Identification Of Moringa Oleifera Leaves Content Fermented By Rhizopus Oligosporus

Asriati Djonu, Sri Andayani, Happy Nursyam

Abstract: The fermentation process aims to improving the content of Moringa oleifera leaves by utilizing Rhizopus oligosporus 10^6 CFU/ml. We used four different treatments in the form of fermentation time consisting of 0, 3, 6 and 9 days. The best fermentation treatments obtained from test result include physical fermentation score test, phytochemical test, Fourier Transform Infra Red Spectrophotometer, total phenol content, protein content and crude fiber. Results showed the best treatment with fermentation time on day 6 with total phenol content of 4.73%, protein content of 29.69% and crude fiber of 10.37%.

Keywords: Crude Fiber, Fermentation, Moringa oleifera, Rhizopus oligosporus, Total Phenol.

1. Introduction

Moringa (Moringa oleifera) is a plant from India and widely spread in Indonesia. Almost every part of moringa plants can be consumed and potentially as medicine [1]. One part that can be use is moringa leaves. Moringa leaves in dry form and powder have protein content of 27,1-28,44% and crude fiber of 12,63-19,2% [2]. The content of crude fiber is quite high so that it can affect and reduce the digestibility; therefore it is necessary to do an effort to improve the quality of moringa leaves, both primary and secondary metabolite quality. The improvement of the quality of moringa leaves can be done by fermentation method. Fermentation is a process that utilizes the ability of microbes to produce primary and secondary metabolites in a controlled environment. Rhizopus oligosporus is one type of mold that can be used in the fermentation process. Utilization of R. oligosporus in the fermentation process can increase protein levels by protease enzyme activity and also can decrease crude fiber at KOHAY concentrate and bran [3]. Then R. oligosporus can also increase the total phenolic content of bran fermentation [4]. The fermentation process with R. oligosporus produces enzymes such as β-glucosidase, cellulase and xylanase that play an important role in biotransformation processes such as modifying primary and secondary metabolites that can enhance active compounds such as polyphenols [1]. Therefore, the aim of this research is to improve the quality of moringa leaf using fermentation.

2. Materials and Method

The research was conducted in April - September 2017 at the Faculty of Fisheries and Marine Sciences, Brawijaya University, East Java, Indonesia.

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Fermentation Moringa oleifera leaves

The material used in fermentation is simplicia moringa (M. oleifera) leaves with fermentor agent is Rhizopus oligosporus 10^6 CFU/ml. Ratio of fermentation materials was 10: 5: 1: 1 (M. oleifera: aquades: R. oligosporus: molasses). Fermentation was carried out for 9 days with observation time on days 0, 3, 6 and 9.

Parameter Analysis

Moringa leaves fermentation parameters include physical fermentation score test. Analysis was done by observing the physical characteristics of each fermentation result which includes texture, aroma, the amount of water vapor and the amount of hyphae fermented product. Scoring was done with a range scoring of 1-4. Total phenol content, protein content, crude fiber, phytochemical test, Fourier Transform Infra Red Spectrophotometer.

Data Analysis

Data analysis was performed by analysis of variance ANOVA (SPSS version 16).

3. Result and Discussion

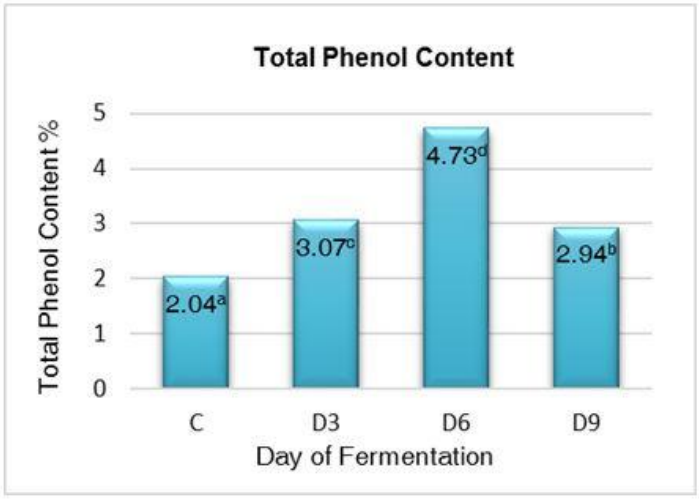
- **Physical Scoring Fermentation M. oleifera Leaves**

Table 1. *Fermented Physical Scoring*

Day	Texture	Aroma	Amount of water vapor	Amount of hyphae
3	3	2	2	2
6	4	3	3	4
9	2	2	3	3

Based on the results of physical scoring fermentation, moringa leaves obtained best treatment on the fermentation day 6. The texture of fermentation produced lumps, the aroma produced is fragrant, there is little water vapor and many hyphae. Higher the scoring, the better it results fermented products.

