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EFFECTS OF KAPPA CARRAGEENAN AND GLYCEROL IN PURPLE SWEET POTATO STARCH BASED EDIBLE FILM

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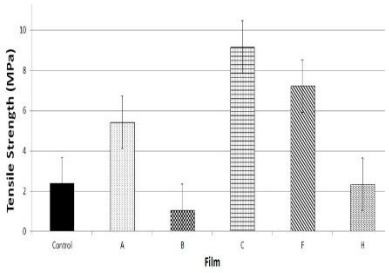


Abstract

The solubility and mechanical properties of purple sweet potato (PSP) starch-based edible films are being investigated. The formulation was prepared using a constant amount of PSP starch (4 % w/v) with five different ratios of carrageenan and glycerol (1:1, 1.5:0.5, 0.5:1.5, 0.5:0.5, 1:0) which act as gelling agents and plasticizers respectively. PSP starch generates 61.79 mg/100 g anthocyanin and retains 16.78 % antioxidant capacity. Carrageenan and glycerol (0.5:1.5) ratio shows the highest solubility in water (53.50 ± 0.1085 %) and highest tensile strength (9.1674 ± 0.5399 MPa) with the lowest elongation at break (20.436 ± 2.826 %).

Keywords: Purple sweet potato, edible film, carrageenan, solubility, mechanical properties

Abstrak

Kebolehlarutan dan sifat mekanikal filem kanji yang boleh dimakan yang berasaskan ubi keledek ungu (PSP) sedang dikaji. Formulasi bahan telah menggunakan jumlah kanji PSP (4 % w / v) yang tetap dengan lima nisbah carrageenan dan gliserol yang berbeza (1: 1, 1,5: 0.5, 0.5: 1.5, 0.5: 0.5, 1: 0) yang masing-masing bertindak sebagai ejen membuat gel dan pemplastik. Kanji PSP mengekalkan 61,79 mg / 100 g antosianin dan menunjukkan 16.78% kapasiti antioksidan. Nisbah carrageenan dan gliserol (0,5: 1,5) menunjukkan kelarutan yang paling tinggi di dalam air (53.50 ± 0,1085 %) dan kekuatan tegangan tertinggi (9.1674 ± 0.5399 MPa) dengan kadar pemanjangan yang paling rendah pada ketetapan rehat (20.436 ± 2.826%).

Kata kunci: Keledek Ungu, Filem boleh dimakan, carrageenan, kebolehlarutan, ciri-ciri mekanikal

1.0 INTRODUCTION

Edible film has becomes one of the crucial forms of food packaging nowadays due to the rising concerns regarding increased environmental pollution, which is a serious environmental issue. The usage of edible film may reduce the amount of non-degradable packaging present in the market. Edible film can be consumed and provides barrier from moisture, oxygen and solute movement into foods [1]. Besides acting as

a barrier, edible film can also be turned into one of the highest potential and highly marketable packaging to reduce the usage and problematic disposal of synthetic packaging. Starch edible film has the highest potential as food packaging as compared to lipid and protein films since starch is a renewable resource, inexpensive and widely available [2].

Purple sweet potato (PSP) contains high levels of anthocyanin pigments in its flesh which makes it a more favourable raw material to make films. Anthocyanin in PSP is a water soluble vacuoles pigment which belongs to flavonoid classes in plants where it appears as purple in colour [3]. Apart from the bright purple colour which can be used as natural colorants in food ingredients, cosmetics and medicine [4], anthocyanins also have high antioxidant agents that can cure severe diseases [5–7].

Starch itself would not form into an optimum edible film without the addition of plasticizer. Various methods, including the addition of glycerol [8–10] and kappa carrageenan [11-13] into starch based matrix have proven to result in a more favourable film. The addition of food grade glycerol into edible film has proven to give a high tensile property [14] and moisture retention [15] as compared to sorbitol and xylitol respectively. Carrageenan however is not a plasticizer but has excellent properties to form gel and films with the presence of complex mixture of several water-soluble galactose polymers [12].

In this study, PSP starch-based edible films were incorporated with kappa-carrageenan and glycerol. PSP starch was extracted from raw materials using solution extraction and forming film using casting method. The objective of this study is to prepare PSP film using different formulations to define the characteristics of the PSP film based on different solubility, tensile strength and elongation at break to achieve optimum mechanical properties.

