Production of Mozzarella Cheese Using Rennin Enzyme from 
*Mucor miehei* Grown at Rice Bran Molasses Medium

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Abstract. The research aimed to study the characteristic and yield of Mozzarella cheese produced by using rennin enzyme from *Mucor miehei* which is grown at rice bran and molasses medium. The popularity of Mozzarella cheese in Indonesia is increased caused by the spreading of western foods in Indonesia such as pizza and spaghetti that use Mozzarella cheese for ingredient. In Italy, Mozzarella and pizza cheeses are dominating 78% of the total Italian Cheese products. In producing Mozzarella cheese, rennin enzyme is always used as milk coagulant. Even now, Indonesia has not produced the rennin enzyme yet. The rennin enzyme from *Mucor miehei* growing at rice bran and molasses medium which have the availability can be managed purposively within short period of time. The completly randomized design methode used to get the best crude extracts of *Mucor miehei* rennin enzyme, then is employed to produce mozzarella cheese. The result of Mozzarella cheese has various characteristics such as the yield’s weight is 9.1%, which consists of 50% moisture content, 36.64% protein levels, 0.1 melting ability and 82.72% stretch ability or 0.79/N. With that characteristic it is concluded that rennin enzyme from *Mucor miehei* grown at rice bran molasses medium has the potential to alternatively substitute calf rennin to produce Mozzarella cheese, and the characteristics fulfill the standart.

1. Introduction

Mozzarella cheese is originally manufactured from high-fat bufallo milk. It is a soft, unripened cheese variety of the Pasta –filata family whose origin is from the Battipaglia region of Italy [1]. In the other European countries and the U.S., Mozzarella cheese is also made from cow milk with certain modification [2].

The recent increasing in global cheese production and the scarcity oncalf rennet which is usually obtained from the fourth stomach of suckling calves, become the driving force to look for some new rennet substitutes. In the recent decades, due to a shortage of calf rennet in world markets, alternative milk-clotting enzymes from different origins have been investigated [3, 4]. *Mucor miehei* is preferred by the industry to produce milk-coagulating enzyme because of its high R factor (milk-clotting activity/proteolytic activity), especially for certain peptide bonds in casein, good cheese quality, and yield [5,6].

Solid State Fermentation (SSF) technique is one of the recent techniques particulary suitable for milk-clotting enzyme production [7, 8], while rice bran and molasses have the needed nutrients [9,10].
to grow *Mucor miehei*. This work is conducted to study the characteristic and yield of Mozzarella cheese produced by using rennin enzyme from *Mucor miehei* which is grown at rice bran and molasses medium.

2. Material and Method

2.1 Material

Fresh cow milk was obtained from the village unit cooperative Junrejo, Malang. *Mucor miehei* was obtained from the chemical laboratory of Universitas Brawijaya. The fungal culture was periodically subcultured and maintained in potato dextrose agar medium. Molasses was obtained from Tasikmadu’s sugar cane factory, Karanganyar, Central Java. Rice bran was obtained from the local market Dinoyo, Malang, East Java.

2.2 Method

This research used completely randomized design method, there are 2 factors and repeated three times. The factors are additional molasses concentration, and incubation time. The data analysed by ANOVA (Analysis of Variance) method and continued with posthoc test used least significance different (LSD) test 5%.

2.2.1 Growing Medium and Condition

This work employed SSF (Solid State Fermentation) system as the growing medium, which is 5 ml spore suspension of *Mucor miehei* was inoculated in petridish containing 1:1 of sterilized rice bran and molasses medium. Molasses was diluted by using 10% of aquadest, and added by 2% of casein. And incubated at 37°C for 96 hours.

2.2.2 Extraction of Rennin Enzyme *Mucor miehei*

After the incubation period, solid state fermentation medium was extracted by using destilled water (1:10 w/v) and 5 ml of tween 80, in low speed blender for 5 minutes. The obtained filtrate was filtered by using filter paper, and centrifuged at 4000 rpm for 20 minutes at 4°C. The supernatant was filtered again with whatman 42 paper and used as crude enzyme source to produce Mozzarella cheese. The obtained enzyme has 21,946 unit/ml/minute of proteolytic activity and has 5,637.251094 unit/mg protein/minute of milk-clotting activity.

2.2.3 Production of Mozzarella Cheese [11]

Firstly, cow fresh milk was pasteurized at 72°C for 5 minute, then cooled down until the milk’s temperature was 40°C. Rennin enzyme from *Mucor miehei* with 0.55% (v/v) and 0.5% (v/v) concentration of citric acid was added to the pasteurized milk, and incubated at 43°C for 5 minutes (or until the curd can be cut). It was then cut and left undisturbed for 15 minutes at 43°C until the curd was separated from the whey, and the whey was separated from the curd. After that, the curd was collected in the bowl. Hot water (75°C) was added to the curd in a bowl, then the curd was mixed and kneaded by hand until homogenous paste was obtained. The curd was soaked into ice water for 1 hour, then soaked into saturated brine for 15-20 minutes. Finally the Mozzarella cheese was made.

