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Development and characterisation of active antioxidant packaging films

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Abstract

The development of food packaging materials from naturally renewable resources and the incorporation of natural preservatives might be one way to address the problem on food packaging wastes and aid in the increasing awareness of consumers on healthy lifestyle. The present work was conducted to develop edible films based on gelatine and carboxymethylcellulose (CMC) incorporated with *Antidesma bunius* (L.) Spreng. (*bignay*) phenolic extract for possible application as active edible film. The optimisation of the antioxidant activity and tensile strength of the films was done using mixture design of experiments using Design Expert (version 10.0.3.1). Results showed that the responses were mainly affected by the amount of *bignay* extract whose total phenolic content was found to be 444.36 ± 25.78 mg gallic acid equivalents/100 g purée, and antioxidant activity of 65.25 ± 0.71% DPPH radical scavenging capacity and 50.74 ± 0.60% ABTS radical scavenging capacity. The optimised proportion of gelatine, CMC and *bignay* was found to be 15% gelatine, 56% CMC and 29% *bignay* extract with 25.89% ± 1.26% DPPH radical scavenging capacity and a tensile strength of 7.45 ± 1.00 MPa. The colour analysis on the optimised film with an average final thickness of 0.10 ± 0.02 mm revealed an *L** value of 79.29 ± 2.38 and positive values for a* (3.09 ± 0.23) and b* (3.13 ± 1.30).

Introduction

Due to the increasing consumers' awareness on the environmental problems arising from the use of synthetic packaging materials and the demand for food products with higher quality, there is thus an increasing demand for packaging materials manufactured from naturally renewable resources like polysaccharides, proteins and lipids (da Silva *et al*., 2009). Due to their biodegradability, biopolymer-based materials might address the problem on the disposal of conventional synthetic polymer food packaging materials to some extent. However, as compared to synthetic polymer-based packaging materials, bioplastics have poor mechanical and water vapour barrier properties. Studies have therefore been conducted to improve their properties. Some techniques developed were chemical modification of biopolymers, addition of plasticiser and combining different biopolymers (Tang *et al*., 2012). Films formed by combining different polysaccharides, proteins and lipids are called composite films, and could be designed such that the components work in synergy. The properties of the composite films, therefore, depend on the characteristics and compatibility of the constituting polymers (Garcia *et al*., 2004).

Gelatine, a protein of animal origin, produces clear, flexible, strong and oxygen-impermeable films making them good oxygen barriers. However, due to the hydrophilic nature of gelatine, films produced have poor moisture barriers. Several techniques have been developed in order to improve the properties of gelatine films. The mechanical properties, water vapour permeability and water solubility of gelatine films were significantly improved by the incorporation of nanoclays like laponite (Li *et al*., 2015). However, potential migration and health risks of nanosized particles are still not fully understood. Another technique developed was blending gelatine with another polymer, pectin. Results showed improved mechanical properties and water resistance in the presence of a crosslinking agent, glutaraldehyde (Farris *et al*., 2011). Carboxymethylcellulose (CMC), on the other hand, is a cellulose derivative used in edible packaging. It is a generally recognised as safe (GRAS) food additive. Preliminary studies conducted

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by Muppalla *et al*. (2014) revealed that 1% CMC had excellent film-forming properties. However, the films showed weak mechanical properties that can be improved when combined with other polymers.

Active packaging is defined by the European Union (European Commission, 2009) as a packaging material that serves an additional function aside from protection. An active package might either absorb or release active substances like preservatives, antioxidants and flavourings. The active components must perform their intended function to modify the food condition and extend its shelf life and improve product safety and quality (Mihindukulasuriya and Lim, 2014). Among the active compounds available, antioxidants and antimicrobials are most frequently studied. The incorporation of antioxidants in food packaging materials prevents the development of off-flavours, colour changes and nutritional losses in fatty foods. These foods are susceptible to spoilage due to lipid oxidation, next to microbial deterioration (Gómez-Estaca *et al*., 2014). Synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been applied to food packaging materials and have shown a delay in lipid oxidation and protein denaturation (Torres-Arreola *et al*., 2007). However, the use of synthetic antioxidants has become questionable as they are suspected to promote the formation of cancer cells when consumed at high levels (Williams *et al*., 1999). As an alternative, researches have been focused on the use of natural antioxidants and plant extracts that are considered to be safer and offer several health benefits (Lopez-de-Dicastillo *et al*., 2012). Antioxidants from *Ugna molinae* Turcz (*murta*) fruit extract (López-de-Dicastillo *et al*., 2016), green tea aqueous extract (López de Lacey *et al*., 2012; Lopez-de-Dicastillo *et al*., 2012; Li *et al*., 2014; Lorenzo *et al*., 2014), cocoa extract (Calatayud *et al*., 2013), oregano essential oil (Lorenzo *et al*., 2014), grape seed extract, ginger and gingko leaf extracts (Li *et al*., 2014), citrus fruit extract and α-tocopherol (Contini *et al*., 2011) have been used in packaging films. The present work was therefore aimed to explore the use of *Antidesma bunius* (L.) Spreng., also known as "*bignay*" in the Philippines. The phenolic extract of *bignay* berries could be a good candidate for active packaging applications. According to Santiago *et al*. (2007), the methanolic extract of *bignay* has 76.06% non-site specific scavenging activity using the deoxy-D-ribose assay. The methanolic extract also showed 75.77% inhibition of lipid peroxidation using the linoleic acid pre-emulsion system, which is comparable to BHT and BHA with 85% and 81.15% inhibition, respectively. Different species of the plant contain varying amounts of phenolic acids, flavonoids and anthocyanins. A study conducted by Jorjong *et al*. (2015) found that the antioxidant activity of the fruit is highly correlated with active compounds gallic acid, ferulic acid and some anthocyanins, mainly cyanidin-3-O-glucoside.