Table 1 The water solubility and elapsed time for different formulation of PSP base edible film

	Ratio of C:G (g)	Thickness (mm)	Water solubility (%)	Elapsed time in 27 ± 2°C (min)	Elapsed time in 95 ± 2°C (min)
Control	-	0.079 ± 0.006a	74.87 ± 1.398a	6.12 ± 0.734a	1.43 ± 1.114a
A	1:1	0.156 ± 0.004b	42.91 ± 2.573b	12.44 ± 0.632b	2.52 ± 0.024b
B	1.5 : 0.5	0.173 ± 0.026c	24.60 ± 4.793c	18.54 ± 1. 092c	4.37 ± 0.924c
C	0.5 : 1.5	0.159 ± 0.006b	53.50 ± 1.108d	8.43 ± 0.103d	2.25 ± 2.497b
F	0.5 : 0.5	0.122 ± 0.007ab	20.78 ± 1.328e	22.17 ± 1.563e	5.03 ± 1.624d
H	1 : 0	0.103 ± 0.007ab	9.02 ± 1.479f	28.55 ± 2.122f	8.42 ± 0.344e

where C= Carrageenan and G = glycerol. Values are mean ± standard deviation (n=3) and A to ǂ F represent significant difference at 5% level of significance

2.0 EXPERIMENTAL

2.1 Materials

Purple sweet potato (Vietnamese culture) was purchased from a local store in Taman Universiti, Johor. Food grade glycerol which acts as a plasticizer was purchased from Merck KGaA, Darnstadt, Germany. Kappa-Carrageenan that helps to strengthen the gelling properties for the film was bought from Sigma-Aldrich, Saint Louis, USA.

2.2 Extraction Preparation

The starch was extracted from PSP as the sources of anthocyanin using solution extraction [16]. The PSP were weighed, washed and sliced and subsequently blended with ratio of 1:1 w/v upon distilled water. The blend was then filtered using muslin cloth and the filtrate was centrifuged using a centrifuge (MPW-352, Poland). The anthocyanin extract was decanted whereby whitish sediments were obtained and dried in freeze dryer (-45^{0}C) for 3 hours prior to grinding. The dried starches were stored at 4^{0}C and total yield percentage was measured by subtracting the amount of sample used to extract with total yield of extraction.

2.3 Total Anthocyanin Content and Antioxidant Activity

The total anthocyanin content was measured on extracted PSP starch using the spectrophotometric pH-differential method described by Sutharut and Sudarat [17], by using two buffer systems: potassium chloride buffer, pH 1.0 and sodium acetate buffer, pH 4.5. The anthocyanin content and antioxidant activity were only measured on PSP starch to determine the initial content of the anthocyanin and antioxidant in the starch before proceeding to film preparation. The absorbance was measured at 520 nm and 700 nm with distilled water as blank using UV-VIS spectrophotometer (Jenway 7300/7305, German). The absorbance difference between the pH 1.0 and pH 4.5 samples was calculated in eq. (1) while the total anthocyanin content was calculated as cyanidin-3-glucoside according to the following eq. (2):

$$A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5} \quad (1)$$

$$\text{Total anthocyanin content (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \quad (2)$$

where MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor; 1= path length in cm; ε = 26,900 molar extinction coefficient in L/mol/cm for cyanidin-3-glucoside; 1000 = conversion from g to mg. All analyses were done in triplicate.

The antioxidant activity was also tested on the extracted PSP starch by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) colorimetric analysis method based on Viña *et al*., [18] with slight modification. The starch (1 mg) was dissolved and diluted in 1 ml absolute ethanol. 1 ml of the diluted solution was eluted and transferred to another tube with 2 ml of DPPH in 0.004 % ethanol. The concentration of anthocyanin present in the sample was considered as 1.0 mg/ml. The sample was left incubated in the dark for 30 min at room temperature. The samples were measured at 517 nm using UV-Vis spectrophotometer (Lambda 25, Perkin Elmer, USA) and ethanol as a blank control. Sanna *et al*., [19] stated the calculation for antioxidant content using UV-Vis measurements in eq. (3):

$$\text{Percentage of Antioxidant: } (A_0 - A_S) / (A_0 - A_i) \quad (3)$$

where A_0 is the absorbance at 517 nm of the DPPH solution without antioxidant, A_S is the absorbance of the sample and A_i is the absorbance of the solution when 100% of DPPH is reduced. The test was performed in triplicate for each batch.