2.2.4 Cheese Evaluation

After the Mozzarella cheese was obtained, the amount of yield is examined by measuring the cheese’s weight. The moisture content was determined by AOAC [12]. The total protein content was determined by measuring the total nitrogen by using the Kjeldahl method [13]. The meltability was determining by using Tunick et al method [14], and the stretchability was determined by using Kuo and Gunasekaran method [15].
3. Result and Discussion
Rennin enzyme from *Mucor miehei* obtained from this research does not belong to a good milk-clothing activity yet. However, when *Mucor miehei*’s enzyme is supported by even a little bit of commercial enzymes it could produce a better yield of cheese than a pure commercial rennin. There is a possibility of synergy between commercial rennin and rennin enzyme from *Mucor miehei*. The commercial renet used in this research to support the clotting activity is Marzyme Supreme, the double microbial-strain rennin manufactured by a USA company called Danisco. The equation used in producing *Mozzarella cheese* is 0.5% of renin enzyme from *Mucor miehei* and supported by 0.01% commercial rennin. The Mozzarella cheese which is employed for comparison (the control cheese) uses 0.25% of the commercial enzyme, according to the company rules.

3.1 Yield Percentage of Mozzarella Cheese
The comparison between the yield percentage of the Mozzarella cheese sample which is repeated three times (R1, R2 and R3) and the control cheese are presented in figure 1. The cheese yield is calculated from 100 gram of cheese per liter of milk times 100%. It is obtained that the average yield percentage of Mozzarella cheese produced by *Mucor miehei*’s rennin (9.1%) is higher than Mozzarella cheese produced by commercial rennin (5%).

3.2 Moisture Percentage in Mozzarella Cheese
The result in figure 2 shows the moisture presentage in Mozzarella cheese. The average value of Mozzarella cheese produced by *Mucor miehei*’s rennin (50.04%) is not significantly different from Mozzarella cheese produced by commercial rennin (49.93%).

![Figure 1. Mozzarella cheese yield](image-url)
The percentage of moisture level from both of the products still fulfill the Mozzarella cheese moisture standard according to USDA specification for Mozzarella cheeses (2012) which mentions that the moisture content in Mozzarella cheese should be more than 45% but not more than 52%.

3.3 Protein Percentage in Mozzarella Cheese

The average of protein content from Mozzarella cheese produced by *Mucor miehei*’s rennin (36.64%) is lower than the protein percentage of the Mozzarella cheese produced by commercial rennin (44.7%). The data is showed at Figure 3.

The lower value of protein content of Mozzarella cheese produced by *Mucor miehei*’s enzyme probably because of high proteolytic activity in rennin enzyme from *Mucor miehei* grown at rice bran and molasses medium.
3.4 Meltability in Mozzarella cheese

Meltability in Mozzarella cheese is one of its important functional features. The comparison of meltability between Mozzarella cheese sample which is repeated three times and the control cheese are presented in figure 4. The data shows that the meltability of the Mozzarella cheese produced by *Mucor miehei*’s rennin is not significantly different from the control cheese. All of them have meltability no more than 10 mm, or have the meltability value of 1.0

![Figure 4. Meltability of Mozzarella cheese](image)

The meltability of cheese is defined as the ease and extent of cheese to melt and spread upon heating [16]. It is found that the melting area is determined by the fat and moisture content [17]. The low meltability in this research might be caused by the low fat content in both of Mozzarella. According to Alexandra et al [18], solid state fermentation of *Mucor miehei* has the highest lipolytic activity compared to the other Mucoromycotina strain. It makes this rennin enzyme have the capability to divide lipid in cheese and decrease the lipid content significantly.

3.5 Stretchability in Mozzarella cheese

The comparison between the stretch ability of the cheese sample and the control cheese are presented in figure 5. Stretch ability percentage of Mozzarella cheese produced by *Mucor Miehe’s* rennin is higher than Mozzarella cheese produced by commercial rennin or control. This is based on Kuo and Gunasekaran [15] who report that stretch ability for pasta filata ranges between 0.5- 1.2 1/N, and the average of 1/N value obtained from this research is 0.79/N or 82.72%.

The functional quality of Mozzarella cheese is defined by its ability to melt and stretch, so the value of melt ability and stretch ability are important.

4. Conclusion

In short, Mozzarella cheese produced by rennin enzyme from *Mucor miehei* has better yield percentage (9.1%), protein percentage (36.64%), and stretchability (82.72% or 0.79/N), but the value in the moisture content (50%) and meltability (0.1) are not significantly different. It is concluded that rennin enzyme from *Mucor miehei* grown at rice bran molasses medium has the potential to alternatively substitute calf rennin to produce Mozzarella cheese, and the characteristics fulfill the standart. The low fat content of the product may even be considered as a benefit for those who prefer low fat diet.
Figure 5. Strechability of Mozzarella cheese

Acknowledgement
The author wishes to thank Dr. Joni Kusnadi, Universitas Brawijaya, and Indofood Research Nurgraaha program for the assistance and financial support in this research.

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