

nano rasayan

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INTRODUCTION

Fruits and vegetables are commodities that are growing rapidly at this time, stimulated by the need for food that can improve health. Fruits and vegetables are known to contain bioactive components such as phenolic compounds, carotenoids, organic acids, vitamins and fibre which are very beneficial for health. The drawbacks are very short shelf life and perishable, requiring proper post-harvest handling to reach customers fresh. Various technologies are used to extend their shelf life, such as modified atmospheric packaging, preservatives, irradiation and ozone radiation¹. One alternative approach that has received great attention for increasing the shelf life of fruits and vegetables in recent years is edible coating. The packaging concept for edible coating originated in eleventh century China, when a wax coating was used on oranges and lemons to avoid moisture loss². Edible coating is environmentally friendly and biodegradable food packaging, in the form of a thin, edible layer^{3,4,5}. Edible coating can also function as a carrier for additives, barrier to chemical, physical and biological changes, barrier to gas exchange of O₂ and CO₂, water vapor and mass transfer^{6,7}. Edible coating application can improve the appearance of bright and shiny colours, retain moisture, prevent weight loss and act as an antimicrobial^{2,3}. According to^{8,9}, edible coating is helpful in maintaining quality and extending the shelf life of fruits and vegetables. The added advantage of using edible coating is that it does not pollute the environment, is preservative-free food products, is able to maintain taste and color retention⁶. Edible coatings are usually developed using lipids, polysaccharides, proteins and their composites^{10,11,12,13}. The polysaccharide based edible coating provides an excellent barrier to oxygen due to its dense hydrogen bond network structure¹⁴. The addition of antioxidant additives such as citric acid and ascorbic acid to the edible coating results in better quality maintenance. The microbiological spoilage of coated fruits and vegetables suggests the importance of adding antimicrobials to the edible coating. Potassium sorbate has been reported to exhibit excellent antimicrobial potential and its incorporation in edible coatings has slowed it down. The type and concentration of additives determine the consistency and stability of the edible coating. One of the natural ingredients that has the potential to be used as an edible coating is aloe vera gel^{3,15,16}.

Aloe vera gel consists of polysaccharides containing more than 75 functional chemical compounds, has antioxidant and antimicrobial properties and is able to retain moisture so that it can inhibit postharvest damage and increase the shelf life of fruits and vegetables^{5,7}. Aloe vera gel has very high enzyme activity, is easily oxidized, changes color and has a bad odour^{3,17}. Its viscosity drastically decreases to close to that of water when stored at room temperature for 24-36 hours¹¹. The physicochemical properties of aloe vera gel are also greatly influenced by air, light, and heat¹⁸. Therefore, early identification of aloe vera gel is very important to produce a good formula for Ecogel or edible coating of aloe vera gel. The advantages of using Ecogel are its biodegradability, permeability to oxygen, antioxidant power, low toxicity effect, cheap and easy to apply, as well as an alternative to the use of chemicals after harvest^{5,7}.

The ability of edible coating as a matrix or additive carrier is strongly influenced by its molecular structure, molecular size and chemical constituents. Small particle size results in a larger surface area so that it has the potential to increase bioavailability, solubility, absorption of active compounds and controlled release¹⁹. Nano-additives are additives whose particles are nano-sized. The application of nanotechnology in a variety of products, including edible coating food packaging, is currently in great demand²⁰. The advantages of using nano-additives include antimicrobial ability, emulsion system stability, barrier properties and bioavailability²¹. Information on the use of nano-additives, especially the type and concentration used in Ecogel, does not yet exist. Therefore, the exact type and concentration of nano-additives need to be studied in order to obtain a good Ecogel formula that is able to maintain the quality of fresh fruit and vegetables. This study aims to determine the type and concentration of nano-additives to produce the best Ecogel formula to extend the shelf life of fruits and vegetables.

EXPERIMENTAL

Material and Methods

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The material used in this research is the leaf part of the aloe vera plant (*Aloe barbadensis*. Miller) which will be taken as the gel as raw material for edible coating, obtained from the village of Taro, Tegallalang District, Gianyar Regency. Ecogel formulation with nano-additives used a completely randomized design

with two factors. Factor I is a type of nano-additive which consists of four types, namely citric acid, ascorbic acid, potassium sorbate, and a mixture of the three. The second factor was the concentration of nano-additives which consisted of three levels, namely 0.15%, 0.30%, and 0.45% in aloe vera gel. Control was prepared as a comparison by means of aloe vera gel without adding nano-additives and repeated three times.