The present work also aimed to provide experimental evidence on the formation of active gelatine/CMC composite films with possibly improved properties; specifically to (1) determine the antioxidant activity of the aqueous phenolic extract of *bignay* fruit, and (2) develop, characterise and optimise the antioxidant activity and tensile strength of films from gelatine, carboxymethylcellulose and *bignay* fruit phenolic extract.

Materials and methods

Sample preparation

Ripe *bignay* berries were harvested in mid-November at the Institute of Food Science and Technology, UPLB. The berries were washed and stored at -4°C until extraction.

Aqueous extraction of bignay phenolic compounds

The berries (together with the seeds) were blended using a blender (model 4172-051, Oster®, Mexico). The purée was extracted with 0.01% hydrochloric acid at a ratio of 1:10 (w/v) purée:solvent in extraction flasks (1 L Erlenmeyer flasks) covered with aluminum foil at 40°C for 2 h with constant agitation (speed 7) using a hot plate stirrer (HTS-1003, LMS Co., Ltd., Tokyo, Japan). The solution was filtered through Whatman No.1 using a vacuum pump, and the supernatant was collected and stored at -4°C until analysis for total phenolic content and antioxidant activity.

Determination total phenolic content

The total phenolic content of *bignay* fruit extract was determined using the Folin-Ciocalteu assay as described by Lizardo *et al*. (2015) with modifications. In a test tube, 0.2 mL diluted extract (dilution factor = 10) was added with 2.8 mL distilled water. Sodium carbonate (1.0 mL) with a concentration of 0.2 M was then added. The mixture was then added with 0.2 mL Folin-Ciocalteu phenol reagent and was mixed thoroughly using a vortex mixer. The test was done in triplicates. The test tubes were placed in a boiling water bath for 15 min and cooled to room temperature. Absorbance was then read at 710 nm using a UV spectrophotometer (CT-2600, ChromTech, Taipei, Taiwan). A standard curve was prepared using different concentrations (0, 0.02, 0.04,

0.06, 0.08, 0.10, 0.12, 0.16 mg/mL) of gallic acid in methanol (R^2 = 0.9981). The results were expressed as mg gallic acid equivalents (GAE) per 100 g purée.

Analysis of antioxidant property

The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of the extract was measured according to Lizardo *et al*. (2015) with modifications. In a test tube, 1.0 mL diluted extract (dilution factor = 10) was added with 4.0 mL distilled water. The solution was mixed, and 1.0 mL of freshly prepared 0.1 mM DPPH methanolic solution (4 mg of DPPH in 100 mL absolute methanol) was added. The absorbance of the resulting solution was read at 517 nm using a UV spectrophotometer (CT-2600, ChromTech, Taipei, Taiwan) after 30 min. A blank containing distilled water and DPPH solution was also run (absorbance = 0.141). The antioxidant activity of the extract was calculated against a calibration curve (0, 5, 10, 15, 20 µM) established with Trolox (R^2 = 0.9910), and expressed as mg Trolox equivalent per 100 g purée.

The ABTS (2,2′-azino-bis-3-ethylbenzothiazoline -6-sulfonic acid) free radical scavenging activity of the extract was measured using the method described by Re *et al*. (1999). The ABTS stock solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubated for 16 h in the dark at 30°C. An aliquot of the stock solution was diluted with methanol to adjust the absorbance to 0.70 ± 0.02 at 734 nm. A 20 µL aliquot of diluted extract (dilution factor = 10) was added with 980 µL ABTS reagent. The absorbance of the resulting solution was read at 734 nm using a UV spectrophotometer (CT-2600, ChromTech, Taipei, Taiwan) after 1 min. The antioxidant activity of the extract was calculated against a calibration curve (0, 5, 10, 15, 20, 25, 30, 35, 40 µM) established with Trolox (R^2 = 0.9898), and expressed as mg Trolox equivalent per 100 g purée. Results were also expressed as % radical scavenging activity using the following formula:

$$\%\ \text{scavenging activity} = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ blank}\right) \times 100$$

Film preparation

Gelatine film-forming solution was prepared by mixing gelatine powder (Gelatine 220 Bloom, GELTECH, Busan, South Korea) with distilled water (containing glycerol at 40% w/w gelatine) at 10% (w/v) ratio. The solution was mixed with mechanical stirring (speed 7) until completely dissolved and heated at 60°C using a hot plate stirrer (HTS-1003, LMS Co., Ltd., Tokyo, Japan) for 60 min to obtain a homogeneous solution (Farris *et al*., 2011). The carboxymethylcellulose film-forming solution was prepared by hydrating sodium CMC with distilled water (40% glycerol w/w CMC) at 1% (w/v) with mechanical stirring (S18520-26, Thermolyne Corporation, Dubuque Iowa, USA) for at least 6 h at 25°C (Muppalla *et al*., 2014).

