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Abstract. Mozzarella cheese is usually packaged using non-biodegradable plastic, and edible films are used as a more environmentally friendly alternative. Furthermore, incorporating bioactive compounds into the edible film helps prevent microorganism damage and oxidation to the product. One of the traditional herbal plants that have the characteristics as an antimicrobial and antioxidant agent is the Purslane (Portulaca oleracea). This study aims to determine the effect of purslane extract in chitosan edible film to inhibit microbiological and oxidative damage to mozzarella cheese. Purslane ethanol extract was added to the chitosan mixture for film making and then the film was applied to wrap the mozzarella cheese. The mozzarella cheese was then stored at room temperature (27°C) and in the refrigerator at a temperature of (4°C). The tests to determine the quality of the cheese were performed on day 0; 3; 6; 9 respectively, during the storage, which involves determining total bacteria and mold yeast using the Total Plate Count test, while oxidation damage was measured using the thiobarbituric acid (TBA) test. Based on data analysis using the Kruskal-Wallis method, during 9 days of storage, the incorporation of purslane extract to the edible film significantly inhibited the growth of microorganisms and oxidative damage in the mozzarella cheese packed, compared to control and the use of chitosan edible film alone. In conclusion, Chitosan edible film with purslane extract can be used as a substitute for plastic packaging to preserve mozzarella cheese during storage at room temperature or in the refrigerator.

Keywords: edible film, food packaging, mozzarella cheese

1. Introduction

Mozzarella is a type of soft cheese that can be easily contaminated by microorganisms and spoil. Furthermore, it is usually sold in vacuum cleaner plastic wrap for storage. The plastic used for the wrapping is usually polyethylene, polyamide, or polypropylene, which are non-biodegradable and can cause environmental problems. Therefore, a more environmentally conscious packaging is required to help maintain the quality of cheeses during their shelf life [1].

Edible film is an environmentally friendly packaging that has been widely applied to various kinds of food products [2,3]. The main ingredients of edible films are polysaccharides, such as chitosan, flavor enhancers and other incorporational compounds [4]. The application of edible films to cheese can also be used as an alternative to prevent damage due to bacterial or fungal contamination during storage. Microbial contamination in cheese products can affect the texture and taste, therefore reducing the value of the cheese [5]. The use of edible films packaging for cheese can be maximized by adding active...
compounds such as antimicrobials or antioxidants, which can have a positive effect on shelf life and food quality.

Purslane (*Portulaca oleracea*) is one of the traditional medicinal plants that is known for its antimicrobial, antioxidants, and other various benefits. The antimicrobial activity of purslane extract has been proven against several tested microbes, including *Eschericia coli*, *Staphylococcus aureus*, *Shigella dysentrica*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Aspergillus niger*, *Trichophyton* sp., and *Candida* sp. [6,7]. In addition to the antimicrobial activity, phytochemical compounds such as flavonoids, alkaloids and other organic acids in purslane extract can also have antioxidant properties [8,9]. The incorporation of antioxidants to the edible film can be beneficial to avoid oxidative rancidity, degradation, and discoloration. Antioxidants can increase stability because they can reduce the respiration rate of the product, and therefore maintaining the nutrition and the color of the product [10].

With these bioactivities, purslane extract is a potential candidate as an additive to edible films for its application in food packaging. This study aims to determine the effect of purslane extract incorporation in chitosan edible films to prevent microbiological and oxidative damage in mozzarella cheese.

2. Material and method

2.1 Extraction

A total of ± 500 g of whole Purslane was washed and then sun dried for ± 1 week and mashed using a blender. The Purslane powder was dissolved in 80% ethanol (Sigma) and was macerated for ± 3x24 hours. Furthermore, the ethanol solvent was evaporated using a rotary evaporator (Buchi) to obtain a dry paste. The paste was then diluted to 10, 20 and 30% concentration in distilled water.

