Improvement of yogurt quality from mushrooms and nuts by the addition of Amyloproteolytic from *Lactobacillus satsumensis* EN38-32

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Abstract. Yogurt made from the vegetable is good for human health. This study focused on improving the quality of yogurt from mushrooms and nuts with the addition of amyloproteolytic enzymes from *L. satsumensis* EN38-32. Pasta flour juice from oyster mushroom (*Pleurotus ostreatus*), button mushroom (*Agaricus bisporus*), soybean (*Glycine soja*), and mung bean (*Vigna radiata*) were used to make yogurt with milk yogurt as a comparison. The α-amylase activity was measured by the DNS method, while the protease activity was measured by formol titration. Reducing sugar was detected by the Bernfeld method, while degraded protein by titration. The AOAC method measured total acid and proximate analysis. The results showed that the increase in reducing sugar and the increase in total acid in oyster yogurt, button yogurt, and soygurt with and without the addition of amyloproteolytic enzyme from *L. satsumensis* EN38-32 was lower than that in mung bean yogurt with each value of 0.0169% and 0.0770% (p <0.01). Carbohydrate (11.30%) and protein (1.12%) in oyster yogurt were higher than button yogurt, while carbohydrate (13.95%) and protein (3.15%) in soygurt were higher than in mung bean yogurt. Based on its nutritional content, soygurt was the best yogurt compared to the others.

1. Introduction

Edible mushrooms and other protein sources such as nuts can be used to substitute for cow's milk. Pasta flour from mushrooms and nuts, which was eaten as non-dairy pasta flour, was used as a base for various food products made from mushrooms and nuts, such as yogurt with ingredients mixed with mushrooms and nuts to produce mushroom yogurt and peanut yogurt [1,2]. However, the pasta flour quality from mushrooms and nuts was not as good as the quality of powdered cow's milk and cow's milk pasta. This is because the protein in mushrooms and nuts does not contain casein, which is a major protein in milk [2,3].

According to Khusniati *et al.* [4], pasta flour from mushrooms and nuts showed it had reducing sugars contents values in the range of 0.0177-0.0908% and degraded protein content in the range 0.3100-4.5200%, while the powdered cow's milk had reducing sugar content about 0.0543% and degraded protein content value of 2.2800%. Furthermore, yogurt production from juice extracted from paste flour of mushrooms and nuts showed it had reducing sugars contents values in the range of 0.0143-0.0357% and the acidity content in the range 0.0730-0.6480%. In comparison, the powdered
cow’s milk had a reducing sugar content of about 0.0615% and an acidity content value of 0.8730%
[4]. The starters used in vegetable yogurt production were Lactobacillus bulgaricus and Streptococcus
thermophilus, which were the starters in making milk yogurt [1].

Lactic Acid Bacteria (LAB) produced amyloproteolytic enzymes [5-8]. These amyloproteolytic
enzymes consisting of α-amylase catalyze amylose in starchy carbohydrates to glucose and maltose [5-
7] and proteases that hydrolyze flour proteins into peptides [8,9]. The enzymes make flour
homogenized, and the flour is more digested [6-8].

Lactobacillus satsumensis EN38-32, as indigenous lactic acid bacteria, produced amyloproteolytic
enzyme [10,11]. The use of this enzyme in pasta flour of mushroom and nuts to produce a more
homogeneous pasta flour so that better juice from the pasta flour was obtained and juice used as a
basic material in vegetable production yogurt had not been reported. The L. satsumensis EN38-44
amyloproteolytic enzymes addition to the pasta flour from mushrooms and nuts aims to produce more
homogeneous pasta flour and increase the quality of extracted juice from the pasta flour. The quality of
the yogurt made from the juice of mushrooms and nuts will then improve. This study focused on
"Improvement of yogurt quality from mushroom and nuts by the addition of amyloproteolytic enzyme
from Lactobacillus satsumensis EN38-32", especially in reducing sugars, total acid, and proximate
analysis

2. Materials and Methods

2.1. Material used
The material used were (1) Edible mushrooms used: commercial oyster mushrooms (Pleurotus
ostreatus) and button mushrooms (Agaricus bisporus) in Bogor city, and (2) Nuts used: commercial
powder of soybeans (Glycine soja) and mung beans (Vigna radiata) in Bogor (3) Milk: milk cow
powder produced by Dancow Fortigro with nutritional compounds in 27 g powder milk product of
carbohydrate (13 g), protein (5 g), lipid (6 g), and energy total (120 kcal) was used as a comparison.