The optimisation of the film antioxidant activity and tensile strength was carried out using the Mixture Design - Response Surface Methodology (RSM). The design consisted of nineteen experimental runs including five replicates at the centre point, ran in random order. The design variables were gelatine (low = 0; high = 100%), CMC (low = 0; high = 100%), and *bignay* fruit extract (low = 0; high = 30%), proportions. The composite films were prepared by mixing the gelatine film-forming solution, carboxymethylcellulose film-forming solution and *bignay* fruit extract at the amounts presented in Table 1. The solutions were mixed and 30 mL of each solution was poured onto a clean Petri dish (diameter = 90 mm) lined with Saran, and dried at 40°C and 50% relative humidity for 24 h. The dried films were manually peeled off the plates and stored at 30°C and 50% relative humidity until analysis.

Table 1. Optimisation of antioxidant capacity and tensile strength.

ID	Run	Gelatine (A), %	CMC (B), %	Extract (C), %	% DPPH Radical Scavenging Capacity	Tensile Strength, MPa
0	1	0.425	0.425	0.15	11.98	12.63
1	2	0.26	0.74	0	4.79	10.07
3	3	0.73	0.27	0	10.78	5.99
7	4	0.85	0	0.15	11.18	6.28
0	5	0.425	0.425	0.15	14.17	14.67
8	6	0.7	0	0.3	17.37	14.21
0	7	0.425	0.425	0.15	13.77	10.02
6	8	0	0.88	0.12	21.36	13.95
0	9	0.425	0.425	0.15	20.36	8.65
10	10	0.20	0.50	0.3	29.14	9.23
0	11	0.425	0.425	0.15	17.56	8.60
2	12	1	0	0	16.37	6.27
6	13	0	0.88	0.12	24.55	17.18
11	14	0	0.7	0.3	54.49	7.02
2	15	1	0	0	12.97	5.84
5	16	0	1	0	6.59	16.32
9	17	0.50	0.20	0.3	16.57	6.19
7	18	0.85	0	0.15	13.37	5.82
4	19	0.48	0.52	0	9.18	8.45

Determination of antioxidant activity

Briefly, 10 mg of each film was mixed with 10 mL 0.01% HCl in a plastic centrifuge tube. The mixture was kept at room temperature for 30 min

with occasional vortexing and then centrifuged for 10 min at 25°C and 3,000 rpm. The supernatant was collected and stored in a clean container. The DPPH radical scavenging activity of the extract was determined using the method described earlier.

Determination of tensile strength

The tensile strength of the film was determined using a universal testing machine (INSTRON® 4411, Singapore) at the Agricultural and Bio-Process Division (ABPROD), Institute of Agricultural Engineering, College of Engineering and Agro-Industrial Technology, University of the Philippines Los Baños. The results were obtained as the average of three determinations using dumbbell-shaped films (type V specimen) based on ASTM D638-02a, ASTM International. The strips were placed in the grips of the Universal Testing Machine and pulled until failure using 0.5 kN load cell and a crosshead speed of 50 mm/min.

Characterisation of the film

The film with the optimum antioxidant activity and tensile strength was characterised by determining the average thickness, colour, tensile strength and antioxidant activity.

Film Thickness: The thickness of the film was measured at five random positions using a micrometre (LSC-012, Lotus® Mansion Tools, Inc., USA). The average of three determinations of film thickness was computed and used for calculations.

Color: The colour parameters (L*, a*, b*) was determined at five random positions using a hand-held chromametre (Pantone CAPSURE™, Pantone LLC, X-Rite, Inc., USA). The average of three determinations was obtained.

Tensile Strength: The tensile strength of the optimised film was determined using the method described earlier.

Antioxidant Activity: The antioxidant activity of the optimised film was determined using the DPPH radical scavenging assay described earlier.

Statistical analysis

The Design Expert (version 10.0.3.1) software was used to analyse the experimental data and determine the response surface model. The validity of the model was determined by comparing the experimental and predicted values for the response variables.

Results and discussion

Total phenolic content and antioxidant capacity of bignay fruit crude phenolic extract

The *bignay* fruit extract yielded 444.36 mg GAE/100 g purée. This value was higher than the value (79.67 mg GAE/100 g fresh sample) reported by Santiago *et al*. (2007) but lower than the value (141.80 g/100 g freeze-dried sample) reported by Lizardo *et al*. (2015). The difference could be attributed to the extraction procedure employed. According to Naczk and Shahidi (2004), the efficiency of extraction of phenolic compounds is affected by the extraction procedure, sample particle size, conditions of storage and presence of interferences. The extraction solvent used influences the extraction yield as differences in the polarities and solubility of different sample constituents exist (Butsat and Siriamornpun, 2016).