General procedure

Initial identification was carried out by looking at the characteristics of the gel for 15 days of storage. For the preparation of aloe vera gel, first is sorting the leaves of aloe vera (*Aloe barbadensis* Miller) which are harvested after 1 year of age, selected leaves that are intact, straight and without blemishes or rot. The position of the leaves is taken the lowest three midribs of the aloe vera plant with a length of about 40-50 cm. Leaves leave for 24 hours at room temperature to remove yellow mucus. Then washed thoroughly with running water to remove yellow mucus residue and unpleasant odours that can reduce the quality of the gel. Then the stripping and filleting process to produce gel filets using a stainless-steel knife. The next treatment was homogenization using Philip HR 2116 at a speed of 2 for 5 minutes to produce aloe vera gel. Heating at a temperature of $70\pm 1^\circ\text{C}$ for 5 minutes to inactivate the enzyme. Then the aloe vera gel is cooled at room temperature for 1 hour until it reaches 27°C . The next step is storage at room temperature ($28 \pm 1^\circ\text{C}$) and cold temperature ($7\pm 1^\circ\text{C}$) to be observed periodically for 15 days. The observation variables included surface tension, pH, viscosity, L* color, and transparency.

The first ecogel production is the process of sorting 1 year old aloe vera leaves, leaving them for 24 hours at room temperature to remove yellow mucus¹¹. Then washed with running water to remove yellow mucus residue and unpleasant odours that can reduce the quality of the gel. The process of stripping and filleting to produce gel filets using a stainless knife. The next process is homogenization for 5 minutes followed by heating at a temperature of $70\pm 1^\circ\text{C}$, 5 minutes¹¹. The gel is cooled for 1 hour to a temperature of 27°C , after that it is filtered with a Rocker 300 vacuum pump, 5340FK1000R flash filter and Whatman filter paper no 42. The gel is then added with each type of additive, namely citric acid, ascorbic acid, potassium sorbate and the three mixtures with concentrations of 0.15%, 0.30%, and 0.45% (w/v). The agitation process uses a sonicate model Q125 Masonic to obtain the additive nanostructures, with a pulse 59-time delay of 30 seconds for 50 minutes. Furthermore, it was stored at cold temperature and the observations were carried out on days 5, 10, and 15. The observation variables included surface tension, pH, viscosity, L* color, and transparency. To ascertain the size of the additive particles in the nano size of the aloe vera gel, the analysis was carried out using a UV-Vis spectrophotometer in the wavelength range of 200 - 500 nm²², stated that at a wavelength of 200-500 nm the maximum absorbance indicates a particle size of 20-110 nm. After obtaining the nanostructures, Ecogel is ready to be used as an edible coating.

RESULTS AND DISCUSSION

Determination of nano-additive size

The choice of the method for making nanoparticles depends on the type of polymer. The manufacture of nanoparticles in this study used a top-down process, reducing the particle size from large particles to smaller particles using high pressure homogenization techniques using a sonicator. According to²⁴, the absorbance analysis can simultaneously show the optical properties of a material. The fields of nanoscience and nanotechnology also use UV-vis spectrophotometer analysis to predict the size and shape of nanoparticles. From the results of UV-vis spectroscopy, the absorbance value can indicate qualitatively the number of nanoparticles formed. Meanwhile, the maximum absorbance spectrum (nm) can indicate the size of the nanoparticles produced. The greater the maximum wavelength, the greater the size of the nanoparticles. In addition, this absorbance analysis is also the fastest and easiest type of analysis to find out whether nanoparticles have formed.