2.2. Lactic Acid Bacteria (LAB) used
The indigenous Enggano Island LAB amylproteolictic, Lactobacillus satsumensis EN38-32, was
used to make pasta flour. Meanwhile, Lactobacillus bulgaricus and Streptococcus thermophilus were
used as the yogurt starter.

2.3. Growth media of lactic acid bacteria (LAB)
The growth media of Lactic acid bacteria was MRS media. MRS (de Mann Rogosa Sharpe) media
used as LAB production media consisted of 1% peptone, 0.8% beef extract, 0.4% yeast extract, 1%
glucose, 0.1% tween 80, 0.5% sodium acetate, 0.2% triammonium citrate, 0.02 % magnesium sulfate
monohydrate, 0.005% manganese sulfate tetrahydrate, and 0.2% disodium hydrogen phosphate
dihydrate. The MRS medium was mixed in 200 ml distilled water, then homogenized using a magnetic
stirrer at high temperature. The medium was then autoclaved at 121°C for 15 min.

2.4. Production of the amylproteolytic enzyme of Lactobacillus satsumensis EN38-32
Production of α-amylase and protease enzymes from L. satsumensis EN38-32 (amylproteolytic
enzyme of L. satsumensis EN 38-32) were carried out by growing 2% L. satsumensis EN 38-32 into 25
mL sterile MRS Broth media, incubated for 24 hours at temperature 37°C. The amylproteolytic
enzyme of L. satsumensis EN 38-32 was obtained using a centrifuge at 9000 rpm, 10 minutes at 4°C
[12,13]. The amylproteolytic enzyme of L. satsumensis EN 38-32 was in the form of a supernatant,
whereas cell biomass was in pellets.

2.5. Activity analysis of α-amylase and protease from L. satsumensis EN38-32 amylproteolytic
enzyme
The activity of α-amylase was calculated using the modified Miller [9,10], while protease activity was
calculated using the modified method of Horikoshi [10,11]. One unit of α-amylase activity was defined
as the number of enzymes in which the reaction produced a product equivalent to 1µmol glucose per minute under measurement conditions. Therefore, one protease unit was defined as the number of mL proteases needed to produce 1 µmol tyrosine per minute with casein as a substrate.

2.6. Reducing sugar analysis
Reducing sugar (%) was determined using the DNS (dinitrosalicylic) method [14]. Mushroom and nuts paste flour with and without adding the amylolproteolytic enzyme of L. satsumensis EN 38-32 were centrifuged at 9000 rpm for 10 min at 4°C. 100 µl Amylproteolytic enzyme L. satsumensis EN 38-32 was added with 100 µl DNS reagent, homogenized, and boiling at 100°C for 5 min. 800 µl distilled water was then added to the mixture and homogenized, the absorption of the samples was analyzed using a spectrophotometer at 560 nm. The method was repeated for the standard solution. The results obtained were plotted in a linear curve.

Reducing sugar content (%) = \[ \frac{[\text{glucose concentration (mg/mL)}] \times \text{reaction total volume (mL)} \times 100\%}{[\text{Sample weight (mg)}]} \] (1)

2.7. Analysis of degraded protein content
Determination of degraded protein concentration was done by formol titration [9]. 10 mL of sample was added with phenolphthalein and neutralized with 0.1 N NaOH solution. The solution was added 10 mL 38% formaldehyde solution and titrated with 0.1 N NaOH standard solution until a pink colour change.

\[ \% \text{ Degraded protein} = \% \text{ Nitrogen} \times \text{Conversion factor} \] (2)

2.8. Vegetable yogurt production process
Pasta flour was made using 1 gram nuts powder and 1 gram blended mushroom, then add with 10 mL water. The mixture then boiled at 70°C to form the pasta. Pasta flour with and without amylolproteolytic enzymes was incubated using a shaker incubator at 37°C for 24 hours, filtered with a sterile gauze cloth to obtain juice extracted from pasta flour. The juice was heated, added 10% sugar and stirred until dissolved, and then added 5% skim milk with stirring to be evenly mixed. The mixture was heated again at 85°C for 10 minutes, then cooled until the temperature reached 45°C. The yogurt starter was put into the mixture and incubated for 5 hours at 45°C. The resulting yogurt was kept at a cold temperature [1].

