Innovation of fruit coating with antifungal yeast to maintain the quality of postharvest strawberries

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Abstract. Postharvest strawberries have a relatively short shelf life and the quality of fruit is rapidly declining, especially with a history of high gray mold fungus attacks during cultivation in the garden. The attack rate of fruit rot is high in the garden, causing the surface of strawberries to be colonized by pathogenic spores which results in strawberries becoming susceptible to postharvest disease. One of the main diseases of postharvest strawberries is gray mold rot caused by Botrytis cinerea. Fruit coating is one of the innovations used to extend shelf life and maintain the quality of fruit stored at room temperature. Coating of fruit with natural ingredients is still little researched, especially made from yeast. The aim of the study was to determine the effectiveness of coating postharvest strawberries with antifungal yeast at various levels of maturity on the quality and shelf life of strawberries. The results showed coating strawberries with yeast Debaryomyces Hansenii was able to maintain the quality of the fruit, increase the shelf life of the fruit, and protect the fruit from the attack of postharvest pathogens with parasitism mechanism, without reducing the quality of fruit, so that it can be applied after the harvest of strawberries.

1. Introduction
Strawberries are one of the horticultural plants that have high economic value, are rich in essential nutrients, vitamins and minerals [1, 2]. In East Java, many strawberries are cultivated in the Batu Malang area [3]. One of the important things in strawberry cultivation is postharvest handling. Strawberry fruit is easily damaged [4]. The loss of economic yield postharvest on strawberries is quite high, one of which is because aggressive pathogens colonize injured plants. Direct infection of the fruit occurs if the fruit is damaged due to physical abrasion by extreme environments, insect feeding activities, or other factors [5, 6]. Pathogen attack is one of the main factors causing a postharvest loss in fruits [7]. Pathogens that are often found to attack strawberry postharvest generally come from the mold group. Mold infection is a major cause of fruit postharvest damage. Mold groups can grow well on fruit even in low acidity conditions [8].

Botrytis cinerea is one of the pathogens that often infects strawberries during storage. Botrytis cinerea is the cause of gray mold disease with soft rot symptoms. This pathogen infects the fruit just before cooking. When the fruit is ripe, the cell wall will soften and the lenticel will easily open, making it easier for pathogens to penetrate and infection [7]. The coating of fruit with antifungal yeast aims to protect the fruit during storage from an attack of the postharvest disease. It also reduces oxygen entering the cell without causing fermentation and reduces evaporation of water from the cells that can extend the product's economic life [6, 9]. A natural coating of fruit using antifungal yeast has several advantages. Fruit yeast besides harmless is also able to produce various functional compounds that have a positive effect on the quality of Postharvest fruit. Yeast mainly inhibits the attack of fruit rot pathogens which commonly attack postharvest fruits [10, 11]. condition of the material used for coating is being
able to resist the permeability of oxygen and water vapor is colorless, harmless if consumed and does not cause changes in the nature of the food [12]. Some yeast strains are a strong inhibitor of the pathogens of gray mold in vitro testing, and significantly reduce the severity of fruit rot in vivo testing. Yeast has a high potential for sustainable strawberry production [13]. The development of natural edible coatings with the antimicrobial agent to minimally processed fruits and preserve fresh is a technological challenge and an active research field worldwide [14].

2. Materials and Methods

The study was conducted at the Biotechnology Laboratory of the University of Muhammadiyah Malang in March - July 2018. Strawberry fruit uses a local type obtained from the city of Batu. Fruit coating material in the form of yeast *Aureobasidium pullulans* and *Debaryomyces Hansenii* (density 10^9 cells/ml), which was isolated from healthy strawberries and identified molecularly.

The study was conducted using a factorial randomized block design, which consisted of two factors. Factor I: type of coating, consisting of negative controls (without yeast and pathogen coatings), positive controls (pathogen inoculation alone without yeast), yeast *Aureobasidium pullulans*, and yeast *Debaryomyces Hansenii*. Factor II: maturity phase of strawberries (50% red; 75% red; and 100% red).

Observations were made for 4 days of storage. Observations included the severity of gray mold fungus, the intensity of disease attacks, weight loss, firmness, total dissolved solids (TPT), vitamin C content, fruit storage time, and yeast interaction test with gray mold pathogenic fungi on the surface of the fruit strawberry. This test is carried out by means of healthy strawberries injured 5 mm wide. The wounded fruit area is coated with sterile cellophane. The suspension of pathogenic spores of 20 µl (10^8 spores/ml) was dropped on cellophane, then 20 µl of yeast cell suspension (10^9 cells/ml) were dropped on the same cellophane. incubated at room temperature in a moist plastic box for 48 hours. Five cellophane slices of each strawberry fruit sample were observed under a microscope with a magnification of up to 400 times. Microscopic observations were carried out on samples both before and after washing with running water containing 1% Tween 20.

Data are analyzed qualitatively and quantitatively. Qualitative data were analyzed descriptively, while quantitative data used variance analysis, to find out whether or not the effect of the treatment was proven using the F test. If the data obtained had a significant effect, then it was continued with the Duncan test 0.05.