The results of the UV-Vis spectrophotometer analysis still need to be strengthened by other analyzes such as SEM. UV-vis spectrophotometer measurements on aloe vera gel were carried out in a wavelength range of 200 - 500 nm. ²³stated that at a wavelength of 200-500 nm the maximum absorbance indicates a particle size of 20-110 nm. The sonicator model Q125 Misonix was used after the vacuum process to obtain the size of the nanoparticles with a pulse 59, pause of 30 seconds for 50 minutes. It is supported by²⁵, that aloe

vera gel can be stable under vacuum without losing biological activity. Ecogel nano-additive gives an absorption peak at a wavelength of around 284 nm so that the transmittance is 0.5012%. Aquadest is used as a comparison because it does not have particles that hold light transmission so that it will continue light passing through it without any light scattering effect so it has a transmittance value of 100%. Aloe vera gel should be stored in a dark coloured glass container to avoid the influence of light on sensitive bioactive agents⁵.

Ecogel surface tension

The surface tension of a liquid occurs because of the difference in the resultant attraction of the molecules on the surface of the liquid or because there are adhesion and cohesion forces. Decreasing interface tension can increase the adhesion force between the surface of fruit and vegetables with the coating material. The surface tension of the coating liquid is directly proportional to the cohesion power between the constituent materials and is inversely proportional to the adhesion power between the coating and the material to be coated,²⁶ stated that the addition of Excessive additives can increase the surface tension of the coating fluid.

The concentration of citric acid nano-additives, potassium sorbate and the mixture of the three greatly contributed to the increase in surface tension of Ecogel on day 5 (Table-1). The addition of nano-additives to Ecogel for stabilization is required at low concentrations, supported by the opinion²⁶, that the addition of the additive is excessive, can increase the surface tension of the coating liquid. The nano-additive resulted in a change in surface tension on day 10, this means that all types of nano-additives contributed to surface tension. The highest increase occurred in the addition of citric acid nano-additive. Increasing the interface tension can reduce the adhesion force between the fruit and vegetable surfaces with the coating material. Adhesion is a bonding phenomenon in a complex surface that is the tensile strength between two different surfaces²⁶. Potassium sorbate nano-additive treatment increased Ecogel surface tension until the end of the 15th day of storage. Meanwhile, citric acid nano-additive produced surface tension which increased until day 10 and decreased again on day 15. Thus, the treatment of the nano-additive mixture resulted in a relatively stable Ecogel surface tension after that followed by ascorbic acid nano-additive. Surface tension occurs due to differences in the resultant tensile strength of the molecules that exist on the Ecogel surface or because there are adhesion and cohesion forces. The number of counter-pull interactions can be increased by enhancing the intermolecular relationship between coating and foodstuffs. The requirement for good adhesion is the importance of the wettability criterion. Wetting is increased when there is a strong contact between the coating solution and the solid, making the coating stickier. If perfect contact is achieved, the intermolecular interaction distance will shorten so that adhesion is achieved²⁷. Low adhesion means the coating may be difficult to adhere to or easily come off. The addition of excessive additives can increase the surface tension of the coating fluid²⁶. The addition of additives to Ecogel is required at low concentrations for stabilization and is not expected to increase its surface tension.

Table-1: Ecogel surface tension (N/m), pH, viscosity (mPa.s) on type and concentration of nano-additives treatment

Type of additives	Surface tension (N/m)			pH			Viscosity ((mPa.s)		
	Concentration (%)			Concentration (%)			Concentration (%)		
	0,15	0,30	0,45	0,15	0,30	0,45	0,15	0,30	0,45
Day 5: CA	0,114	0,120	0,128	3,81	3,47	3,18	84,89	77,76	80,34
AA	0,121	0,120	0,118	4,17	3,97	3,93	91,21	87,81	84,53
PS	0,121	0,121	0,131	4,61	4,54	4,64	93,94	103,91	103,99
MA	0,121	0,122	0,125	3,98	3,78	3,51	97,65	77,76	85,80
Day 10: CA	0,123	0,138	0,135	3,78	3,16	3,07	76,27	86,07	81,31
AA	0,112	0,125	0,117	4,19	3,85	3,87	98,55	96,90	77,86
PS	0,113	0,123	0,123	4,46	4,55	4,57	96,57	97,09	101,98