2.9. Titratable acidity content
The 10 mL of yogurt samples were put into an Erlenmeyer to be titrated with 0.1 N NaOH. The phenolphthalein indicator was used to evaluate the change from colourless to pink, and 0.1 N NaOH was standardized using oxalic acid (COOH) 2 0.1 N [15]. Titratable acidity expressed as lactic acid as follow:

\[ \% \text{ Lactic Acid} = \frac{[\text{NaOH volume used (mL)} \times \text{NaOH concentration} \times 90]}{[\text{Volume sample analyzed (mL)}]} \times 100\% \] (3)

2.10. Proximate analysis
Proximate analysis was detected by the AOAC method [15]. Proximate analysis of yogurt-based on mushroom and nuts paste flour consists of carbohydrate, protein, fat, water, and ash contents.

2.11. Statistical analysis
Data were statistically analyzed by using Analysis of Variance (ANOVA) with three replications. The significant difference in the data was further analyzed by the Duncan method.
3. Results and Discussion
The Amyloproteolytic enzyme from *L. satsumensis* EN 38-32 was used to make mushroom and nuts pasta flour. The activity of α-amylase and protease of amyloproteolitic enzyme from *L. satsumensis* EN 38-32 showed in Table 1. The activity of *L. satsumensis* EN 38-32 α-amylase with a value of 0.4545 U/mL was lower than that of *L. satsumensis* EN 38-32 amylase with a value of 0.5000 U/mL. The lower α-amylase activity compared to protease was due to the different types of enzymes produced. It has been reported that the various types of enzymes affected the activity of the enzyme produced [7-9].

**Table 1. Activity of Amyloproteolytic enzyme of *L. satsumensis* EN 38-32**

| Activity of Amyloproteolytic enzyme | (U/mL) |
|-------------------------------------|--------|
| α-Amylase*                          | 0.4545a|
| Protease**                          | 0.5000b|

Note: * Temperature of optimum incubation 45°C, pH 5.5 [10]  
**Temperature of optimum incubation 40°C, pH 7 [11]

The increase in reducing sugar content in pasta flour from oyster mushroom (0.0278%), button mushroom (0.0208%), and soybeans (0.0908%) with and without the addition of Amyloproteolytic enzyme from *L. satsumensis* EN38-32 was lower than the powdered cow’s milk used as a comparison, whereas in mung bean paste flour (0.1076%), the increase in reducing sugar content was the same as powdered milk (p<0.05) (Table 2). The increase in reducing sugar content in oyster mushroom pasta flour with and without the addition of enzyme was the same as in button mushroom, and the increase in reducing sugar content in soybeans pasta flour with and without the addition of enzyme was lower than in mung bean (p<0.05). The increase in reducing sugar content in the pasta flour of mushroom and nuts with and without the addition of Amyloproteolytic enzymes was shown in table 2.

The increase in reducing sugar content in pasta flour from oyster mushrooms, button mushrooms, and soybeans with and without the addition of Amyloproteolytic enzymes compared to powdered cow’s milk (table 2) was caused by the different α-amylase activity in the paste flour in hydrolyzing amylose into glucose. The increase in reducing sugars in powdered cow's milk showed the highest value compared to mushrooms and nuts because powdered cow's milk contains amylose and lactose hydrolyzed glucose, respectively. In contrast, mushrooms and nuts contain only amylose. Mushrooms, nuts, and powdered cow's milk were reported to have different nutritional content [16-18].