Phenolic compounds are one of the most well-known antioxidants. The antioxidant capacity of the diluted (10-1) *bignay* fruit extract obtained by the DPPH and ABTS radical scavenging assays were 65.25% and 50.74%, respectively. These values were lower as compared to the value obtained by Lizardo *et al*. (2015) using the DPPH radical scavenging assay (87.10%). The difference could be attributed to the dilution of the sample which was done to eliminate colour interferences during spectrophotometric analysis. It was also observed that a higher scavenging capacity was obtained using the ABTS (7.01 mg Trolox/100 g purée) assay than the DPPH (3.46 mg Trolox/100 g purée) assay in Trolox equivalents. Although these assays work on the principle of free radical scavenging as exhibited by a decrease in solution absorbance, these radicals might react differently with the phenolic compounds present in the sample. Stoichiometric coefficients of DPPH and ABTS for some phenols was shown to indicate that the reaction of ABTS with the phenols proceeds to almost completion while that of DPPH exhibits reversibility (Lissi *et al*., 1999).

Gelatine/CMC/bignay extract composite films

The formation of gelatine-CMC composite films with antioxidant was achieved using 10% gelatine and 1% CMC film-forming solutions. Glycerol was added at 40% w/w polymer to serve as plasticiser. The addition of plasticiser produced more elastic films. The films produced were generally wrinkled, which might be due to the drying process. According to the theory of wrinkling, a surface skin (a layer that is stiffer than the interior) is produced when water is lost during the dehydration process. This layer contracts more rapidly than the underlying layers

(Rizzieri *et al*., 2006). Some films also contained bubbles which might be due to the stirring/mixing and dispensing of the film-forming solutions.

The films were prepared with the same volume of film-forming mixtures but the resulting films varied in the final thickness. It was observed that the addition of CMC reduced the final thickness of the resulting films. This was obvious especially for films 5, 6 and 11. This result is in agreement with Asma *et al*. (2014). The differences in the final film thickness might be attributed to the differences in the formulation of the film-forming solutions. According to Han and Krochta (1999) the film thickness is influenced by the solid content of the film-forming solutions. Films with higher solid content form thicker films. Since gelatine film-forming solution contained higher amount of solids (10% w/v) than CMC film-forming solution (1% w/v), increasing the gelatine concentration in the film formulation produced thicker films.

The resulting films also exhibited variation in the final colour. As the concentration of *bignay* fruit extract increased, the resulting film became more purple in colour. The purple colour of the extract was mainly due to their anthocyanin, a flavonoid (Butkhup and Samappito, 2008). The total flavonoid content of *bignay* fruit extract was found to be 2.75 mg quercetin equivalents (QE)/g fresh berries (Santiago *et al*., 2007), and was shown to be significantly ($p < 0.05$) and positively correlated ($R = 0.182$) with its total antioxidant activity (Belina-Aldemita *et al*., 2013). Figure 1 shows the films formed from the 12 formulations.

Optimisation of film antioxidant activity and tensile strength

The responses for antioxidant activity (AOX) and ultimate tensile strength (UTS) were analysed using the design expert version 10.0.3.1. Analysis of Variance ($\alpha = 0.05$) revealed a significant model ($p < 0.05$) for both responses. Non-significant lack-of-fit tests ($p > 0.05$) indicate the applicability of the models generated. The ANOVA results are presented in Table 2. A special quadratic model for AOX ($R^2 = 0.9662$) and cubic model for UTS ($R^2 = 0.8098$) are described by equations 1 and 2, respectively, with the non-significant ($p > 0.05$) terms eliminated.

$$AOX = 15.14A + 5.07B + 200.34C - 248.22AC - 1858.35\,ABC^2 \quad (1)$$

$$UTS = 6.20A + 15.85B - 9202.10C \quad (2)$$

where A, B, and C denote the amount (in mL) of gelatine, CMC, and *bignay* fruit extract, respectively. Positive values of coefficients indicate a positive effect on the response variable (response increases as the component increases) while negative coefficients indicate the opposite. Higher coefficient values indicate greater contribution to the response. Based on the equations, *bignay* fruit extract had the greatest effect on both the antioxidant capacity and tensile strength of the films among the three components.

The antioxidant activity of the films was expected to be greatly affected by the amount of *bignay* fruit extract since it contained the active components. According to Butkhup and Samappito (2008), some bioactive compounds found in *bignay*