MA	0,121	0,114	0,122	3,99	3,60	3,45	97,13	87,30	94,03
Day 15: CA	0,124	0,108	0,111	4,11	3,27	3,09	98,76	94,55	87,38
AA	0,122	0,113	0,111	4,36	4,01	3,89	102,78	100,80	91,95
PS	0,122	0,127	0,127	4,36	4,47	4,48	123,76	121,61	121,33
MA	0,122	0,116	0,114	4,22	3,51	3,49	96,03	65,53	71,81
Note: CA (Citric acid), AA (Ascorbic acid), PS (Pottasium sorbate), MA (Mix additives)									

Acidity of Ecogel (pH)

The level of acidity or pH is a parameter used to indicate the acidity of a substance or solution. Potassium sorbate at all concentrations increased pH compared to other treatments. This is because the enzyme activity can be suppressed so that the formation of less organic acids. The use of sorbic acid and its salt for food varies²⁸. On day 5 of the citric acid additive treatment produced the lowest pH, this is because citric acid is an acidulant compound that can lower pH. The acidity of citric acid is obtained from the three COOH carboxyl groups which can release protons in solution²⁹. Citric acid can be used to regulate acidity or food preservatives, and also to prevent browning of fruits. The 10th day of storage is the same as the 5th day that citric acid is an acidulant and chelating compound, it can complex copper ions which act as a catalyst. Citric acid can inhibit browning by lowering the pH so that the polyphenol oxidase enzyme becomes inactive. Citric acid is a natural preservative that is safe for consumption³⁰.

Ecogel formulation with ascorbic acid and additive mixture produces a stable pH and according to the pH of the fruit to be coated, namely 3.5-4.5. Ecogel with citric acid additive has the lowest pH compared to the others. This is because citric acid is an acidulant compound²⁹. The addition of citric acid and ascorbic acid to Ecogel resulted in a pH of around 4²⁵, the higher the concentration of the Ecogel additive the lower the pH. Aloe vera gel is known to be stable at low pH. The addition of nano-additives prolongs shelf life, due to enzyme inactivation, preventing the breakdown of polysaccharid⁴ into arachidonic acid, γ -linoleic acid and other organic acids^{2,7}. The ecogel function is most effective if it has a pH similar to coated fruits and vegetables. This is related to the stickiness or the ability to form cross-links with polymers and pectin compounds in fruits and vegetables.

Viscosity of Ecogel (mPa.s)

Viscosity is a measure that states the thickness of a liquid or fluid. Increasing the concentration of potassium sorbate resulted in a higher viscosity of Ecogel than other nano-additives. This is because the higher the concentration of nano-additives, the viscosity increases. It is in line with the opinion of²³, that additive accumulation is thought to strengthen the cross-linking of polysaccharide polymers. Acemanan and glucomannan were identified as the main functional components of aloe vera gel. Specifically, acetylated-glucomannan is a polysaccharide consisting of mannose units located in the protoplasm of parenchyma cells²⁴. The nano-additive with a concentration of 0.15% also produced the lowest viscosity value and was relatively stable. Migration of nano-additives into acetyl glucomannan bonds increases the molecular weight of Ecogel. The greater the molecular weight, the slower the solution flow rate, and this increases the viscosity value. The enzymes in aloe vera gel are very active and also have an effect on the viscosity of the gel⁷.

The results of day 5 showed that the viscosity of Ecogel was higher with the addition of potassium sorbate nano-additive. According to³², the enzymes in aloe vera gel are very active which affect the bonds of the compound and also the gel viscosity. Likewise on day 10, migration of nano-additive potassium sorbate into acetyl glucomannan bonds increased the molecular weight of Ecogel. The greater the molecular weight, the slower the flow rate of the solution and increase the viscosity⁷. The addition of the nano-additive mixture on the 15th day resulted in a relatively stable viscosity of Ecogel during 15 days of storage. The addition of additives can strengthen Ecogel so that the viscosity can be maintained. The nano-additive mixture, namely citric acid, ascorbic acid, and potassium sorbate, is able to synergize and strengthen the cohesion bond of Ecogel polymer. Supported by the opinion¹⁷, the stability of Ecogel is maintained by the addition

of additives. Research³³ stated that increasing the concentration of the filler in solution tends to increase the viscosity.