**Table 2. Increase in reducing sugar content of mushroom and nuts pasta flour without and with addition Amyloproteolytic enzyme from *L. satsumensis* EN 38-32**

| No | Type of paste flour | Reducing sugar content (%) without addition enzyme | Content (%) with addition enzyme | Increase in Reducing sugar (%) |
|----|---------------------|-------------------------------------------------|---------------------------------|--------------------------------|
| 1  | Oyster mushroom     | 0.0177                                          | 0.0455                          | 0.0278b                        |
| 2  | Button mushroom     | 0.0242                                          | 0.0450                          | 0.0208b                        |
| 3  | Soybean             | 0.0660                                          | 0.1568                          | 0.0908a                        |
| 4  | Mung-bean           | 0.0908                                          | 0.1984                          | 0.1076f                        |
| 5  | Powdered cow’s milk as comparison | 0.0543 | 0.1985 | 0.1442c |

Note: * Khusniati et al. [4]; Mean value followed by the same letter in the same column was not significantly different according to Duncan's test at the 5% significance level (p<0.05)
The increase of degraded protein content in oyster mushroom pasta flour with and without the addition of amylproteolytic enzymes from *L. satsumensis* EN38-32 (2.6900%) was higher than powdered cow’s milk. In contrast to pasta flour from button mushroom (0.0700%), soybean (0.2500%) and mung bean (0.0400%) was lower than powdered cow’s milk (p<0.05) (table 3). The increase in degraded protein content in oyster mushroom pasta flour with and without the addition of enzymes was higher than in button mushroom. On the contrary, the increase in degraded protein content in soybean paste flour was higher than in mung bean (p<0.05) (table 3). The increase in degraded protein content in the pasta flour of oyster mushrooms, button mushrooms, soybeans, mung beans, and cow’s milk powder with and without the addition of enzyme was shown in table 3.

**Table 3. Increase in degraded protein content of mushrooms and nuts pasta flour without and with addition amylproteolytic enzyme from *L. satsumensis* EN 38-32**

| No | Type of paste flour | Degraded protein content (%)* | Increase in degraded protein (%) |
|----|---------------------|-------------------------------|--------------------------------|
| 1  | Oyster mushroom     | 1.3000                        | 2.6900b                        |
| 2  | Button mushroom     | 0.3100                        | 0.0700a                        |
| 3  | Soybean             | 4.5200                        | 0.2500d                        |
| 4  | Mung-bean           | 3.8700                        | 0.0400e                        |
| 5  | Powdered cow’s milk as comparison | 2.2800 | 0.5200b |

Note: * Khushniati *et al.* [4]; Mean value followed by the same letter in the same column was not significantly different according to Duncan's test at the 5% significance level (p<0.05)

The increase of degraded protein showed oyster mushroom pasta flour was higher than powdered cow’s milk (table 3) due to the higher protein content in oyster mushroom than in powdered cow’s milk. The lower increase of degraded protein in the pasta flour of button mushroom, soybean, and mung bean compared to powdered cow’s milk (table 3) showed lower protease activity in protein degradation in all three pasta flour compared to powdered cow’s milk. It was reported that the protein content and protease activity of the materials used in the production of pasta flour affected the degraded protein in pasta flour [16-18].

An increase in reducing sugar content in yogurt from the juice of flour paste from oyster mushroom (0.0050%), button mushroom (0.0016%), and soybean (0.0021%) with and without the addition of the Amylproteolytic enzyme of *L. satsumensis* EN38-32 was lower than the powdered cow’s milk yogurt used as a comparison, while the soygurt made from the juice of mung bean paste flour (0.0169%) was higher than that of powdered cow’s milk yogurt (p<0.01) (table 4).

**Table 4. Increase in reducing sugar content of yogurt from mushrooms and nuts without and with addition amylproteolytic enzyme from *L. satsumensis* EN 38-32**

| No | Type of paste flour | Reducing sugar content (%)* | Increase in reducing sugar (%) |
|----|---------------------|----------------------------|--------------------------------|
| 1  | Oyster mushroom     | 0.0143                      | 0.0050a                        |
| 2  | Button mushroom     | 0.0150                      | 0.0016a                        |
| 3  | Soybean             | 0.0297                      | 0.0021b                        |
| 4  | Mung-bean           | 0.0357                      | 0.0169c                        |
| 5  | Powdered cow’s milk as comparison | 0.0615 | 0.0117d |

Note: * Khushniati *et al.* [4]; Mean value followed by the same letter in the same column was not significantly different according to Duncan's test at the 5% significance level (p<0.05)


The increase in reducing sugar content in yogurt from the juice of oyster mushroom pasta flour (oyster yogurt) with and without the addition of enzyme was the same as in yogurt from button mushroom pasta flour (button yogurt). The increase in reducing sugar content in yogurt from the juice of soybean paste flour (soy yogurt) in the same treatment was lower compared to yogurt from the juice of mung bean paste flour (mung bean yogurt) (p<0.05) (table 4). The increase in the reducing sugar content of mushrooms yogurt, nuts yogurt and milk yogurt with and without Amyloproteolytic enzyme was shown in table 4.

