Synthesis and characterization of aloe vera-based edible film incorporated with shellac resin and hydrocolloids

IOP Conf. Series: Materials Science and Engineering **557** (2019) 012076 doi:10.1088/1757-899X/557/1/012076

Synthesis and characterization of aloe vera-based edible film incorporated with shellac resin and hydrocolloids

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Abstract. Aloe vera-based edible film usually has weak structure, thus it has low strength. Incorporation of shellac resin and gelatine/guar gum might create composite edible film with preferable properties. This research aimed to synthesis edible film of aloe vera gel that was incorporated with shellac resin and gelatine or and guar gum. The initial concentration of shellac resin was kept constant at 6% w/w; meanwhile the initial concentrations of gelatine were 4% and 6% w/w and of guar gum were 1% and 2% w/w. The ratio between SHGE/SHGG and aloe vera gel in the suspension (ALSHGE/ALSHGG) was 1:1. The suspension was kept in the fridge for 4 days and the film was prepared from this suspension at day 0, 2 and 4. The suspension was characterized in terms of its viscosity, sedimentation and microbial activity during storage. The film was characterized in terms of its thickness, solubility, moisture content and water vapor transmission rate (WVTR). The results showed that ALSHGE and ALSHGG suspension were pseudoplastic, depending on their concentrations, with sedimentation occurred in ALSHGG suspension after 4 days. Microbial growth in the suspension was inevitable in both concentrations of ALSHGG, but there was no microbial growth observed in both concentrations of ALSHGE. After 20 g of suspension was casted into film, ALSHGE4% and 6% resulted in thicker films, with lower moisture content, than ALSHGG1% and 2%. These could be the reason for high solubility of ALSHGG films. The WVTR of the films varied, independent of storage time of the suspension, with the least WVTR was observed in the film from ALSHGE.

1. Introduction

Horticultural products such as fruits and vegetables are perishable; therefore, they have relatively short shelf life. The basic methods to extend shelf life of fresh horticultural products are by retarding their metabolisms, including respiration, transpiration, evaporation, and preventing microorganism contamination [1]. This might be achieved by applying, one amongst others, an edible film to fresh horticultural products. Aloe vera gel has great potential to be applied as edible film in Indonesia because aloe vera is widely cultivated in the country and it is cheap [2]. Aloe vera gel is rich of polysaccharide compounds which would make the film has good barrier properties against O_2 at low to moderate humidity condition. The film would also have ability to maintain moisture content of coated products by controlling evaporation and exchange of water-soluble compounds with environment [3]. However, this particular ability also becomes the drawback of aloe vera-based edible film, i.e., it adsorbs water from its environment due to its hydrophilicity [4]. Imbalance water activity between the coated product, the film, and their environment might lead unexpected water transport to the products that leads to spoilage. Therefore, incorporation of other materials that improve the drawback of film properties shall be considered. Shellac resin was then selected in this research to add certain hydrophobicity to aloe vera-based edible film. Nevertheless, shellac alone has poor mechanical property and unstable; therefore, a hydrocolloid, i.e., guar gum or gelatine was added as a stabilizer.

This research aimed to synthesize aloe vera-based edible film which was incorporated with shellac resin and a hydrocolloid. The suspension containing those materials was characterized in terms of viscosity, sedimentation and its microbial activity during storage. Further, the resulted composite film was characterized in terms of its physical properties, i.e., thickness, solubility, moisture content and water vapor transmission rate (WVTR).

2. Materials and Methods

2.1. Materials

Aloe vera leaves which were the main material to prepare aloe vera gel were cultivated in Bogor and harvested at 8-10 month after planting. Other materials used were yellow shellac resin, obtained from Lubuk Linggau, South Sumatera, guar gum (Premium Guar Gum, Bob's Red Mill), gelatine (Halal Gelatine Powder, Naturich), citric acid, ascorbic acid, alcohol 96%, and aquadest (local chemical stores around Bogor).

