Evaluation of the Effect of *Curcuma longa* L. Essential Oil in Chitosan-starch Edible Coating

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**Abstract.** *Curcuma longa* L. is one of the commercially planted herbs in Malaysia for its phytochemical benefits. In this study, the effect of chitosan-starch at different concentration of *Curcuma longa* L. (CUR) were examined. The application of edible coating by using dipping technique has been analysed for their effectiveness in extending shelf life of fruits through several analyses include the microstructure observation, disk diffusion and appearance. Chitosan-starch coating incorporated with 10 uL of CUR had the best appearance and resulted a layer of homogeneous surface about 37.45 μm as can be seen in microstructure observation. The disk diffusion analysis proves that the chitosan with 10uL of curcuma longa had the highest inhibition zone which is 17 mm. Based on the present study, chitosan-starch with the addition of (CUR) is believed to have a major potential in extending shelf life of fruits with high antimicrobial activity from local herbs.

1. Introduction

Strawberries are very sensitive to high temperature that can be easily damage due to the handling processes once they were harvested. It can lead to produce rotten fruits at very minimal time even though the right storage utilized on them. Several methods have been proposed to overcome this including the application of technology by physical, chemical and biological means to prevent deterioration. By applying physical treatment like drying for example, lots of drawback may arise from this treatment such as the heat being applied may alter the quality of product such as nutrient loss, change in texture, colour and odour. Other than that, if chemical treatment being applied the main concern among all these synthetic chemicals is the residues that will remains in the product and can be very harmful. Thus, the application of edible coating enriched with essential oils using curcuma longa can be used as alternative to prolonging shelf life of fruits. Application of edible film from chitosan and starch enriched with curcuma longa essential oil can hold off the actual deterioration of strawberries as well as keep up with the freshness for a longer period.

Chitosan is derivation of chitin component via deacetylation in alkaline media and it is a natural, nontoxic copolymer consist of β- (1-4)-2-acetamido-D-glucose and β-(1-4)-2-amino-D-glucose units. It is abundant as it naturally can be found in the exoskeleton of crustaceans, fungal cell walls and other biological materials [1]. Chitosan coating preserve the quality of fruit by regulating gas exchange and decrease the respiration. It reduces the oxidative process due to reduction of reactive oxygen species.
accumulation by lowering the respiration rate [2]. It has been proven by applying the chitosan as coating on postharvest fruit including logan, pear, plum and apple to maintain its quality [3].

Edible coating is a thin layer of materials that offers a barrier to oxygen, microbes that come from environment, moisture loss and solute movement for food plus, it can be consumed by consumers [4]. It also does not add unfavourable properties to the fruits and environmental friendly due to its natural biodegradable source. It works by delaying the rate of respiration, decrease weight loss and extend the shelf life of fruits and vegetable during the postharvest storage [5]. The addition of essential oil would give significant effect on antimicrobial activity against bacteria, yeast and moulds and higher safety to environment and consumer [6].

The use of Curcuma longa L. (CUR) essential oil which abundantly available in Malaysia as one of the natural antimicrobial meet the needs. According to Wang [7], the antimicrobial, antibacterial and antifungal activities of CUR against foodborne pathogens are very promising to be use as the preservative in food industry. These properties attributed from the existence of phenolic compounds composed mainly of curcumin, demethoxy curcumin and bis-demethoxy curcumin [8].

2. Materials and method

2.1. Materials
Starch was purchased from local market, Curcuma longa L. being used as the essential oil was bought from BF1 Sdn. Bhd., 0.1M sodium hydroxide, Tween 80, acetic acid glacial and glycerol were bought from R&M Chemicals while medium molecular weight chitosan was purchased from Sigma Aldrich.

2.2. Edible coating preparation
Chitosan-starch edible coating was developed based on the procedures by [9] with some modifications.

2.2.1. Chitosan solution. Chitosan with 1% (w/v) was gelatinized in an aqueous solution of 0.5% (w/v) acetic acid at room temperature. 0.1 % (w/v) of Tween 80 was added as a surfactant to increase wettability [10]. To ensure that the chitosan is completely dissolved, the solution was stirred for 24 hours at 350 rpm until all the chitosan flake disappear. pH of the solution was adjusted by using 0.1 M sodium hydroxide until it reach pH of 5.6 [1] to decrease the acidic condition of chitosan solution. The solution was made up to 100 mL by adding distilled water up to 100 mL.

2.2.2. Starch solution. Starch solution at 4% (w/v) was gelatinized at 80°C. The solution was stirred for 30 minutes at 350 rpm to overcome the starch from being coagulated. The solution was made up to 100 mL by add up distilled water until reach to 100 mL. To enhance the application of solution formed by the combining chitosan and CUR essential oil, 2 mL of glycerol was added as a plasticizer.