2.4 Edible Film Preparation

The edible film base suspension (50 ml) was prepared with 2.0 g of purple sweet potato starch (4% w/v) and added with five different ratios of carrageenan to glycerol (A= 1:1, B= 1.5:0.5, C= 0.5:1.5, F= 0.5:0.5, H =1:0) respectively. The preparation was based on Abdou and Sorour, [11] using casting method with slight modification. The solution was constantly stirred using a magnetic stirrer. The starches were gelatinized at 65^{0}C and added with glycerol and carrageenan. After the solution thickened and was fully homogenized, 15 ml of solution was measured and casted thoroughly onto a 9 cm diameter plate and allowed to cool at room temperature before drying in ~~a~~n convection oven at 60^{0}C overnight (12 hours). The yield films with a range of thicknesses from 1.0 mm to 0.5 mm were manually peeled off and stored in airtight containers at room temperature. The purple sweet potato with the starch-alone base edible film was labelled as control film.

2.5 Water-solubility

The water solubility test was based on a research by Kim and Ustunol, [20], with slight modification. The water solubility of a film helps to determine the time for the film to solubilize in hot or cold water. Approximately 15 mm x 15 mm film samples were cut and dried in the drying oven (100 ± 2^{0}C 24 h) to obtain the initial dry matter weight of the films. The dried films were immersed into 10 ml of deionized water (27 ± 2^{0}C, 30 min) and agitated at 100 rpm. The films in water were visually inspected every minute until 30 min to determine the final solubility of the film. At the end of 30 min, the insolubilized films were taken out of the water and dried (100 ± 2^{0}C) to determine the weight of the dry matter which was not solubilized in water. The weight of dry matter solubilized was calculated as follows in eq. (4) and reported as percent water solubility of the films:

$$\text{Water solubility (\%)} = \frac{\text{wt.of initial dry matter} - \text{wt.of dry matter not solubilized}}{\text{wt.of initial dry matter}} \text{ x } 100 \quad (4)$$

The water solubility test also included testing the solubility of the film in hot water (95 ± 5^{0}C) and measuring the elapsed time. The elapsed time will show the duration taken for the film to fully solubilize in a solution. Approximately 15 mm x 15 mm film samples were cut and dried in the drying oven (100 ± 2^{0}C 24 h) to obtain initial dry matter weight of the films. The dried films were immersed into 10 ml of boiled distilled water (95 ± 2^{0}C) and agitated at 100 rpm. The films in water were visually inspected until the films fully solubilize. The time taken was measured using a stopwatch.

2.6 Tensile Strength

The mechanical properties which include~~s~~ the tensile strength, were measured using the Tensile tester (LRX 2.5 KN LLOYD, China) [21]. The average thickness of the film packaging was approximately less than 1.0 mm. Tensile strength (TS), elongation at break (EAB) and Young's modulus (YM) were determined according to ASTM Standard D-882 using rectangular (80 x 20 mm) specimen with the test speed of 10 mm min^{-1} using the given eq. (5), (6) and (7) respectively.

$$\text{Tensile strength (MPa)} = \frac{\text{maximum load}}{\text{(original width x thickness)}} \quad (5)$$

$$\text{Elongation (\%)} = \frac{\text{elongation at rupture x 100}}{\text{initial gauge length}} \quad (6)$$

$$\text{Young's modulus (MPa)} = \frac{\text{tensile stress}}{\text{extensional strain}} \quad (7)$$

Data was analysed with NEIX software. Film thickness was measured using digital micrometer having 0.001 mm resolution.

2.8 Statistical Analysis

Data were analysed using analysis of variance (ANOVA) in order to determine the significant differences among the samples. The means which were statistically different from each other were compared using Duncan's comparison tests at 5 % confidence level of $p < 0.05$. SPSS (version 16.0) software was used to perform the statistically analyses.

3.0 RESULTS AND DISCUSSION

The PSP extracted starch was analysed for its anthocyanin content and antioxidant activity before proceeding to film formation. The total anthocyanin content retained in the PSP starch extracted was approximately 61.79 mg/100 g. The anthocyanin content usually varies depending on the different methods for anthocyanin analysis. According to Reyes *et al.*, [22] the anthocyanin content in purple-fleshed tubers ranged from 11 to 174 mg CY-3-Glu/100 g fresh weight. Meanwhile as reported by Han *et al.*, [7] the anthocyanin content in a type of PSP named 'Ayamurasaki' variety contains of 59 mg/100 g fresh weight. The results indicated that the sample film content of anthocyanin varied widely in the purple sweet potato.