2.2. Methods

2.2.1. Preparation of aloe vera gel. Aloe vera leaves were cleaned under running tap water and then trimmed. The pulp was filleted, cleaned with water and then pulverized using a blender for 10 s. The pulverized pulp was further homogenized at 10,000 rpm for 30 min at 5 ºC. The homogenized pulp was filtered, pasteurized at 70-75 ºC for 15 min, rapidly cooled, and then citric acid and ascorbic acid were added into the homogenized pulp. The pulp exposed to these treatments was then referred as aloe vera gel.

2.2.2. Preparation of composite suspension. The composite suspension consisted of aloe vera gel, shellac resin solution and guar gum or gelatine solution. Solution of shellac resin was prepared by dispersing shellac flakes into alcohol with concentration of 6%w/w. The dispersion was stirred for 24 h and then centrifuged at 10,000 rpm, 5 ºC, for 20 min. The supernatant was harvested as shellac solution. Guar gum solution was prepared by dispersing guar gum powder in aquadest at 1% and 2% w/w and then stirring at 650 rpm was performed until the powder was completely dissolved. Gelatine solution was prepared in similar way as guar gum solution but heating at 50 ºC was applied during stirring to speed up solubilization of gelatine. Concentrations of the solution were 4% and 6% w/w. The composite suspension was prepared as follows, guar gum or gelatine solution (40%) was mixed with shellac resin solution (60%) for 3 h. Aloe vera gel was added into the mixed solution with a ratio of 1:1 and then homogenized at 10,000 rpm for 1 min. This composite suspension containing guar gum was coded as ALSHGG and the one containing gelatine was coded as ALSHGE. The percentage following the codes represented concentration of the guar gum or gelatine.

2.2.3. Characterization of the composite suspension. The suspension was kept for 4 days in the fridge and then, its viscosity, as well as its flow behaviour, and number of its microbial contaminants were measured at day 0, 2 and 4. Viscosity and flow behaviour of the suspension was measured using a rotational test (rheometer MCR301, Anton Paar, Austria) at shear rate range of 0.1 – 500 s^{-1} for up-ramp shear rate sweep and vice versa for down-ramp shear rate sweep, at a temperature of 30 ºC. Each sample was measured in triplicate. Number of microbes that contaminated the suspension was examined by performing total plate count (TPC) method [5]. The composite suspension was diluted 100-fold and 1000-fold, spread over the surface of agar plates, and then the plates were incubated at 35 – 37 ºC for 48 h. Number microbe colonies appeared on the surface was counted to indicate extent of microbial growth in the composite suspension. Number of colonies per ml of the suspension was calculated using Equation (1). Sedimentation that occurred in the suspension was observed every day during storage. The suspension was placed in sample tubes and the suspension height was between 5.5 cm and 6.6 cm. If sedimentation took place, the height of the sediment was measured.

$$Number\ of\ colonies\ per\ ml\ suspension = \frac{Number\ of\ colonies\ on\ the\ plate}{dilution\ factor} \qquad (1)$$

2.2.4. Preparation of the film and its characterizations. Aloe vera-based edible film was prepared by casting 20 g of the composite suspension, which was stored for 0, 2 and 4 days, onto plastic plates of diameter 85 mm. The casted solution was dried at room temperature for 2 days and then the resulting film was peeled off of the plate. Thickness of the film was measured using a thickness gauge with 0.005 mm precision. Ten points on the film was measured and the results were presented as an average thickness. The film solubility was measured according to the method of Khoshgozaran-Abras et al. [6].