The lower increase of reducing sugar content in juice-based yogurt from pasta flour from oyster mushrooms, button mushrooms, soybeans compared to powdered cow’s milk yogurt (table 4) indicated that the amylase activity in the degradation of carbohydrates in the three yogurts was lower than that of powdered cow’s milk yogurt. The higher increase in reducing sugar content in mung bean yogurt compared to milk yogurt was due to the higher amylase activity in hydrolyzing amyllose in mung bean pasta flour than in powdered cow’s milk. It was reported that the carbohydrate content and amylase activity in the degradation of carbohydrate from the materials used in making of yogurt affected increasing the reducing sugar content of the yogurt produced [19-21].

Increase in total acid content in oyster yogurt (0.0310%), button yogurt (0.0230%), soy yogurt (0.0630%), and green bean yogurt (0.0770%) with and without the addition of amylproteolytic enzymes from Lactobacillus satsumensis EN38-32 was higher than milk yogurt used as a comparison (p<0.05) (table 5). The increase in total acid content in oyster yogurt with and without the addition of enzyme was the same as in button yogurt. In contrast, the increase in total acid content in soy yogurt with and without the addition of enzyme was lower than mung bean yogurt (p<0.05) (table 5). The increase in total acid content in mushrooms yogurt, nuts yogurt, and milk yogurt used as a comparison, with and without the addition of Amyloproteolytic enzyme, was shown in table 5.

The higher increase in total acid content in mushrooms and peanut yogurt than milk yogurt (table 5) was due to the reduced sugar content in the pasta flour from mushrooms and nuts, which was higher than in the reducing sugar content in powdered cow's milk. Therefore, the organic acids resulting from the reducing sugar catalysis in mushroom and peanut yogurt were higher than in milk yogurt. It was reported that the higher content of reducing sugar catalyzed into organic acids from the materials used in the making of yogurt affected the increase in the total acidity of the yogurt produced [19-21].

### Table 5. Increase in acidity content of yogurt from mushrooms and nuts without and with addition amylproteolytic enzyme from L. satsumensis EN 38-32

| No | Type of paste flour | Acidity without addition enzyme* | Content (%) with addition enzyme | Increase in Total acid (%) |
|----|---------------------|----------------------------------|---------------------------------|---------------------------|
| 1  | Oyster mushroom     | 0.0730                           | 0.1040                          | 0.0310^a                  |
| 2  | Button mushroom     | 0.1400                           | 0.1630                          | 0.0230^a                  |
| 3  | Soybean             | 0.4230                           | 0.4860                          | 0.0630^b                  |
| 4  | Mung-bean           | 0.6480                           | 0.7250                          | 0.0770^c                  |
| 5  | Powdered cow’s milk as comparison | 0.8730                           | 0.8950                          | 0.0220^d                  |

Note: * Khusniati et al. [4]: Mean value followed by the same letter in the same column was not significantly different according to Duncan's test at the 5% significance level (p<0.05)

The proximate analysis of oyster yogurt, button yogurt, and milk yogurt with the addition of amylproteolytic enzyme from L. satsumensis EN38-32 was shown in table 6. The carbohydrate and protein content in oyster yogurt with the addition of amylproteolytic enzyme was higher than the carbohydrate and protein content in button yogurt (p<0.05) (table 6). The content of carbohydrate, protein, and fat in oyster yogurt and button yogurt was lower than the content of the three nutritional compounds in milk yogurt (p<0.05) (table 6).
Table 6. Proximate analysis of oyster yogurt, button yogurt, and milk yogurt with addition amyloproteolytic enzyme from \textit{L. satsumensis} EN38-32

| Proximate analysis (%) | Oyster yogurt | Button yogurt | Milk yogurt |
|------------------------|---------------|---------------|------------|
| Carbohydrate           | 11.30\textsuperscript{a} | 10.60\textsuperscript{a} | 12.85\textsuperscript{c} |
| Protein                | 1.12\textsuperscript{b}  | 0.80\textsuperscript{a}  | 2.55\textsuperscript{c}  |
| Lipid                  | 0.29\textsuperscript{a}  | 0.25\textsuperscript{a}  | 1.56\textsuperscript{b}  |
| Water                  | 87.05\textsuperscript{b} | 88.17\textsuperscript{b} | 82.47\textsuperscript{a} |
| Ash                    | 0.24\textsuperscript{a}  | 0.18\textsuperscript{a}  | 0.57\textsuperscript{b}  |

