
gelatin6% oil1%	6.86±0.14^{cd}	56.17±4.54^{bc}	0.53±0.13^{c}	19.70±2.62^{ab}	1.80±0.04^{cde}

Different letters in the same column indicates the statistically different ($p < 0.05$)

F. *The Scanning Electron Microscopy of the Films*

Scanning electron microscope (SEM) pictures of the surface of gelatin films incorporated with TPOE are presented in Fig. 2. The film, without TPOE, had a compact, smooth, transparent, and homogeneous surface structure due to the ordered-phase and homogeneous network structure [6]. The addition of the TPOE increased roughness, opaqueness, and bubble distribution in the film matrix. The roughness could be due to the distribution if oil droplets throughout the film matrix [7]. The number of bubbles increased with an increase in the oil content. The spaces were filled by oil that had evaporated from the film's surface [9]. The results were similar to the gelatin with *Zataria multiflora* oil which had bubbles in the film [15]. The high amount of oils might be segregated from the aqueous phase. This was indicated by the heterogeneous distribution of oils as is usual when crystals are formed [14].

G. *The Antioxidant Activities of the Films*

The anti-oxidant activity is expressed as DPPH scavenging activity (Table III). The major constituent in TPOE is lycopene. In general, the lycopene content of films, incorporated with tomato, increase as the amount of TPOE is increased. The lycopene in tomatoes is an antioxidant substance. It can inhibit the oxidation reaction in the film and the food that is protected by the film [5]. Thus, the film, incorporated with TPOE, showed a higher antioxidant activity than the control film ($p<0.05$). The DPPH radical scavenging activities of films showed no significant difference between 2% and 4% gelatin films with TPOE, which were higher than the 6% gelatin film with 0.5% tomato oil extract. In the present study, the control film, without TPOE, had no DPPH radical scavenging activity which was similar to the results from Tongnuanchan, et al. [13]. While, Kavoosi, et al. [15] reported that films, prepared from gelatin, showed antioxidative activity as determined by ABTS. The DPPH of the results was 0.08-0.2 µmol Trolox equivalents (TE)/ g dried film which was similar to the results of gelatin film that had incorporated citrus essential oils (0.02-0.42 TE/g dried film) [13], but it was lower than the results of the gelatin film incorporated with root essential oil (0226-2.39 TE/ g dried film) [14]. However, the results suggested that gelatin, incorporated with TPOE, could be further utilized in active films processing because of its anti-oxidant activity.

TABLE III. LYCOPENE AND ANTIOXIDANT OF GELATIN FILMS

Films	Lycopene (ug/100g)	Antioxidant (%)
gelatin2% oil0%	0	0
gelatin2% oil0.5%	0.71 ±0.02[bc]	17.21 ±4.86[b]
gelatin2% oil1%	0.81 ±0.12[c]	19.07 ±9.56[b]
gelatin4% oil0%	0	0
gelatin4% oil0.5%	0.22 ±0.06[a]	12.60 ±1.84[b]
gelatin4% oil1%	0.70 ±0.08[c]	13.60 ±2.08[b]
gelatin6% oil0%	0	0
gelatin6% oil0.5%	0.20 ±0.04[a]	1.52 ±0.29[a]
gelatin6% oil1%	0.50 ±0.10[b]	16.35 ±1.01[b]

Different letters in the same column indicates the statistically different ($p < 0.05$)

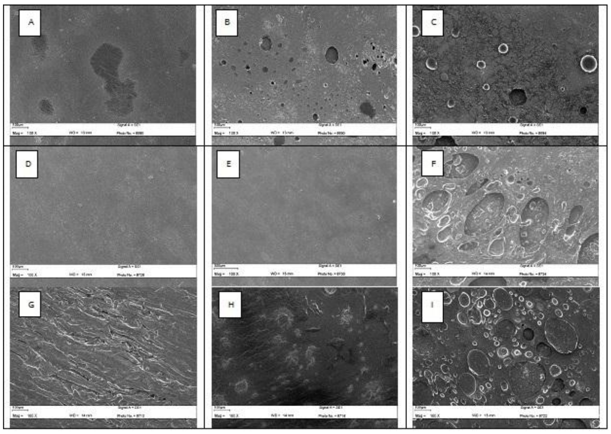

Figure 2. Scanning electron microscope micrographs of surface gelatin films; A= gelatin2% oil0%, B= gelatin2% oil0.5%, C= gelatin2% oil1%, D= gelatin4% oil0%, E= gelatin4% oil0.5%, F= gelatin4% oil1%, G= gelatin6% oil0%, H= gelatin6% oil0.5% and I= gelatin6% oil1%

IV. CONCLUSIONS

The gelatin content caused the tensile strength and the lightness of the film to increase. The addition of TPOE in gelatin film caused roughness, tensile strength, and lightness, as well as caused a decrease in the solubility of the film. However, the redness and yellowness were increased. While, the elongation and water vapor permeability were slightly changed. When comparing the antioxidant activities, the TPOE affected the film's antioxidant activity in terms of both lycopene and DPPH. The suitable conditions for the physical and antioxidant properties of the film were 4% gelatin with 0.5% TPOE.

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