The film was cut into pieces with a dimension of 1x4 cm^2 and its dry weight was measured. The film was then immersed in 50 mL of aquadest for 24 h. After 24 h, the soaked film was filtered and then dried in an oven at 105 ºC for 24 h before measuring its final dry weight. Solubility of the film is defined in Equation (2). Moisture content of the film was measured according to Costa et al. [7]. About 50 mg of film was weighed and then it was dried in an oven at 105 °C for 24 h. The dried film was weighed, and the moisture content was calculated using Equation (3). Gravimetric method according to Warkoyo et al. [8] was applied to measured WVTR of the film. The film with a designated area was weighted and then, it was placed exactly on the mouth of sample container. The sample container with the film on it was kept in a desiccator at 25 ºC and 75% relative humidity for 24 h. To maintain a 75% RH gradient across the film, anhydrous calcium chloride (0% RH) was placed inside the sample contained; meanwhile saturated solution of sodium chloride (75% RH) was placed at the bottom of desiccator. In this way, RH inside the sample container was always lower than that outside of the container. WVTR was determined from the weight gain by the film [9] and calculated with Equation (4).

$$Solubility\ (\%) = \frac{W_0 - W_t}{W_0} \times 100\% \quad (2)$$

where W_0 is the initial dry weight (g) and W_t (g) is the final dry weight of the film.

$$Moisture\ content\ (\%) = \frac{m_i - m_f}{m_i} \times 100\% \quad (3)$$

where m_i is the initial weight (g) and m_f (g) is the final weight of the film after drying.

$$WVTR\ (g/m^2h) = \frac{b}{A} \times 100\% \quad (4)$$

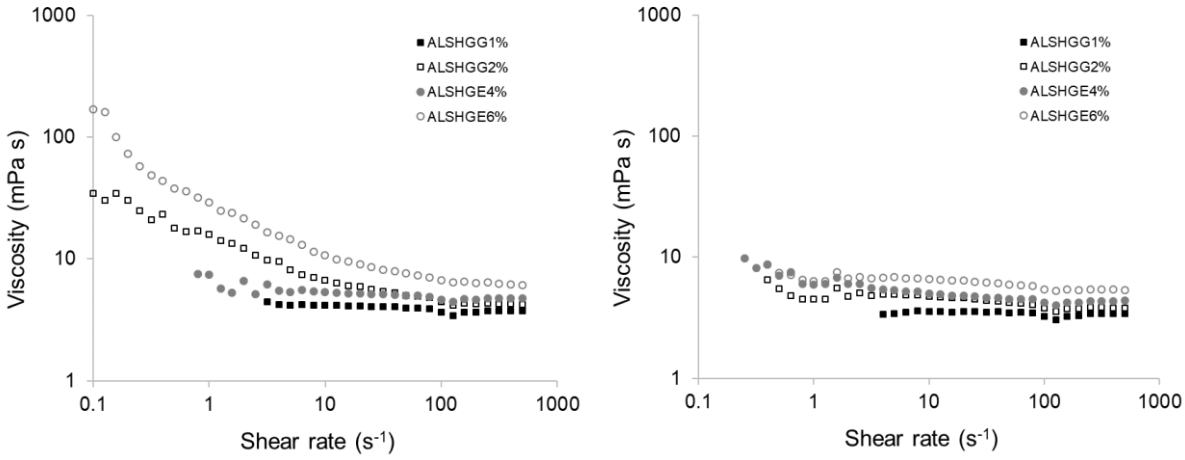
where b is the rate of weight gain (g/h) and A is the film area (m^2).