2.2 Antibacterial and antifungi assay of the extract

The determination of the antimicrobial activity of the Purslane extract was carried out using the disk diffusion method. The mozzarella cheese sample was diluted for 10 times, and the dilution suspension was rubbed with a cotton bud evenly on the surface of the PCA or PDA medium (HiMedia) for antibacterial and antifungi test respectively. The paper disks (Oxoid) were immersed in 10%, 20%, and 30% purslane extract and were placed on the surface inoculated media. The positive controls used for the antibacterial and anti-fungal tests were ampicillin (Phapros) and ketoconazole (Sigma) respectively, while the negative control was distilled water. The inhibition zone formed was observed after 24 hours of incubation at 37° and the diameter was measured.

2.3 Antioxidant assay of the extract

The antioxidant activity test was performed using the DPPH method. The IC50 was calculated based on the equation obtained from the standard curve.

2.4 Preparation of chitosan edible film

The procedure for making edible films was based on the research by Purwanti [11] and Katili et al [12] with modifications. Two percent (v/v) chitosan (Pharma grade) solution was mixed with 2% glycerol (Sigma) solution as a plasticizer using a magnetic stirrer for 10-15 minutes and heated at 50-60°C. The edible film solution was added with 10% (v/v) purslane extract and stirred with a magnetic stirrer for 30-45 minutes at a temperature of 50-60°C. The mixed edible film solution was then poured on the surface of a petri dish with a thickness of ± 2mm and dried in an oven (Memmert) for ± 48 hours. For the control, edible film without purslane extract incorporation was prepared.

2.5 Application of chitosan edible film as mozzarella cheese packaging

The mozzarella cheese cuts (3 cm x 3 cm x 0.5 cm) were coated with the previously prepared edible film. Each treatment was carried out in 3 replicates. The packaged cheese were then divided into two different treatments, namely storage at 4°C and 27°C using incubators (Memmert). The analysis of microbiological and oxidative damage was carried out on day 0; 3; 6; 9 during storage. The appearance of the edible films was visually observed, including the color, texture, and aroma.
2.6 Total Bacterial and fungi count
The total bacteria and fungi were determined using the total plate count method. Ten grams of cheese sample was mashed and dissolved in 90 ml of peptone buffer (Merck) or PDB diluting media (HiMedia). The sample was then serially diluted to $10^{-3}$ dilution. Each 1 ml dilution of the sample was mixed with melted PCA or PDB medium (HiMedia) and then the mixture was poured into a petri dish. The buffer was used as a control. After incubation at a room temperature for 1-5 days, the colony growth was calculated in colony forming units per gram sample (cfu / g).

2.7 Thiobarbituric acid assay (TBA)
A total of 2 grams of cheese slice per treatment were mixed and mashed with 18 ml of 5% TCA (Merck). The mixture was filtered and centrifuged for ± 30 seconds. The supernatant of about 5 ml was taken and added with 5 ml of TBA (Merck) solution. Furthermore, the sample was heated for 30 minutes at a temperature of 90-100°C using a water bath, then cooled at a temperature of 20°C. The test is determined based on the measurement of the absorbance value of each sample using a spectrophotometer at a wavelength of 532 nm. The blank solution was prepared from a mixture of 5 ml of TBA solution with 5 ml of TCA solution.

2.8 Data Analysis
The data from each test were analyzed using SPSS software with Kruskall-Wallis non-parametric analysis. The analysis was carried out to determine if there was a significant difference/effect of the results of the data for each test on giving variations in the treatment of cheese.

3. Result and Discussion
Purslane extract had moderate to strong antibacterial and antifungal activities against microbes from the mozzarella as shown in Table 1. The antimicrobial activity of purslane extract increased with increasing concentration. Although it showed quite good antimicrobial activity, purslane extract showed low antioxidant activity as indicated by IC50 value of extract, which was much higher compared to IC50 of vitamin C (Table 2.). It is possible that the extraction process using 80% ethanal can not extract the active antioxidant compounds from purslane or the compounds are unstable in such a way that it requires sufficient preservation procedures to maintain its activity. Several antioxidant compounds have been identified in purslane, such as phenolic compounds, ascorbic acid and beta carotene, however the composition of these compounds varies depending on the stages of plant development, environmental conditions, harvest seasons, and extraction methods [13]. The differences in the sample or sample processing may result in different complex bioactive compounds. The types of antioxidant present were more responsible for the biological activity than their concentrations [14]. Different compounds may have different mechanisms of action. Subsequently, the DPPH method only measure antioxidants action to inhibit lipid peroxidation. Some phenolic compounds are good inhibitors of lipid peroxidation, while others are poor inhibitors [15].

