Determination of rice expiration time based on microbiological contaminant

Mulyana Hadipernata1,2, Nikmatul Hidayah1 and Sigit Nugraha1
1Center for Agricultural Postharvest Research and Development, Agency for Agricultural Research and Development, Indonesian Ministry of Agriculture, Bogor, Indonesia.
2Rice and Cereal Postharvest Laboratory, Center for Agricultural Postharvest Research and Development, Karawang, Indonesia.

E-mail: mulyana.hadipernata@litbang.pertanian.go.id

Abstract. Information on expiration time of rice on packaging labels is needed to provide quality assurance to consumers. The research aimed to find out the expiration time of rice from the microbiological aspect of rice. Rice samples used was Ciherang varieties. Rice was packed using 3 types of packaging, sacks, non-vacuum plastic and vacuum plastic. Rice was stored in two different places, air-conditioned rooms and room temperature. The results showed that mold and yeast contamination in Ciherang Rice up to 8 months’ storage are still below the required standard.

1. Introduction
Indonesia's rice production reaches 79.14 million tons of dry unhusked rice [1]. This rice production is sufficient for the consumption of Indonesian people. Occasionally, Indonesian government imports rice with the aim for government rice reserves and stabilizing rice prices. Not only the amount of production, but also the quality of rice began to come to the attention of the government and Indonesia people. At present there is a Minister of Agriculture Regulation No. 31 of 2017 concerning rice quality classes [2]. There are two quality classes, namely premium rice and medium rice. In addition, there are also specialty rice. Rice producers and the Indonesian people who initially did not pay much attention to rice quality classes, began to need high quality rice.

The decline in the quality of rice can be physical and biochemical damage, thus affecting the appearance of rice, changes in texture, taste and aroma of rice. The main factors causing damage during storage are water content, temperature and duration of storage, where water content is the most determining factor for rice damage [3]. An increase in temperature and water content during rice storage will result in chemical and biochemical changes including changes in the rice texture thereby accelerating the damage to the material and reducing its shelf life [4,5] Changes in the texture of soft (fluffy) rice to hard (dry) rice during storage are thought to be caused by an enzymatic biochemical reaction that results in breaking of the 1.6 β glucosidic chain link in amylopectin to a 1,4 α- glucosidic long chain of amylose by the enzyme 1,6 β amylase or pullulansae [6].

The expiration time of rice can give an overview to the producers and consumers of rice related to the quality of rice stored in a certain period so that it can provide the quality assurance on rice trading. Determination of rice expiration time are important for consumer protection and support the regulation issued by the government that was Indonesian Ministry of Agriculture regulation no. 38, 2018 [7]. The expiration time for rice are still not known clearly, that can be assessed based on physical quality aspects,
cooking quality, nutrition quality, or microbiological contaminant. The research aimed to find out the expiration time of rice based on microbiological aspect of rice.

2. Materials and Methods

The research was conducted in Karawang Laboratory of Rice Quality and Cereal Postharvest and Center for Agricultural Postharvest Research and Development at Bogor. Research activities on rice storage and packaging technology was carried out from January-December 2018.

The material research in this study using rice from Indonesian Rice Institute. The variety of rice was Ciherang. The chemicals used include methylene blue, eosin, ethanol, methanol and aquades. While the equipment used includes mini husker, mini polisher, moisture tester, miling meter, and laboratory equipment for analysis such as petri dishes, test tubes, test tube racks, 50 ml measuring flask, micropipette, 150 ml beak fiberglass, water bath, and centrifuge.

The determination of shelf life by empirical methods carried out at certain temperature conditions until the rice is damaged and unfit. Storage was carried out for 8 months. The experimental design used was a Completely Randomized Design with two treatment factors namely the type of packaging and storage temperature. The types of packaging used in this study consisted of three types, namely plastic sacks, non-vacuum polypropylene plastic bags, and vacuum polypropylene plastic bags, while storage temperatures used consisted of two levels, namely air conditioner temperatures (18-20 ºC) and room temperature (28-31ºC), Critical parameters used in determining the shelf life of rice are water content, and mold/yeast contamination.

Rice storage activity starts from the process of grinding rice into rice with a milling degree of 95%. Milled rice was sorted and sifted first, then packed and stored in a clean room at room temperature and cold temperature. A complete flow diagram of the process of storing rice is presented in Figure 5. Observation parameters made for rice after storage include physicochemical characteristics, cooking quality, food safety or microbiological quality and organoleptic properties. The physicochemical properties of rice observed included water content, head rice, broken rice, total other rice grains, degree of silence, density, level of hardness, starch, amylase/amylopectin, fat content, and minerals. For the observed cooking quality, consist of gel consistency, ratio of water absorption, and ratio of volume development ratio. While in terms of the quality of food safety observed, among others were mold/yeast contamination and insect contamination. The organoleptic properties of rice after storage include color, aroma and texture. The organoleptic properties of rice were tested using a hedonic test, in which the samples used were rice samples that had been cooked with 2 times the addition of water.