3. Results and Discussion

The composite suspension containing aloe vera gel, shellac resin and guar gum/gelatine was fairly stable during storage. ALSHGE at 4% and 6% did not show any precipitation during 4-day storage but ALSHGG at both concentrations of 1% and 2% w/w started to precipitate at the fourth day of storage. Nevertheless, the maximum thickness of the sediment was only about 4% of the suspension's initial height. Low concentration of ALSHGG and ALSHGE was prone to Newtonian-like behaviour. At the highest concentration tested, both ALSHGG and ALSHGE suspension were pseudoplastic (Figure 1a). After 4-day storage, these suspensions were Newtonian-like behaviour similar as those ALSHGG and ALSHGE at low concentrations (Figure 1b). Pseudoplastic behaviour of the suspensions at high concentrations was probably contributed by guar gum, aloe vera, and into certain extent by gelatine and shellac. Guar gum as low as 1% w/v showed Pseudoplastic behaviour measured at 25 ºC. At low shear rate, the apparent viscosities slightly reduced during storage up to 37 days, but overall flow behaviour remained Pseudoplastic [10]. The mucilaginous fresh aloe vera gel was also reported to be Pseudoplastic behaviour, regardless harvesting time of the leaves, irrigation status of the leaves in the field, or gel treatment [11-12]. On the other hand, gelatine solution was often reported as Newtonian-like behaviour, even at high concentration [13-14]. Nevertheless, Pseudoplastic behaviour of gelatine solution could also be found depending on gelatine sources [14-15]. In this research, the gelatine was originated from bovine and its solution has Newtonian behaviour. Flow behaviour of shellac solution measured at the range shear rates tested in this research would be Newtonian behaviour as has been shown by Gao et al. [16]. At shear rates between 1 s^{-1} and 100 s^{-1}, sodium shellac solution at 0.1-15% w/w was Newtonian and deviation from Newtonian occurred at lower and higher shear rates than 1 s^{-1} and 100 s^{-1}, respectively. Considering flow behaviour of each component in the suspension, significant change on flow behaviour of ALSHGG2% and ALSHGE6% after 4-day storage was most probably contributed by aloe vera gel. Composition within aloe vera gel underwent significant changes during storage for 48 h

at 40 ºC that led to Newtonian behaviour [11]. Glucose content in the gel dropped up to 75% of its original content; meanwhile, mannose content increased up to 44% of its original content during storage. Overall, polysaccharide content of aloe vera gel tended to decrease during storage. In addition, imperceptible precipitation of remaining fibre in the gel might contribute to the change of Pseudoplastic to Newtonian.

Microbial contamination was inevitable in the suspension containing guar gum of both concentrations (ALSHGG1% and ALSHGG2%). The contamination took place already after suspension preparation and increased during storage, except for ALSHGG1% at day 2 and 4 but the numbers are not significantly different (Figure 2). Number of colonies per ml suspension could be suppressed with increasing guar gum concentration. Contrarily to ALSHGG suspension, ALSHGE suspension at both concentrations did not show significant contamination of microorganism. Guar gum has been proven as a good media to replace agar for microbial culture media [17]. This gum was shown to act as prebiotics for the growth of *Lactobacillus reuteri* at 37 ºC for 16 h [18]. It is also the case for gelatine because protein is a good source for microbial growth. Various bacteria such as *Staphylococcus aureu*s and *Listeria monocytogenes* grew well on gelatine-based film [19]. On the other hand, shellac was not reported to have certain support towards microbial growth nor against it. Therefore, application of shellac-based film for retarding microbial activity was often in combination with other antimicrobial agents such as lemongrass oil [20]. The only component that owns antimicrobial activity in the suspension was aloe vera gel. Various methods have been tested to screen antimicrobial properties of aloe vera gel and most of the methods indicated that aloe vera gel is capable of inhibiting gram-positive and gram-negative bacteria to grow [21]. In this research, microbial activity remained occur in ALSHGG suspensions. The reason might be the fact that guar gum is better food source for microbes than gelatine; therefore, the existence of aloe vera gel in the suspension is not enough to suppress their activities.



a. Flow behaviour at day 0 b. Flow behaviour after 4-day storage

Figure 1. Flow behaviour and apparent viscosity values of ALSHGG and ALSHGE suspension during 4-day storage.

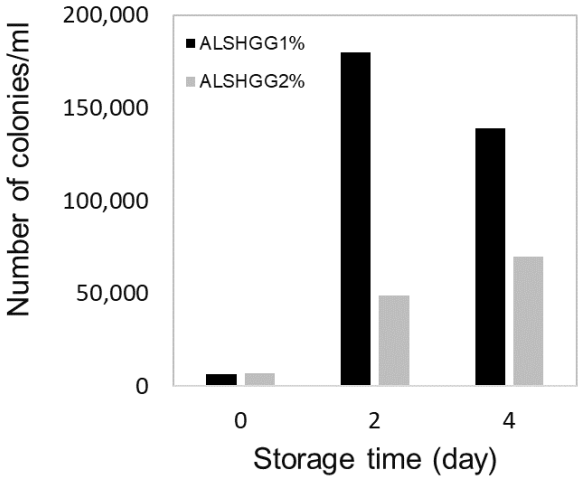


Figure 2. Microbial growth in ALSHGG suspension during storage.