The antioxidant activity of the PSP starch is 16.78 % of total DPPH scavenging activity. Based on Peng *et al.*, [23], the DPPH radical scavenging activity of purple sweet potato flour shows approximately 54.30 % of antioxidant activity for 0.3 mg/ml whereas 300 µg/ml concentration of PSP anthocyanin shows more than 90 % DPPH radical scavenging activity as reported by Cho *et al.*, [24]. The DPPH readings vary depending on the different methods of DPPH analysis and no research has been done yet regarding the antioxidant capacity in PSP starch-base edible film.

3.1 Water Solubility

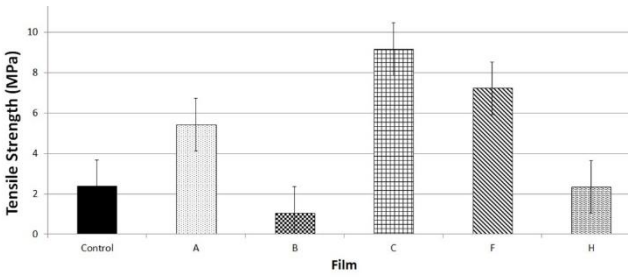
Table 1 shows the percentage of water solubility and the elapsed time to solubilize PSP base edible film. The control film had solubilized more than half in percentage of its initial dry weight in 27 ± 2^{0}C water for 5 minutes followed by sample film C (53.50 ± 4.0085 %) whilst the rest of the films with solubility below than 50 % in water were only partially soluble. The differences between each film were in the varying amounts of plasticizer (glycerol) and kappa carrageenan used to produce them.

Haq *et al.*, [14] examined the solubility of starch films, in which findings showed that solubility increased in line with the increased concentration of plasticizer. Film C which had the highest amount of plasticizer showed the highest solubility in water after the control film. Plasticizer gives high impact solubility in water as it reduces the intramolecular hydrogen bonding along the polymer chain to increase the film's flexibility [15]. The lowest solubility can be seen in film H which did not contain any plasticizer. Carrageenan which is known for its ability to form rigid and strong films [11] had made the film harder to solubilize and took a longer time to solubilize in room temperature water.

The elapsed time were divided into two classes in which the first was to determine the solubility of film in 27^{0}C agitated distilled water, whereas the second was to determine the time elapsed for all the films to solubilized in 95^{0}C agitated distilled water. These two measurements were conducted to determine the solubility of the film in moderate temperature of distilled water and in hot boiled distilled water within a set amount of time. As clearly indicated in Table 1, the higher the percentage of film solubility, the faster the elapsed time to solubilize in water. This is because a higher water solubility film consists of large porous structure which enables the film to easily penetrate into water in a shorter time. Film C shows the shortest elapsed time for the film to be solubilized in normal temperature and boiled distilled water. Meanwhile Film H with the highest amount of carrageenan shows that the duration for the film to fully solubilize is approximate to 28 minutes of elapsed time. Different formulations of films show different solubility and elapsed times in different conditions.

3.2 Tensile Strength

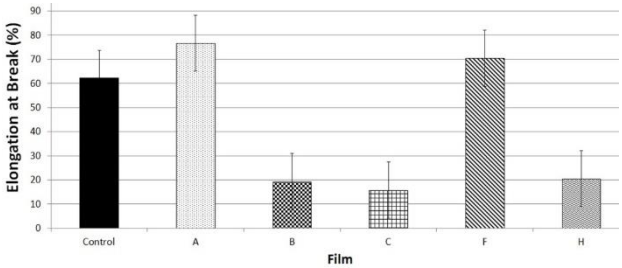
The mechanical properties such as tensile strength (TS), elongation at break (EAB) and Young's modulus (YM) from the PSP base edible film were evaluated. Film C (carrageenan 0.5:1.5 glycerol) exhibited better TS (9.1674 ± 0.539 MPa) as shown in Figure 1 followed by Film F (carrageenan 0.5:0.5 glycerol), Film A (carrageenan 1:1 glycerol) and Film H (carrageenan 1:0 glycerol) (C > F > A > H > B). Film B (carrageenan 1.5:0.5 glycerol) shows the lowest TS due to the high amount of carrageenan which made the film to crack and cut easily. Film C consisted of the highest amount of glycerol which plasticized the film as well as increased its tensile ability. Glycerol also results in a higher ability of tensile strength when incorporated in amylose and amylopectin films compared to films with starches only [9]. TS for Film F is significantly different than Film A due to the incorporation of PSP starch in which the lower amount of carrageenan in Film F produced higher tensile strength compared to the incorporation of higher carrageenan in Film A.