2.2.3. Chitosan and starch coating solutions. Prepared starch solution at 2% (w/v) was added to 100 mL of prepared chitosan solution. The combined solution was stirred at room temperature for 18h to enable the solution well mixed. For the combination of CUR into chitosan and starch, different concentration of CUR essential oil was used which are 20, 10, 5, and 0 µL. The CUR essential oil was added into the prepared chitosan and stirred at room temperature until complete mixing. The coating solution formulations were added with chitosan concentrations of: 20, 15, 10 and 5 µL; glycerol concentrations of 2.0% and (v/v); Tween 80 concentrations of 0.1% (w/v).

2.2.4. Chitosan-starch with CUR essential oil solution. 100 mL of chitosan solution was taken, and 2 mL of prepared starch solution was added into the chitosan solution. After that, 5 µ L of CUR was
added to the mixture. The process was repeated for different concentration of CUR which is 10, 15 and 20 μL for every 100 mL of prepared chitosan and 2 mL of starch solution [11].

2.3. Microstructure observation
The microstructure of coated sample was analysed using a field emission-scanning electron microscopy (FESEM) Zeiss Supra 35VP. The strawberry sample were mounted onto metal stubs and coated with Au using an accelerating voltage of 5 kV.

2.4 Disk diffusion analysis
A sterile swab was used to take fungal culture from strawberry sample. Then, the culture was streak onto the Potato Dextrose Agar plate. The plate was stark with the swab in one direction and then rotate the 90° and streak the plate again in that direction to obtain uniform growth. The rotation was repeated for 3 times. After that, antibiotic disc that had been immersed with the chitosan samples of different concentration of turmeric oil were dispensed onto the agar. The forceps were used to gently press each of the disc to the agar to ensure each of the disc was attached to the agar. The forceps were sterilized by using flame. The plates were incubated at 25°C for 48-72 hours [12].

2.5 Physical appearance
The laboratory assays were done using fresh strawberries with same the maturity stage, 75% red colour, uniform weight with no sign of mechanical or microbiological injury.

3. Results and discussion

3.1. Microstructure observation
Field emission scanning electron microscopy (FESEM) was carried out to observe the microstructure and surface characteristics of coating film to establish a relationship between coating and strawberry. Figure 1 shows FESEM micrograph of cross section of strawberry coated with chitosan-starch coating plus 10 μL of CUR essential oil. As can be seen the coating had a quite smooth and homogeneous surface with a thickness about 37.45 μm. It suggests that the blending of chitosan and starch with the addition of CUR essential oil are highly miscible and homogeneous.

3.2. Disk diffusion analysis
The inhibition zone was recorded through disk diffusion analysis as can be seen in Figure 2. The inhibition zone indicates the antifungal properties for each of the chitosan coating that had been added with different concentration of CUR essential oil. After three day of incubation period, the inhibition zone for chitosan coating added with 10 ul of turmeric oil showed the highest inhibition zone which is 17 mm while the chitosan coating with 5ul turmeric oil give 10mm. For other samples, there were no sign of antifungal activity. Chitosan-starch coating added with 10ul of turmeric oil has the highest antifungal properties thus prolong the shelf life of strawberry samples. As proven by the investigation by Perdones [13], the use of chitosan coating would result to reduction in growth of Botrytis cinerea thus, it showed the degree of antimicrobial activity. Moreover, curcumin found in CUR has fairly good antimicrobial effect against large number of microorganisms and give a good antiseptic effect when the concentration is above 0.05% [14], [15].
Figure 1. FESEM micrographs of the cross section of the strawberry coated with chitosan-starch based coating containing CUR essential oil.

Figure 2. The inhibition zone after 3 days of incubation period.
3.3. Physical appearance
The appearance of strawberry sample was observed for five days at room temperature of 29 °C. From Figure 3, the non-coated strawberry sample showed unsatisfactory of appearance after one day of storage period as juice leakage happened on the second day of storage. The strawberry samples coated with chitosan and addition of 10 μL of turmeric oil could last up to three days. On the other hand, for the other strawberry samples, their appearances were satisfied for two days before wound, black spot and juice leakage could be seen on their surface.

![Figure 3. Appearance of control and coated strawberries at different concentrations CUR essential oil: a) control, b) 0 μL, c) 5 μL, d) 10 μL, e) 15 μL and f) 20 μL in chitosan-starch based coating.](image)

4. Conclusion
Incorporating *Curcuma longa* L. (CUR) essential oil into chitosan-starch coating significantly affected the ripening process and reduced physicochemical changes of strawberry fruit. Chitosan-starch coating incorporated with 10 μL of CUR had the best appearance and resulted a layer of homogeneous surface about 37.45 μm as can be seen in microstructure observation. The disk diffusion analysis proves that the chitosan with 10 μL of curcuma longa had the highest inhibition zone which is 17 mm. The addition of turmeric essential oil improves the antimicrobial properties of the chitosan coating to inhibit the growth of spoilage for reducing postharvest deterioration that make them suitable as a coating for fruit and vegetable products.
5. References

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