After casting the suspension into films, different film colours were obtained. Originally, film made of aloe vera gel was transparent as shown in Figure 3a. Incorporation of shellac resin turned all film colours with certain yellow tone. Shellac flakes had yellow golden colour initially. As the results, ALSHGE films (Figure 3d and e) had bold yellow colour without significant difference between the two concentrations (colour data and its statistical analysis are not shown). ALSHGG films (Figure 3b and c) had less yellow tone with significant difference between the two concentrations.

a. Film of aloe vera gel

b. ALSHGG1%

c. ALSHGG2%

d. ALSHGE4%

e. ALSHGE6%

Figure 3. Appearance of ALSHGG and ALSHGE films. Film of aloe vera gel was depicted as the reference.

Various film thickness was also obtained. ALSHGE suspension at both concentrations of gelatine resulted in thicker films than ALSHGG suspension (Table 1). Higher viscosity of ALSHGE suspension than ALSHGG suspension, as shown in Figure 1, could be the reason. At the same weight, casted ALSHGE suspension would result in thicker layer than ALSHGG suspension, leaving thicker film when it was dried. This thicker film took longer time to dissolve in water and had low WVTR as shown in Table 1. Some researches indicated anomalous relationship between film thickness and WVTR. WVTR of a hydrophilic film increased linearly with the thickness [22-23]; however, other research suggested differently. Relatively constant WVTR of film was found over a thickness range of 0.046 - 0.061 mm (methyl cellulose film) and a thickness range of 0.023 – 0.14 mm (hydroxypropyl cellulose film) [24]. Molecular weight of the film components and their hydrophilicity dictated degree of WVTR. In ideal polymer film, thickness does not have any effect on WVTR [23]. Degree of WVTR might vary depending on the film materials, hence, film solubility might change in accordance with the WVTR. ALSHGG and ALSHGE films had similar basic materials, i.e. aloe vera which is highly hydrophilic and shellac resin which is highly hydrophobic, while, both guar gum and gelatine are hydrophilic materials. If WVTR is related to molecular weight of the film material as stated by Park et al. [24], thus, results of this research are inline with that theory. Molecular weight of guar gum is much higher than that of gelatine, therefore, WVTR of ALSHGG films is higher than that of ALSHGE films.

Table 1. Physical characteristics of various aloe vera-based edible films.

Film	Thickness (mm)	Moisture content (%)	Solubility (%)			WVTR (g/m^2 h)
			day 0	day 2	day 4	
ALSHGG1%	0.04 (cv. 6%)	19.1 (cv. 2%)	31.5	37.2	42.5	8.3
ALSHGG2%	0.06 (cv. 3%)	17.4 (cv. 6%)	27.8	41.4	32.0	7.7
ALSHGE4%	0.13 (cv.1%)	10.1 (cv. 3%)	23.6	25.3	25.6	4.6
ALSHGE6%	0.14 (cv. 2%)	11.0 (cv. 5%)	23.0	24.3	27.2	6.6

4. Conclusion

This research showed that aloe vera-based edible film was possible to be formed by incorporating shellac resin and guar gum or gelatine. Suspension of the composite film containing gelatine (ALSHGE) seemed to be a better option for forming the film because the suspension withstood sedimentation process and the microbial growth during 4-day storage. This would allow longer time of film preparation because new and freshly prepared suspension might not be necessary. Further, higher viscosity of the ALSHGE suspension allowed formation of thicker film, which consequently, resulted in low solubility and low WVTR of the film. Selection upon the best gelatine concentration to be incorporated into the composite suspension subjects to further statistical analysis.

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