3. Results and Discussion

3.1. Severity and Intensity of Disease Attack

The disease that is predominantly found attacking strawberries is gray mold caused by the pathogen *Botrytis cinerea*. The treatment of various types of coating has a very significant effect on the severity and intensity of the disease. The mean severity and intensity of disease attacks are presented in Table 1.

Table 1 shows that the coating of strawberries with yeast *A. pullulans* and *D. hansenii* can protect the fruit from the attack of fruit rot pathogens, but white yeast groups such as *D. hansenii* are more effective in protecting the fruit. Both yeasts do not cause the quality of the fruit to decline so that at the end of storage the fruit is still safe for consumption. In the treatment without coating yeast without pathogenic inoculation (K-) it turned out that the disease still appeared. This shows that the fruit orchard has been invested/infected with gray mold fungus spores. Yeast is effective in inhibiting the growth and development of diseases in Postharvest fruit because yeast is a good competitor of space and nutrition [15-17]. Yeast *D. hansenii* has the ability to release toxic volatile compounds [18] which inhibit the formation of pathogenic spore fungus sprout tubes.

Strawberry fruit coated with yeast *D. hansenii* looks healthier and fresher for longer. The attack of gray mold by *Botrytis cinerea* is characterized by the appearance of fungi on the fruit, initially, the fruit is moist and softened then the surface of the fruit is overgrown with gray fungus mycelium. On storage until the 7th day, it appears that gray ash mold fungus grows heavily on strawberries, while most of the fruit with *D. hansenii* yeast coating is still good not affected by gray mold (Figure 1).
Table 1. The average severity and intensity of gray mold for 4 days of strawberry fruit storage.

| Treatments of coating types                        | Severity (%) | Disease intensity (%) |
|---------------------------------------------------|--------------|-----------------------|
| Control - (without yeast and pathogen coatings)   | 11.07 b      | 19.00 c               |
| Control + (pathogen inoculation, without yeast)   | 33.97 c      | 30.67 d               |
| Aureobasidium pullulans                          | 8.40 b       | 11.00 b               |
| Debaryomyces hansenii                            | 2.47 a       | 2.67 a                |

| Treatment of strawberry fruit maturity level      |              |                       |
|---------------------------------------------------|--------------|-----------------------|
| 50% red                                           | 12.45 a      | 14.00 a               |
| 75% red                                           | 13.43 a      | 16.25 a               |
| 100% red                                          | 16.05 a      | 17.25 a               |

Numbers followed by the same letters in the column and the same treatment factors were not significantly different according to the Duncan test 0.05.

According to Hjeljord et al. [19], the conducive temperature to inhibit the growth of the postharvest disease is 10-15 °C. Another disease that attacks strawberries is *Rhizopus stolonifer*. Generally, the cause of the postharvest disease is a group of pathogenic fungi [20].

3.2. The Shelf Life of Strawberry Fruit
The maturity level of strawberries with fruit coating using antifungal yeast has a significant effect on the shelf life of strawberries. The average shelf life of strawberries is presented in Table 2.

Coating of strawberries with yeast *D. hansenii* at 50% maturity level can increase the shelf life of fruit for 6 days. In the treatment without yeast coatings, the shelf life lasts for 2-3 days. The increase in shelf life is due to the ability of *D. hansenii* yeast to inhibit the development of *Botrytis cinerea* pathogens that cause fruit rot and the optimal maturity level of strawberries [18].

3.3. Test of The Yeast Interaction Against Gray Mold Disease Pathogen
Microscopic observations showed that there was a very strong attachment of *D. hansenii* cells to the hyphae of *Botrytis cinerea* (Figure 2). The yeast cells of *D. hansenii* remain firmly attached to the pathogenic fungal hyphae despite washing with running water containing Tween 20. The attachment of *D. hansenii* cells to pathogenic hyphae causes hyphae damage. Hifa is unable to develop further, becomes vacuolar, disconnects and lysis. Hyphae which are parasitic to yeast cell *D. hansenii* are not able to produce spores, which means that in the next cycle there is a reduction in the number of inoculums. On the other hand, in samples that were not washed with running water containing Tween 20.
20, it appeared that all parts of the cellophane were packed with *D. hansenii* cells and formed a matrix covering the hyphae, resulting in stagnant hyphal growth. Yeast *D. hansenii* is also reported to be able to produce toxic volatile compounds that inhibit the growth and development of pathogens that cause fruit rot in some commodities such as apples, oranges, and chili [18].

**Table 2.** The average shelf life of strawberries is due to various types of coatings and the level of fruit maturity.

| Treatments of coating types | The maturity level of strawberries |
|-----------------------------|-----------------------------------|
|                            | 50% red | 75% red | 100% red |
| Control - (without yeast and pathogen coatings) | 3.6 c    | 3.7 c    | 3.3 c    |
| Control + (pathogenic inoculation, without yeast) | 2.6 b    | 1.9 a    | 2.0 a    |
| *Aureobasidium pullulans* | 3.4 c    | 4.6 d    | 3.6 c    |
| *Debaryomyces hansenii*   | 5.7 e    | 4.9 d    | 4.7 d    |

Numbers followed by letters are not significantly different according to Duncan's 0.05 test.