Color different (L*) Ecogel

Color plays an important role in the choice of food ingredients by consumers before considering nutritional value and taste. This parameter is used as a subjective indicator of product quality, although the frequently used edible coating is transparent and tends not to affect the display color. The synergy of the additive mixture of citric acid, ascorbic acid and 0.15% potassium sorbate on the 0th day resulted in the brightest color of Ecogel. This is because the addition of 0.15% nano-additive mixture is able to suppress color changes, maintain brightness which leads to its stability and effectiveness as an edible coating. The stabilization process is carried out by proper processing techniques, heating treatment, adding preservatives and other additives such as potassium sorbate, citric acid and ascorbic acid³².

On the 5th and 10th days there was also the addition of a nano-additive mixture capable of suppressing color changes, maintaining brightness which led to the stability and effectiveness of Ecogel as an edible coating. Aloe vera gel has the potential to be biopreservative, forming a waxy coating that can increase the shelf life of fruits³. This is because the proportion of the nano-additive mixture of citric acid, ascorbic acid and potassium sorbate at a concentration of 0.15% is ideal for maintaining the L* value.¹⁷, stated that the consistency and stability of aloe vera gel can be maintained by adding additives. The L* values for all treatments showed a slight decrease until the 10th day and increased again on the 15th day, but the gel looked cloudier. Turbidity is a manifestation of the result of polymer gel hydrolysis, which occurs after enzymatic reactions and also physicochemical properties which are strongly influenced by air, light and heat. On direct contact with air the gel color becomes pink and finally brown¹⁸. Citric acid is a browning inhibiting agent. This compound also lowers pH and inactivates the polyphenol oxidase enzyme. Heat and light catalyze the reaction supported by the high sugar content³⁴.

Transparency of Ecogel

Transparency refers to the ability of a material to emit light. Ecogel on storage has the highest transparency value in the potassium sorbate nano-additive treatment, this is because potassium sorbate is an unsaturated fatty acid in the form of powder and granules from white to yellowish brown, and has no smell³⁵. Increasing the concentration of potassium sorbate increases turbidity so that transparency increases. Day 5, the addition of potassium sorbate nano-additive causes Ecogel to look cloudier than the others. On the 10th day, the lowest Ecogel transparency of 85.38 was obtained from the treatment of 0.15% nano-additive mixture. One of the special requirements for edible coatings according to⁹ is that it has a transparent sensory property. According to³⁶, the antioxidant citric acid and ascorbic acid incorporated into a methylcellulose-based edible coating resulted in a transparent coating on the apricots and extended shelf life.

The results of the 15th day study showed an increase in the transparency value of Ecogel with various additives over a longer storage period. High transparency values are indicated by the appearance of increased turbidity. This is because storage is assumed to facilitate the breaking of the acetyl bond of the glucomannan polymer into smaller components, which then affects the level of gel transparency³. Aloe vera gel is part of the clear parenchyma cells and has decreased stability during storage³⁷. reported that the level of transparency of an edible coating usually increases with the addition of additives. The addition of an additive at high humidity causes the polymer to expand the network and decrease the forces between the molecules, thereby increasing the transparency value. The ascorbic acid type and the nano-additive mixture responded almost equally to Ecogel's transparency during storage. Increasing the concentration of nano-additives increases the transparency value. Ecogel transparency with nano-additive blends is relatively stable up to 15 days when compared to others. The combination of the three nano-additives produces a clear, transparent white Ecogel appearance. The nano-additive concentration yields the lowest transparency value at 0.15%, and produces the most transparent Ecogel coating without changing the appearance of coated fruit and vegetables. In accordance with the opinion⁹, the characteristics of edible coating are colorless and transparent. According to³⁸, the special characteristics of edible coating are as follows: natural material which is waterproof, can function as a barrier, permeable to moisture and solutes, melting point

not more than 40 ° C, easy to form emulsions, non-sticky, no impact on fruit quality, low viscosity, transparent, tasteless, odorless and able to tolerate pressure. Ecogel formulation is also expected to be safe from hazardous additives, and technology costs and raw materials must be relatively cheap.