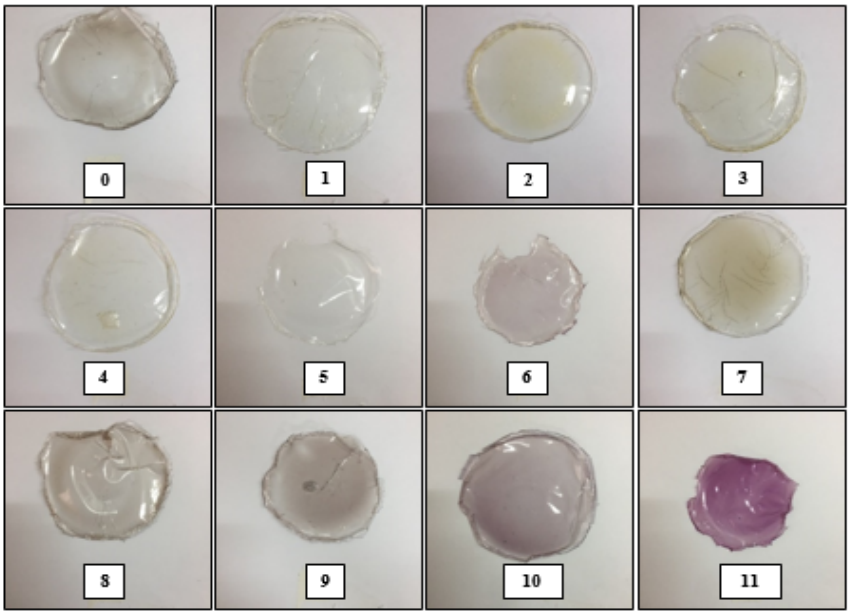

Figure 1. Films with different gelatine/CMC/bignay extract blends: (0) 0.425/0.425/0.15; (1) 0.26/0.74/0; (2) 1/0/0; (3) 0.73/0.27/0; (4) 0.48/0.52/0; (5) 0/1/0; (6) 0/0.88/0.12; (7) 0.85/0/0.15; (8) 0.7/0/0.3; (9) 0.5/0.2/0.3; (10) 0.2/0.5/0.3; (11) 0/0.7/0.3.

Table 2. Optimisation of film antioxidant activity and tensile strength.

Analysis of variance for antioxidant activity (%).

Source	DF	Sum of Squares	Mean Square	F	P	
Model	8	2033.71	254.21	35.70	< 0.0001	*
Linear Mixture	2	972.97	486.49	68.32	< 0.0001	*
AB	1	3.88	3.88	0.55	0.4772	ns
AC	1	66.34	66.34	9.32	0.0122	*
BC	1	2.37	2.37	0.33	0.5768	ns
A^2BC	1	9.42	9.42	1.32	0.2768	ns
AB^2C	1	0.52	0.52	0.073	0.7925	ns
ABC^2	1	68.25	68.25	9.58	0.0113	*
Residual	10	71.21	7.12			
Lack of Fit	3	12.93	4.31	0.52	0.6834	ns
Pure Error	7	58.29	8.33			
Total	18	2104.92				

Analysis of variance for ultimate tensile strength (MPa).

Source	DF	Sum of Squares	Mean Square	F	P	
Model	9	212.18	23.58	4.26	0.0210	*
Linear Mixture	2	105.61	52.80	9.54	0.0060	*
AB	1	12.50	12.50	2.26	0.1673	ns
AC	1	13.77	13.77	2.49	0.1492	ns
BC	1	13.54	13.54	2.45	0.1523	ns
ABC	1	13.51	13.51	2.44	0.1527	ns
AB(A-B)	1	0.57	0.57	0.10	0.7553	ns
AC(A-C)	1	13.92	13.92	2.51	0.1473	ns
BC(B-C)	1	13.18	13.18	2.38	0.1573	ns
Residual	9	49.84	5.54			
Lack of Fit	2	16.06	8.03	1.66	0.2562	ns
Pure Error	7	33.77	4.82			
Total	18	262.02				

Comparison of experimental and predicted values for model validation.

A	B	C	Response	Experimental value	Predicted value	% Error
0.15	0.56	0.29	AOX	25.89 ± 1.26	32.70	20.81
			UTS	7.45 ± 1.00	17.18	56.64

fruit are catechin and procyanidins B1 and B2. These phenolic compounds are able to donate hydrogen to radicals like DPPH•, thus changing the colour from purple to yellow. The DPPH radical scavenging capacities of the films obtained ranged from 4.79% to 54.49%. The lowest antioxidant activity was observed from the film with 26% gelatine, 74% CMC and 0% *bignay* fruit extract, while the highest was at 0% gelatine, 70% CMC and 30% *bignay* fruit extract. However, it was also observed that in the presence of gelatine, the antioxidant activity of the films was reduced even when the amount of *bignay* fruit was at the highest (30%). This was evident with films ID 8, 9, and 10. The presence of protein might have reduced the antioxidant activity of the extract as phenolic compounds are able to bind with proteins, which also explains the significant interaction of gelatine**bignay* (AC). This effect was also observed by Heinonen *et al*. (1998).

On the other hand, the tensile strength of the films was greatly affected by the amount of *bignay* fruit extract due to the dilution of the solutions. An increase in moisture causes a reduction in the ultimate tensile strength (Shen and Springer, 1977; Li, 2004). Water also has a plasticising effect to biopolymers by disrupting the intra- and intermolecular forces of attraction between polymers (Matveev *et al*., 2000). These effects are also illustrated in the contour plots and 3-dimensional graphs for the responses presented in Figure 2.