Note: The mean value followed by the same letter in the same column was not significantly different according to Duncan's test at the 5% significance level (p<0.05)

The higher carbohydrate and protein content in oyster yogurt than in button yogurt (p<0.05) was due to the higher carbohydrate and protein content of oyster mushroom than button mushroom [16-21]. The lower carbohydrate, protein, and lipid content of oyster yogurt and button yogurt compared to milk yogurt showed lower nutritional compounds in the two mushroom yogurts than in milk yogurt. It was reported that the carbohydrate and protein content of oyster mushroom as the material used for making yogurt was higher than button mushroom [16-21], and the type of material used to make yogurt affected the nutritional content of the produced yogurt [20-22].

The proximate analysis of soygurt, mung bean yogurt, and milk yogurt with the addition of amyloproteolytic enzyme from \textit{L. satsumensis} EN38-32 was shown in table 7. The carbohydrate and protein content of soygurt with the addition of amyloproteolytic enzyme was 13.95\% and 3.15\%, respectively. The carbohydrate and protein content in the soygurt was higher than the mung bean yogurt at 12.25\% and 2.42\%, as well as for the milk yogurt at 12.85\% and 2.55\%. Also, the fat content in soygurt was lower than that in milk yogurt (table 7).

Table 7. Proximate analysis of soygurt and mung bean yogurt with addition amyloproteolytic enzyme from \textit{L. satsumensis} EN38-32

| Proximate analysis (%) | Soygurt | Mung-bean yogurt | Milk yogurt |
|------------------------|---------|------------------|------------|
| Carbohydrate           | 13.95\textsuperscript{c} | 12.25\textsuperscript{a} | 12.85\textsuperscript{b} |
| Protein                | 3.15\textsuperscript{b}  | 2.42\textsuperscript{a}  | 2.55\textsuperscript{a}  |
| Lipid                  | 0.63\textsuperscript{b}  | 0.51\textsuperscript{a}  | 1.56\textsuperscript{c}  |
| Water                  | 81.76\textsuperscript{a} | 84.36\textsuperscript{b} | 82.47\textsuperscript{a} |
| Ash                    | 0.51\textsuperscript{a}  | 0.46\textsuperscript{a}  | 0.57\textsuperscript{a}  |

Note: The mean value followed by the same letter in the same column was not significantly different according to Duncan's test at the 5% significance level (p<0.05)

The higher carbohydrate and protein content in soygurt with the addition of amyloproteolytic enzymes compared to mung bean yogurt and milk yogurt and lower fat content in soygurt than milk yogurt indicated a higher nutritional content in soygurt with the addition of amyloproteolytic enzymes than in mung bean yogurt and milk yogurt. The higher carbohydrate and protein content in soygurt with the addition of amyloproteolytic enzyme compared to the other two yogurts was due to the higher carbohydrate and protein content of the materials used in making soygurt than in the other two yogurts. It was reported that the carbohydrate, protein, and lipid content and the amyloproteolytic enzyme activity in the materials used in making yogurt affected the nutritional quality of the yogurt produced [21-23].
4. Conclusion
The addition of amylloproteolytic enzymes from *L. satsumensis* EN38-32 increased reducing sugar and total acid in mung bean yogurt. Mung bean yogurt had a higher reducing sugar and total acid value than oyster yogurt, button yogurt, and soycurd (p <0.05). The carbohydrates and protein in oyster yogurt with the addition of the enzyme had a higher value than that of button yogurt, while the carbohydrate and protein in soycurd with enzyme had a higher value than that of mung bean yogurt. Based on its nutritional content, soycurd with the addition of amylloproteolytic enzyme from *L. satsumensis* EN38-32 was the best nutritious yogurt compared to mung bean yogurt, oyster yogurt, button yogurt, and milk yogurt. Further research is needed for organoleptic evaluation and micronutrient analysis in soycurd.

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