Testing of mold/yeast contamination from rice samples was carried out using a cup technique and the spread plate method was two replicated. A total of 1ml of sample dilution suspension was poured on the surface of the PDA media and flattened with the aid of a spreader glass. As a control used media that has been added to chloramphenicol and diluent solution. The petri dish was then incubated at 25C for 5 days. Calculation of the number of mold/yeast contamination colonies refers to the standards or regulations established by the Ministry of Health in 1992. Petri dishes are chosen from one dilution which shows the number of colonies between 40-60. The number of colonies from the two petri dishes is calculated then multiplied by the dilution factor.

3. Results and Discussion

The microbiological quality testing was carried out to determine changes in the microbiological quality of rice during storage, where the microbiological quality parameters tested included mold/yeast contamination in rice during storage. Test results for mold contamination during storage of Ciherang rice at room temperature and air conditioner temperature are presented in Figures 1 and 2.

Based on storage conditions, rice stored in air-conditioned rooms has the ability to withstand the rate of mold development compared to rice stored at room temperature. The mold contamination in Ciherang rice was tended to increase with the storage duration. After 8 months’ storage of mold contamination in Ciherang rice was in the range of 50-60 cfu/g. Overall, the results of testing, mold contamination on rice after 8 months’ storage are still below the required threshold standard for mold contamination.
### Table 1. Mold contaminant on rice for eight months’ storage at room temperature (28-31°C, relative humidity 65-75%).

| Packaging type       | Storage time (month) |
|----------------------|----------------------|
|                      | 0        | 1        | 2        | 3        | 4        | 5        | 6        | 7        | 8        |
| sacks                | 1x10^1   | 2x10^1   | 1.5x10^2 | 9x10^1   | 1x10^2   | 9x10^1   | 2x10^1   | 9x10^1   | 9.1x10^1 |
| non-vacuum plastic  | 1x10^1   | 2x10^1   | 9.5x10^1 | 9x10^1   | 4x10^1   | 5x10^1   | 6x10^1   | 9x10^1   | 9.2x10^1 |
| vacuum plastic       | 1x10^1   | 2x10^1   | 9x10^1   | 7x10^1   | 8x10^1   | 5x10^1   | 3x10^1   | 7x10^1   | 7.3x10^1 |

Based on the type of packaging used, rice stored using vacuum plastic packaging has the lowest mold value compared to rice stored in sack packaging and non-vacuum plastic packaging. By using vacuum packaging, oxygen conditions in the rice vacuum packaging will be limited, so that it will withstand the growth rate of microbes and mold.

### Table 2. Mold contaminant on rice for eight months’ storage at air conditioner temperature (18-20°C, relative humidity 52%).

| Packaging type       | Storage time (month) |
|----------------------|----------------------|
|                      | 0        | 1        | 2        | 3        | 4        | 5        | 6        | 7        | 8        |
| sacks                | 1x10^1   | 2x10^1   | 8x10^1   | 4x10^1   | 4x10^1   | 5x10^1   | 3x10^1   | 4x10^1   | 4.2x10^1 |
| non-vacuum plastic  | 1x10^1   | 2x10^1   | 1.1x10^2 | 5x10^1   | 4x10^1   | 1x10^1   | 3x10^1   | 3x10^1   | 3.1x10^1 |
| vacuum plastic       | 1x10^1   | 2x10^1   | 7x10^1   | 2x10^1   | 2.5x10^1 | 4x10^1   | 2.5x10^1 | 2x10^1   | 2.5x10^1 |

Yeast contamination in rice after 8 months of storage shows a value that exceeds the yeast contamination threshold. Ciherang rice stored at room temperature already shows a yeast threshold contamination after 7 months of storage (Figure 3). Based on SNI 7388: 2009 the mushroom contamination standard for whole cereal products is 104 colonies/g. According to BPOM the standard for yeast contamination for food products is 50-104 (cells g), while for yeast contamination is 50 (cells/g) (2004).

Based on the type of packaging used, vacuum packaged rice has the lowest yeast contamination value when compared to non-vacuum packaged rice and sack packaging. Rice that was packaged in plastic bags only has a shelf life of 5 months, whereas those that were stored using non-vacuum plastic packaging and vacuum plastic have a shelf life of 6 months and 7 months.

### Table 3. Yeast contaminant on rice for eight months’ storage at room temperature (28-31°C, relative humidity 65-75%).

| Packaging type       | Storage time (month) |
|----------------------|----------------------|
|                      | 0        | 1        | 2        | 3        | 4        | 5        | 6        | 7        | 8        |
| sacks                | <10      | <10      | 3x10^1   | 3x10^1   | 3x10^1   | 5x10^1   | 6x10^1   | 9x10^1   |
| non-vacuum plastic  | <10      | <10      | 3x10^1   | 3x10^1   | 3x10^1   | 3x10^1   | 3.2x10^1 | 6x10^1   | 7.9x10^1 |
| vacuum plastic       | <10      | <10      | 2x10^1   | 2.5x10^1 | 3x10^1   | 3.1x10^1 | 3x10^1   | 3x10^1   | 7x10^1   |