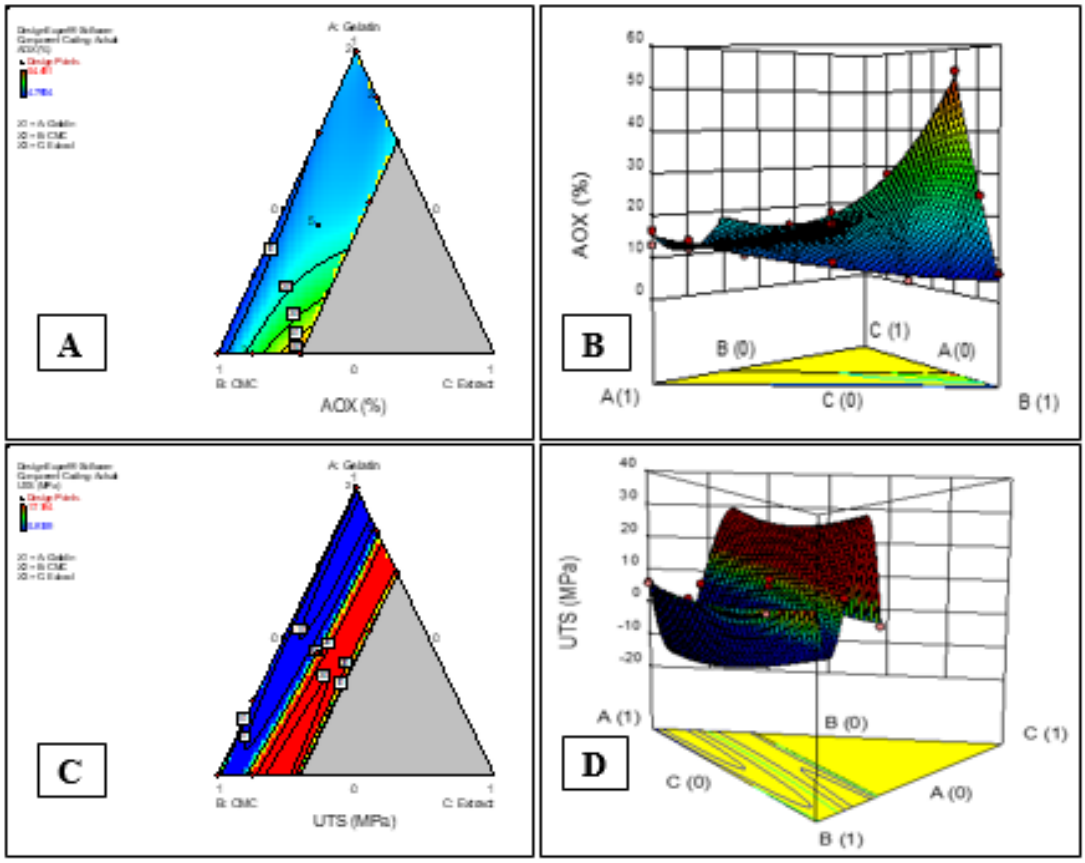

Figure 2. Contour plot for antioxidant activity (A) and tensile strength (C) and response surface plots for antioxidant activity (B) and tensile strength (D).

To verify the validity of the models, a checkpoint formulation with the maximum desirability (0.805) was prepared and tested for the responses. The antioxidant activity and tensile strength were found maximum at 15% gelatine, 56% CMC and 29% *bignay* fruit extract (Table 2). The experimental values were within the acceptable range. However, % error values were high especially for UTS. The large difference between the experimental value and the predicted value might be due to the sensitivity of the method for determination of UTS.

Characterisation of the film

The characteristics of the checkpoint formulation are summarised in Table 3. The colour analysis gave positive values for a* (3.09 ± 0.23) and b* (3.13 ± 1.30) which indicates that the film was in the red and yellow region. Visually, the film exhibited reddish to purplish colour, primarily due to the added *bignay* fruit extract. Compared to most edible/biodegradable wrappers that are usually clear or colourless, the added colour to the film might make it more appealing to the consumers.

Table 3. Characteristics of the optimised film formulation.

Parameter	Mean ± SD
L*	79.29 ± 2.38
a*	3.09 ± 0.23
b*	3.13 ± 1.30
Thickness, mm	0.10 ± 0.02
Tensile strength, MPa	7.45 ± 1.00
DPPH radical scavenging capacity, %	25.89 ± 1.26

Pouring 30 mL film solution into a 90 mm diameter Petri dish and drying at 40°C produced films with an average thickness of 0.10 mm. This thickness value was between those of pure gelatine film (0.56 mm) and pure CMC film (0.04 mm). The difference in the final thickness could be explained by the difference in the total solid content of the film formulations. The resulting film thickness is comparable to that of sago starch films (0.12-0.16 mm) by Abdorreza *et al*. (2011), corn zein films (0.13 mm) by Arcan and Yemenicioglu (2011), and pectin films (0.1 mm) by Azeredo *et al*. (2016).

The tensile strength of the gelatine-CMC-*bignay* fruit extract film plasticised with glycerol was found

to be 7.45 MPa which was also between those of pure gelatine film (6.06 MPa) and pure CMC film (16.32 MPa). When compared with available data, this value was lower than that of pure 1% CMC film plasticised with 0.1% v/v glycerol (31.3 MPa) obtained by Muppalla *et al*. (2014), and that of pure 14% gelatine plasticised with 7% w/w glycerol (8.22 MPa) obtained by Farris *et al*. (2011). However, the value obtained was 11 times higher than the value obtained by Asma *et al*. (2014) for gelatine/CMC film plasticised with glutaraldehyde. The differences in the tensile strength values could be attributed to the composition of the films. Increasing the concentration of plasticiser would reduce the tensile strength as observed by Sobral *et al*. (2001). Also, the addition of antioxidants might have interfered with the polymer network causing a reduction in tensile strength as observed by Li *et al*. (2014). The tensile strength of the active film produced in the present work was lower than that of common synthetic plastics which is usually >20 MPa (Klein, 2011).