Table-2: Colour L* and transparency of Ecogel

Type of additives	Colour L*			Transparency		
	Concentration (%)			Concentration (%)		
	0,15	0,30	0,45	0,15	0,30	0,45
Day 5: CA	15,37	15,39	16,21	82,69	82,69	81,84
AA	16,62	15,37	15,11	81,48	82,69	82,93
PS	13,47	11,29	15,42	84,52	86,61	82,63
MA	16,73	16,86	15,76	81,31	83,36	82,33
Day 10: CA	12,65	8,71	9,78	89,43	89,25	88,17
AA	7,57	9,02	10,87	90,50	88,98	87,10
PS	3,87	6,60	7,71	94,23	91,45	90,25
MA	14,73	7,64	8,36	85,38	90,44	89,62
Day 15: CA	20,86	23,17	25,20	87,10	92,29	89,87
AA	21,87	20,31	21,31	88,42	86,43	86,92
PS	20,67	20,85	18,23	86,81	87,29	86,25
MA	19,51	21,88	21,78	84,40	88,87	88,12

Note: CA (Citric acid), AA (Ascorbic acid), PS (Pottasium sorbate), MA (Mix additives)

Micrograph of Ecogel

The morphology, shape and surface state of the nanoparticles can provide information about the properties of edible coatings on fruits and vegetables. The results of Ecogel micrograph observations before application to the surface of fruits and vegetables showed that the addition of nano-additives resulted in different micrographs as shown in Figure-1 (C). The nano-additive particles are between the cross-linking bonds of the aloe gel polymer and contribute to its stability. This micrograph is different from the micrograph on Ecogel without nano-additives (A) and Ecogel with additives that are not nano-sized (B). The advantages of nano-additive particles are antimicrobial ability, improved barrier properties, flexibility and durability. Nano-additive acts as a reinforcing agent because it reduces the matrix plasticization mechanism. This is related to the interaction between nano-additive particles and the matrix, the transfer of water vapor and gas becomes increasingly difficult due to the tortuous pathway³⁹. The use of edible coating by application to food surfaces aims to provide a modified atmosphere, inhibit gas transfer, reduce moisture and aroma loss, delay discoloration, and improve appearance¹⁵. The ability of edible coatings depends on molecular structure, molecular size and chemical constituents. The application of edible coatings for fruit and vegetables should overcome the difficulty of adhesion of materials to hydrophilic surfaces¹⁶. Ecogel with nano-additives can reduce weight loss, soften texture, improve color appearance and maintain the nutritional components of fresh cut tropical fruit. Several studies have successfully applied Ecogel to the minimal processing of kiwifruit, mango, apples. Opinion⁴⁰, states that the addition of nano-additives improves the polymer composition of the Ecogel matrix

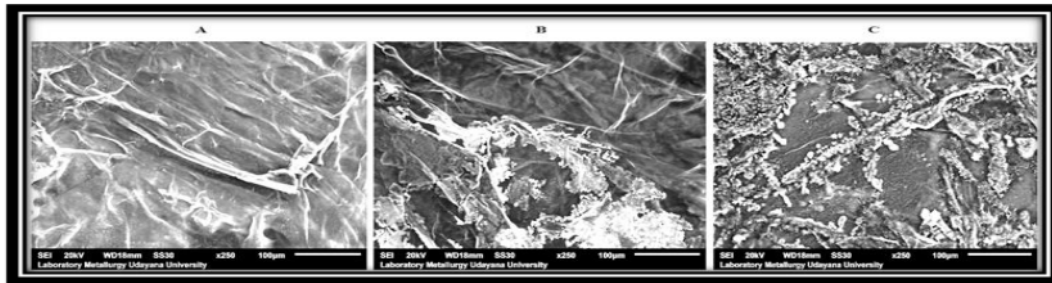


Fig.-1
Micrograph of Ecogel without nano-additive (A), Ecogel with additive (B), and Ecogel with nano-additives (C)

CONCLUSION

The best result of the effect of the type and concentration of nano-additives on aloe vera gel as the base material for edible coating (Ecogel) for 15 days of storage was a mixture of nano-additives (citric acid, ascorbic acid and potassium sorbate) at a concentration of 0.15. %. This is based on the fact that in this treatment Ecogel still has a relatively stable pH and viscosity surface tension, a relatively high brightness L^* and a low transparency value.

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