![Figure 2](image-url) **Figure 2.** The parasitism of *Botrytis cinerea* hyphae by *D. hansenii* cells. Y = yeast; H = hypha; Bar = 20 µm.

### 3.4. Quality of Strawberry Fruit

The maturity level of strawberries has a significant effect on the quality of fruit in storage. This is indicated by the observation of fruit weight loss, fruit color changes, fruit hardness, and total dissolved solids (TDS). The mean of each observation parameter is presented in Table 3.

Table 3 shows that the application of coating strawberries with *yeast A. pullulans* and *D. hansenii* does not affect fruit quality. The quality of strawberries is shown by weight loss, changes in fruit color, fruit hardness, and total dissolved solids as well as the negative control treatment. Changes in fruit quality are more influenced by the level of fruit maturity. In addition, the loss of fruit weight that occurs due to several factors including temperature and respiration rate. Factors that affect the rate of loss of water are the age or maturity of the fruit. Young fruit products have natural holes that still function in larger numbers and a simpler cuticle layer compared to full-aged products, so the rate of water loss can occur faster.

During storage, strawberry color changes. This color change shows that the fruit experiences the fruit ripening process, which is characterized by changes in white, pink, and then becomes dark red [1]. The color changes that occur during the cooking process are caused by chlorophyll in the degraded fruit so that the faded young colors are replaced by red because of the increasing number of carotenoids. Strawberry with 50% red maturity has higher hardness. This is due to the fact that the fruit is still dominated by green / young, showing that it is not perfectly cooked even though it is physiologically ripe. Analysis of total dissolved solids (TDS) showed the maturity of 100% red having higher TDS.
content. This is due to the process of cooking fruit, starch hydrolysis occurs into sugar, as a result, the content of TDS fruit increases gradually after harvesting during the ripening process [21].

Table 3. The average shrinkage of fruit weight, changes in fruit color, fruit hardness, and total dissolved solids for 4 days of storage.

| Treatments of coating types | Shrinkage weights | Fruit Color Change | Fruit hardness | Total Dissolved Solids |
|-----------------------------|-------------------|--------------------|----------------|------------------------|
| Control - (without yeast and pathogen coatings) | 2.08 a | 4.11 a | 1.95 a | 7.35 a |
| Control + (pathogen inoculation, without yeast) | 1.88 a | 4.13 a | 1.82 a | 7.46 a |
| Yeast *Aureobasidium pullulans* | 1.95 a | 4.17 a | 1.86 a | 7.28 a |
| Yeast *Debaryomyces hansenii* | 1.81 a | 4.12 a | 2.24 a | 7.78 a |
| Treatment of fruit maturity level | | | | |
| 50% red | 2.83 b | 4.04 a | 1.49 b | 7.06 a |
| 75% red | 1.71 a | 4.60 b | 1.38 ab | 7.34 b |
| 100% red | 1.80 a | 5.00 b | 1.27 a | 7.45 b |

Numbers followed by the same letters in the column and the same treatment factors were not significantly different according to the duncan test 0.05.

3.5. Content of Vitamin C

The level of fruit maturity has a very significant effect on the levels of vitamin C strawberries. The average fruit vitamin C contents at the beginning and end of storage are presented in Table 4.

Table 4 shows that before strawberries were stored (fresh condition was harvested), the fruit maturity level of 100% red had the highest vitamin C content. After being stored for 4 days, the content of vitamin C both in the type of coating treatment and the level of maturity of the fruit has decreased. So that it can be said that the consumption of strawberries is better in a fresh state before storage to get better quality. According to Bari *et al.*, [22] the more mature the fruit the higher the content of vitamin C in the fruit. The vitamin C content in the early maturity stage increases then decreases in the fruit that is stored until it is near rotten.

Table 4. The average content of vitamin C fruit strawberries for 4 days of storage.

| Treatments of coating types | Vit. C content (Early storage) | Vit. C content (Final storage) |
|-----------------------------|-------------------------------|-------------------------------|
| Control - (without yeast and pathogen coatings) | 9.61 a | 3.68 a |
| Control + (pathogen inoculation, without yeast) | 9.65 a | 3.89 a |
| *Aureobasidium pullulans* | 9.63 a | 3.87 a |
| *Debaryomyces hansenii* | 9.68 a | 3.97 a |
| Treatment of fruit maturity level | | |
| 50% red | 6.53 a | 3.61 a |
| 75% red | 11.22 b | 3.35 a |
| 100% red | 12.71 c | 3.47 a |

Numbers followed by the same letters in the column and the same treatment factors were not significantly different according to the duncan test 0.05.
4. Conclusion
Fruit coating with antifungal yeast *D. hansenii* is able to maintain fruit quality, increase the shelf life of strawberries, and protect against postharvest pathogens without reducing the quality of fruit so that it can be applied to postharvest strawberry fruit.

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