The addition of *bignay* fruit extract added functionality to the film produced. The antioxidant capacity determined using the DPPH assay was 25.89 ± 1.26%. As discussed earlier, the reduction in the antioxidant capacity of the extract might be attributed to inadequate extraction of the phenolic compounds from the film. Difficulty in extraction could be due to the interaction of the polyphenolic compounds with the polymeric matrix.

Conclusion

Aqueous extract of *Antidesma bunius* (L.) Spreng. (*bignay*) was obtained and analysed for its total phenolic content and antioxidant capacity. Results showed that the diluted extract had excellent antioxidant activity with 65.25% DPPH radical scavenging capacity and 50.74% ABTS radical scavenging capacity. Edible films based on gelatine, CMC and *bignay* fruit extract were produced using the casting procedure, and optimised for antioxidant activity and tensile strength using the mixture design of experiments. Mathematical models for both responses indicate that the *bignay* fruit extract yielded the greatest effect on both the antioxidant activity and the tensile strength of the film. Analysis of variance ($\alpha = 0.05$) revealed a significant model and a non-significant lack-of-fit indicating the adequacy of the model to describe the relationship of the film components and the responses. Coefficients of determination (R^2) suggest that the variables explain 96.62% of the variation in antioxidant activity and 80.98% of the variation in tensile strength. In order to better assess the applicability of the film, it is henceforth recommended to test its barrier properties which were not tested in the present work due to limitations in resources. The use of the film as a primary package for ready-to-eat food products or in combination with a secondary packaging material could be further explored in the near future.

References

Abdorreza, M. N., Cheng, L. H. and Karim, A. A. 2011. Effects of plasticizers on thermal properties and heat sealability of sago starch films. Food Hydrocolloids 25(1): 56–60.

Arcan, I. and Yemenicioğlu, A. 2011. Incorporating phenolic compounds opens a new perspective to use zein films as flexible bioactive packaging materials. Food Research International 44: 550–556.

Asma, C., Meriem, E., Mahmoud, B. and Djaafer, B. 2014. Physicochemical characterization of gelatine-CMC composite edible films from polyion-complex hydrogels. Journal of the Chilean Chemical Society 59(1): 1–5.

Azeredo, H. M. C., Morrugares-Carmona, R., Wellner, N., Cross, K., Bajka, B. and Waldron, K. W. 2016. Development of pectin films with pomegranate juice and citric acid. Food Chemistry 198: 101–106.

Belina-Aldemita, M. D., Sabularse, V. C., Dizon, E. I., Hurtada, W. A. and Torio, M. A. O. 2013. Antioxidant properties of *bignay* (*Antidesma bunius* (L.) Spreng.) wine at different stages of processing. Philippine Agricultural Scientist 96(3): 308–313.

Butkhup, L. and Samappito, S. 2008. An analysis on flavonoids contents in Mao Luang fruits of fifteen cultivars (Antidesma bunius), grown in Northeast Thailand. Pakistan Journal of Biological Sciences 11(7): 996–1002.

Butsat, S. and Siriamornpun, S. 2016. Effect of solvent types and extraction times on phenolic and flavonoid contents and antioxidant activity in leaf extracts of *Amomum chinense* C. International Food Research Journal 23(1): 180–187.

Calatayud, M., López-de-Dicastillo, C., López-Carballo, G., Vélez, D., Hernández-Muñoz, P. and Gavara, R. 2013. Active films based on cocoa extract with antioxidant, antimicrobial and biological applications. Food Chemistry 139: 51–58.

Contini, C., Katsikogianni, M. G., O'neill, F. T., O'sullivan, M., Dowling, D. P. and Monahan, F. J. 2011. Development of active packaging containing natural antioxidants. Procedia Food Science 1: 224–228.

Da Silva, M. A., Bierhalz, A. C. K. and Kieckbusch, T. G. 2009. Alginate and pectin composite films crosslinked with Ca2+ ions: Effect of the plasticizer concentration. Carbohydrate Polymers 77(4): 736–742.

European Commission. 2009. EU guidance to the commission regulation on active and intelligent materials and articles intended to come into contact with food. Belgium: European Commission.

Farris, S., Schaich, K. M., Liu, L., Cooke, P. H., Piergiovanni, L. and Yam, K. L. 2011. Gelatine-pectin composite films from polyion-complex hydrogels. Food Hydrocolloids 25(1): 61–70.

Garcia, M. A., Pinotti, A., Martino, M. N. and Zaritzky, N. E. 2004. Characterization of composite hydrocolloid films. Carbohydrate Polymer 56: 339–345.

Gómez-Estaca, J., López-de-Dicastillo, C., Hernández-Muñoz, P., Catalá, R. and Gavara, R. 2014. Advances in antioxidant active food packaging. Trends in Food Science and Technology 35: 42–51.

Han, J. H. and Krochta, J. M. 1999. Water vapour permeability and wetting properties of whey protein coating on paper. American Society of Agricultural Engineers 42: 1375–1382.

Heinonen, M., Rein, D., Satue-Garcia, M. T., Huang, S. W., German, J. B. and Frankel, E. N. 1998. Effect of protein on the antioxidant activity of phenolic compounds in a lecithin-liposome oxidation system. Journal of Agricultural and Food Chemistry 46: 917–922.