Plastic sacks have the highest thickness among other types of packaging, while PP vacuum packaging bags have a higher thickness than vacuum PP plastic bags, but the rate of water vapor transmission rates lower than vacuum PP plastic bags. For plastic sacks, the rate of transmission of water vapor cannot be
analyzed because of the specific structure of the density of the plastic sacks. Water vapor transmission rate (WVTR) is the amount of water vapor that passes through a surface area of unity or slope of the amount of water vapor divided by area. The value of water vapor transmission rate of a material is influenced by the structure of the forming material and the concentration of the plasticizer [8]. Moisture migration generally occurs in the hydrophilic film. The ratio between the hydrophilic and hydrophobic parts of the film component will affect the rate of transmission of the film's water vapor. The greater the hydrophobicity of the film, the value of the film's water vapor transmission rate will decrease. So it can be concluded also, the greater the hydrophilicity of the film, the value of the rate of water vapor transmission of the film will increase. The rate of transmission of water vapor determines the permeability of edible film vapor. Water vapor permeability (WVP) is the ability of a film to hold the rate of water vapor through it. Film permeability is influenced by different concentrations between one side and the other. The greater concentration difference, the faster mass transfer occurs. Permeability is also influenced by the thickness of the film. The higher concentration of dissolved solids, the thickness of the film will increase so that the ability of edible films to retain the lost moisture is increasing [9].

**Table 4.** Yeast contaminant on rice for eight months' storage at air conditioner temperature (18-20ºC, relative humidity 52%).

| Packaging type    | Storage time (month) |
|-------------------|----------------------|
|                   | 0        | 1        | 2        | 3        | 4        | 5        | 6        | 7        | 8        |
| sacks             | <10      | <10      | 3x10^1   | 4x10^1   | 3x10^1   | 4x10^1   | 4.5x10^1 | 9.2x10^1 |
| non-vacuum plastic| <10      | <10      | 2x10^1   | 1.3x10^1 | 2.3x10^1 | 3x10^1   | 4.3x10^1 | 6.9x10^1 |
| vacuum plastic    | <10      | <10      | <10      | 2x10^1   | 1.5x10^1 | 3x10^1   | 3.3x10^1 | 6x10^1   |

The results of organoleptic quality testing of Ciherang rice with the hedonic test method are presented in Tables 5 and 6. The observed organoleptic quality parameter is texture of rice after cooking with the same cooking process and water ratio. Test results on texture of Ciherang rice with various types of packaging after eight months of storage showed that in general rice was disliked by the panelists because of changes in the texture from soft had changed to hard.

**Table 5.** Hedonic test of rice texture for eight months' storage at room temperature (28-31ºC, relative humidity 65-75%).

| Packaging type     | Storage time (month) |
|--------------------|----------------------|
|                    | 0        | 1        | 2        | 3        | 4        | 5        | 6        | 7        | 8        |
| sacks              | 4.30     | 3.27     | 3.13     | 3.02     | 2.89     | 2.75     | 2.65     | 2.62     | 2.68     |
| non-vacuum plastic | 4.30     | 3.43     | 3.32     | 3.20     | 3.08     | 2.97     | 2.88     | 2.86     | 2.91     |
| vacuum plastic     | 4.30     | 3.66     | 3.58     | 3.48     | 3.39     | 3.28     | 3.23     | 3.16     | 3.19     |

* test result for cooked rice
1 = very unlike     3 = neutral 5 = very like
2 = unlike          4 = like

At the beginning of the storage of Ciherang rice, the evaluation scores were quite high above 4 in terms of texture parameter. After 8 months of storage based on texture parameter, the score for texture of Ciherang rice was in the range of values of 2-3, indicating that rice was no longer favored by panelists. In general, the score for the texture of Ciherang rice that stored at air conditioner room after 8 months of storage showed a higher value compared to Ciherang rice that stored at room temperature. Some of the results of previous studies show that the comparison of starch composition, especially amylose and amylopectin in rice, determines the color (transparent or not) and the texture of rice (sticky, soft, hard). Sticky rice is almost completely dominated by amylopectin so it is very sticky, while hard rice has an amylose content exceeding 20% which makes the rice grains scattered (not attached) and hard [10].
Table 6. Hedonic test of rice texture for eight months' storage at air conditioner temperature (18-20ºC, Relative humidity 52%).

| Packaging type       | Storage time (month) |
|----------------------|----------------------|
|                      | 0        | 1        | 2        | 3        | 4        | 5        | 6        | 7        | 8        |
| sacks                | 4.30     | 3.36     | 3.24     | 3.13     | 3.01     | 2.88     | 2.78     | 2.75     | 2.79     |
| non-vacuum plastic   | 4.30     | 3.72     | 3.64     | 3.57     | 3.49     | 3.40     | 3.35     | 3.34     | 3.39     |
| vacuum plastic       | 4.30     | 3.91     | 3.86     | 3.80     | 3.73     | 3.67     | 3.64     | 3.60     | 3.61     |

* test result for cooked rice  
1 = very unlike  
3 = neutral  
5 = very like  
2 = unlike  
4 = like

4. Conclusion.
Ciherang rice has not reached its expiration time until eight months of storage, but based on yeast contamination, Ciherang rice has expired in the seventh month of storage.

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