Edible films with and without extract incorporation had significant differences in texture and color (Figure 1). The chitosan edible film with the incorporation of purslane extract texture was relatively smoother and more flexible than the chitosan edible film without the incorporation of purslane extract. The incorporation of purslane extract can affect the amount and density of chitosan bonds in the edible film, disrupt the bonds between the chitosan molecules as the base material and may reduce the elasticity of the film or decrease the bond density in the film [16].

| Sample          | Bacterial inhibition zone (mm) | Fungi inhibition zone (mm) |
|-----------------|-------------------------------|---------------------------|
| Positive control| 13,5                          | 14,5                      |
| Negative control| 0                             | 0                         |
| 80% Ethanol     | 2                             | 0                         |
| 10% extract     | 7                             | 5,5                       |
Table 2. Antioxidant activity of Purslane extract

| Sample              | IC50 (µg/ml) |
|---------------------|--------------|
| Vitamin C           | 12.57        |
| Purslane Extract    | 173.35       |

Figure 1. The appearance of edible film. Left: Chitosan edible film with Purslane extract added. Right: chitosan edible film.

The physical properties of chitosan edible film with the incorporation of purslane during storage showed no significant visible differences. The appearance of the color, texture, and aroma of the two edible films did not change over the storage period. The absence of visual changes indicates that the chitosan edible film with the incorporation of purslane extract meets one of the requirements as edible films, namely that they do not experience damage during the storage period of food products [17].

The incorporation of purslane extract into the edible film when stored at room temperature significantly inhibited the growth of microorganisms compared to using the edible chitosan film alone (Figure 2 and 3). However, in cold storage, the growth of microorganisms in all packed cheese were relatively similar, possibly due to the inhibition of microorganism growth by cold temperatures. The shelf life of food products stored at low temperatures is longer than those stored at room temperature, and a longer period of observation is needed for low temperature storage.

Figure 2. Total Bacteria (log CFU/g) of Mozzarella Cheese Packaged with Chitosan Edible Film and Chitosan Edible Film with the Addition of Purslane Extract on Day 0; 3; 6; and 9
The incorporation of antimicrobial compounds to edible films can affect the quality of food products in two ways [18]. First, the compounds reside in the edible film matrix and inhibit or prevent contamination of external food products. The second mechanism occurs when the edible film matrix comes in contact with food products and slowly transfers antimicrobial compounds to the food surface. Kalaycioglu et al. [19] showed that antimicrobial compounds in edible film pieces can spread out on the surface of agar inoculated by microorganisms. In this study, the antimicrobial mechanism of the edible film added with purslane extract was not observed.

The packaging using edible film also had a significant effect on the inhibition of TBA levels compared to no packaging (Figure 4.). Meanwhile, the incorporation of purslane extract treatment also had a significant effect on the inhibition of oxidative damage in mozzarella cheese synergistically with chitosan edible film, although purslane extract did not have very high antioxidant activity.

The suppression of MDA formation by chitosan edible film with the incorporation of purslane extract can be caused by the content of antioxidant in purslane extract. The phenol and alkaloid content of the porcelain extract binds to free radicals in food and prevents lipid oxidation reactions. This prevents rancidity in the cheese, which can change its taste and aroma. The microorganisms in mozzarella cheese
also play an important role in the formation of free radicals. The higher the number of microorganisms in mozzarella cheese, the more reactive oxygen species (ROS) are also formed. The ROS is a natural product resulting from the metabolism of organisms. The induction of ROS synthesis can form free radicals, which are highly reactive and affect the quality of food products [20]. Therefore, packaging with an edible chitosan film supplemented with porcelain extract inhibits the growth of microorganisms in mozzarella cheese, this packaging can also reduce the synthesis of free radicals by microorganisms in order to prevent oxidative damage.

4. Conclusion
In conclusion, it was shown that the use of an edible chitosan film with the incorporation of porcelain extract prevents damage to the mozzarella cheese during storage and can therefore be used as a substitute for plastic packaging. Further research is needed to see the effect of this packaging over a longer period of time and also consumer acceptance of the product packaged with edible film.

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