Where Control: Starch only
A: Carrageenan 1:1 Glycerol
B: Carrageenan 1.5:0.5 Glycerol
C: Carrageenan 0.5:1.5 Glycerol
F: Carrageenan 0.5:0.5 Glycerol
H: Carrageenan 1:0 Glycerol

Figure 1 Chart shows the tensile strength for various type of film. Values are mean ± standard deviation (n=3) and the result represent significant difference at 5% level of significance

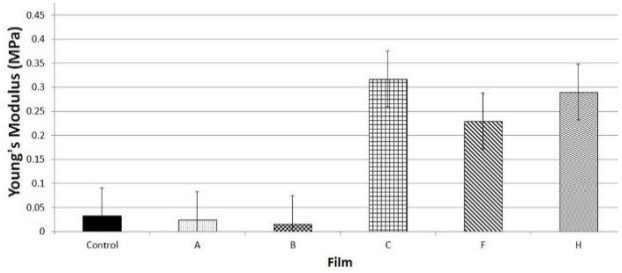
Figure 2 shows the EAB for the different formulation of films where high TS results in low EAB. High tensile strength and Young's modulus showed low in elongation at break [14]. Film A (carrageenan 1:1 glycerol) shows the highest EAB (76.658 ± 9.577 %) while Film C (carrageenan 0.5:1.5 glycerol) exhibits the lowest EAB (20.436 ± 2.826 %). Incorporation of carrageenan and glycerol into PSP starch resulted in~~to~~ varied EAB where starch-alone film consists of high EAB. A research by Muscat *et al*., [15] indicated that the lowest EAB was shown by starch-alone film compared to film added with plasticizer. High EAB in varied film formulations indicate that every film experience~~d~~ different molecular reaction between hydrogen bonds as well as between amylose and amylopectin molecules during film formation which result in different EAB. Lower amount of glycerol has proven to lower the percentage of elongation at break based on the research by Tanada-Palmu *et al*.,[25].



Where Control: Starch only
A: Carrageenan 1:1 Glycerol
B: Carrageenan 1.5:0.5 Glycerol
C: Carrageenan 0.5:1.5 Glycerol
F: Carrageenan 0.5:0.5 Glycerol
H: Carrageenan 1:0 Glycerol

Figure 2 Chart shows the percentage of elongation at break for various type of film. Values are mean ± standard deviation (n=3) and the result represent significant difference at 5% level of significance

The Young's modulus (YM) of different films were evaluated and depicted in Figure 3. YM of each film shows the rigidity and stiffness ability of the film as a reflection of the tension and compression experienced by the film. The YM results for all types of films were lower than 1 MPa due to the thin PSP films produced, which were in the range of 0.1 to 0.5 mm and thereby unable to withstand strong force or tension. A research done by Kuorwel *et al*.,[26] stated that starch incorporated with glycerol film and others also showed low YM in the range of less than 1 MPa. Film C (carrageenan 0.5:1.5 glycerol) with the highest glycerol showed the highest YM (0.3176 ± 0.092 MPa) compared to other film. High amounts of glycerol make~~s~~ the film more resistible to strain and force. The results varied according to the different formulation of film with respect to the material's structure.



Where Control: Starch only
A: Carrageenan 1:1 Glycerol
B: Carrageenan 1.5:0.5 Glycerol
C: Carrageenan 0.5:1.5 Glycerol
F: Carrageenan 0.5:0.5 Glycerol
H: Carrageenan 1:0 Glycerol

Figure 3 Chart shows the Young's Modulus for various type of film. Values are mean ± standard deviation (n=3) and the result represent significant difference at 5% level of significance

4.0 CONCLUSION

Based on the solubility and mechanical properties of the film forming, formulation of 4 % w/w PSP starch incorporated with ratio of (0.5:1.5) carrageenan and glycerol has proven to be the optimum purple sweet potato starch based edible film. Time dependency of the starch, carrageenan and glycerol blends were evaluated for all formulation which was influenced by the hydrophobic behaviour as the solubilisation time is shortened with a higher amount of glycerol. Mechanical properties (tensile strength and elongation) of the starch, carrageenan and glycerol films were evaluated. The behaviour of tensile strength and elongation at break vary according to the carrageenan and glycerol contents whereby tensile strength significantly increases with the increase of glycerol content in the blend. Also elongation at break increases with the increase of glycerol content in the blend. The water solubility values of starch, carrageenan and glycerol films show that solubility significantly increases with increasing glycerol ratio.

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