Jorjong, S., Butkhup, L. and Samappito, S. 2015. Phytochemicals and antioxidant capacities of Mao-Luang (*Antidesma bunius* L.) cultivars from Northeastern Thailand. Food Chemistry 181: 248–255.

Klein, R. 2011. Laser Welding of Plastics: Materials, Processes and Industrial Applications, 1st eds. United States: John Wiley and Sons, Inc.

Li, J., Miao, J., Wu, J., Chen, S. and Zhang, Q. 2014. Preparation and characterization of active gelatine-based films incorporated with natural antioxidants. Food Hydrocolloids 37: 166–173.

Li, X., Liu, A., Ye, R., Wang, Y. and Wang, W. 2015. Fabrication of gelatine-laponite composite films : Effect of the concentration of laponite on physical properties and the freshness of meat during storage. Food Hydrocolloids 44: 390–398.

Lissi, E. A., Modak, B., Torres, R., Escobar, J. and Urzua, A. 1999. Total antioxidant potential of resinous exudates from *Heliotropium* species, and a comparison of the ABTS and DPPH methods. Free Radical Research 30: 471–477.

Lizardo, R. C. M., Mabesa, L. B., Dizon, E. I. and Aquino, N. A. 2015. Functional and antimicrobial properties of *bignay* (*Antidesma bunius* (L.) Spreng.) extract and its potential as natural preservative in a baked product. International Food Research Journal 22(1): 88–95.

López de Lacey, A. M., Giménez, B., Pérez-Santín, E., Faulks, R., Mandalari, G., López-Caballero, M. E. and Montero, P. 2012. Bioaccessibility of green tea polyphenols incorporated into an edible agar film during simulated human digestion. Food Research International 48(2): 462–469.

López-de-Dicastillo, C., Bustos, F., Guarda, A. and Galotto, M. J. 2016. Cross-linked methyl cellulose films with *murta* fruit extract for antioxidant and antimicrobial active food packaging. Food Hydrocolloids 60: 335–344.

López-de-Dicastillo, C., Gómez-Estaca, J., Catalá, R., Gavara, R. and Hernández-Muñoz, P. 2012. Active antioxidant packaging films: Development and effect on lipid stability of brined sardines. Food Chemistry 131: 1376–1384.

Lorenzo, J. M., Batlle, R. and Gómez, M. 2014. Extension of the shelf-life of foal meat with two antioxidant active packaging systems. LWT - Food Science and Technology 59(1): 181–188.

Matveev, Y. I., Grinberg, V. Y. and Tolstoguzov, V. B. 2000. The plasticizing effect of water on proteins, polysaccharides and their mixtures. Glassy state of biopolymers, food and seeds. Food Hydrocolloids 14(5): 425–437.

Mihindukulasuriya, S. D. F. and Lim, L. T. 2014. Nanotechnology development in Food Packaging: A review. Trends in Food Science and Technology 40: 149–167.

Muppalla, S. R., Kanatt, S. R., Chawla, S. P. and Sharma, A. 2014. Carboxymethyl cellulose–polyvinyl alcohol films with clove oil for active packaging of ground chicken meat. Food Packaging and Shelf Life 2(2): 51–58.

Naczk, M. and Shahidi, F. 2004. Extraction and analysis of phenolics in food. Journal of Chromatography A 1054: 95–111.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26(9–10): 1231–1237.

Rizzieri, R., Mahadevan, L., Vaziri, A. and Donald, A. 2006. Superficial wrinkles in stretched, drying gelatine films. Langmuir 22: 3622–3626.

Santiago, D. M. O., Garcia, V. V. G., Dizon, E. I. and Merca, F. E. 2007. Antioxidant activities, flavonol and flavanol content of selected Southeast Asian indigenous fruits. The Philippine Agricultural Scientist 90(2): 123–130.

Shen, C. and Springer, G. S. 1977. Effects of moisture and temperature on the tensile strength of composite materials. Journal of Composite Materials 11: 1–5.

Shimada K., Fujikawa K., Yahara K. and Nakamura T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 40: 945–948.

Sobral, P. J. A., Menegalli, F. C., Hubinger, M. D. and Roques, M. A. 2001. Mechanical, water vapour barrier and thermal properties of gelatine based edible films. Food Hydrocolloids 15(4-6): 423–432.

Tang, X. Z., Kumar, P., Alavi, S. and Sandeep, K. P. 2012. Recent advances in biopolymers and biopolymer-based nanocomposites for food packaging materials. Critical Reviews in Food Science and Nutrition 52(5): 426–442.

Torres-Arreola, W., Soto-Valdez, H., Peralta, E., Cardenas-Lopez, J. L. and Ezquerra-Brauer, J. M. 2007. Effect of a low-density polyethylene film containing butylated hydroxytoluene on lipid oxidation and protein quality of sierra fish (*Scomberomorus sierra*) muscle during

frozen storage. Journal of Agricultural and Food Chemistry 55: 6140–6146.

Williams, G. M., Iatropoulos, M. J. and Whysner, J. 1999. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. Food and Chemical Toxicology 37: 1027–1038.