- **Total Phenol Content**



(Difference letters shows significant difference in level of confidence 95%)

Figure 1. *Results of Total Phenol Content*

Total phenol content of moringa leaves fermentation treatment showed an increase compared to the control treatment. The highest treatment was on day 6 (4.73%) and the lowest was in the control treatment (2.04%). The longer the fermentation processes the total phenol content increases. Increased total phenols content of moringa leaves is due to the activity of enzyme at R. oligosporus that capable of forming a phenol compound. Results of previous studies have shown an increase in total phenol by β-glucosidase activity, which plays a key role in breaking the glycoside bond to release the total bound phenol found in guava leaves during the fermentation process [1]. ß-glucoside is key in the bioconvection process to improve the quality of a product during the fermentation process [7,8,9].

- **Protein Content**

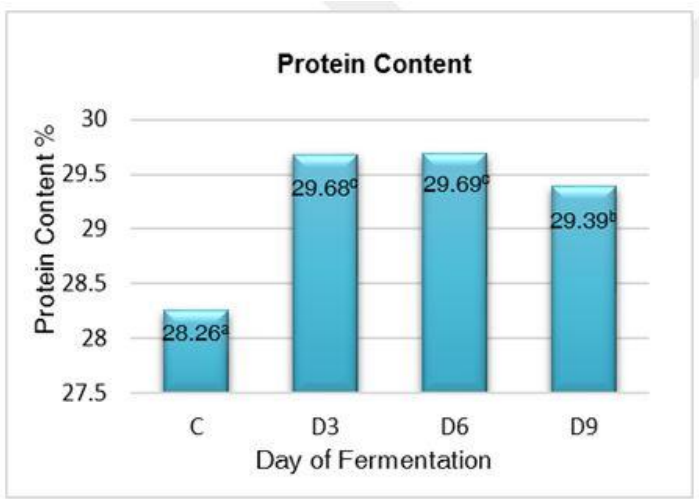


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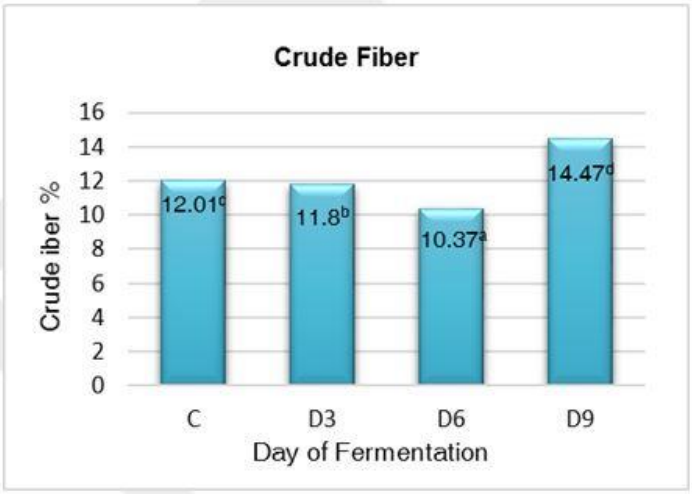
Figure 2. *Results of Protein Content*

Protein content in this study tended to increase. The highest protein content was found in the 6th day of fermentation treatment at 29,69% and the lowest was in the control treatment with the level of 28,26%. Increased protein levels caused by protease enzyme activity produced by R. oligosporus. Protease enzyme can break down carbohydrates, especially C atoms on substrate into various group functional bundles that can disfigure a protein [10]. Increased protein may be caused by mold mycelium on substrate; this is because mold itself contains nucleic acids that can contribute to nitrogen that is the sole source of a single cell protein. More mold grows, the higher protein content, because some of the mold is protein [11].

- **Crude Fiber**



(Difference letters shows significant difference in level of confidence 95%)

Figure 3. *Results of Crude Fiber*

Results of analysis of crude fiber showed highest value found in treatment of 9th day fermentation with content of 14.47% and the lowest crude fiber value found in the 6th day fermentation treatment with content of 10.37%. The decrease in crude fiber during the fermentation process is due to enzyme in R. oligosporus which breaks the cell wall of cellulose of M. oleifera leaves. This is because R. oligosporus is capable of producing cellulase enzymes capable of degrading crude fiber during fermentation process making it easier to digest [12]. The decrease in crude fiber in fermentation results due to enzyme produced by R. oligosporus capable of breaking cellulose into glucose. Cellulase enzyme is a complex enzyme that works gradually in breaking cellulose into glucose, and then glucose generated from the substrate will be used as a source of carbon and energy [13].

- **Phytochemical Moringa oleifera Leaves**

Table 2. *Phytochemical Moringa oleifera Leaves*

Parameter Analysis	Analysis Results
Phenol	+
Flavonoid	+
Saponin	+
Alkaloid (P. Mayer)	-
Alkaloid (P. Dragendrof)	-

Results of phytochemical moringa leaves showed the

presence of active compounds such as phenols, flavonoids and saponins. The result of phytochemical studies of moringa leaves also contain secondary metabolite compounds such as flavonoids, alkaloids, phenols that play a role in inhibiting bacterial activity [5]. Phytochemical content of moringa leaves extracted with water includes gallictannin steroids, triterpenoids, flavonoids, saponins, antraquinones, catecol tannins, alkaloid and reducing sugar [6].

- **Fourier Transform Infra Red Spectrophotometer**

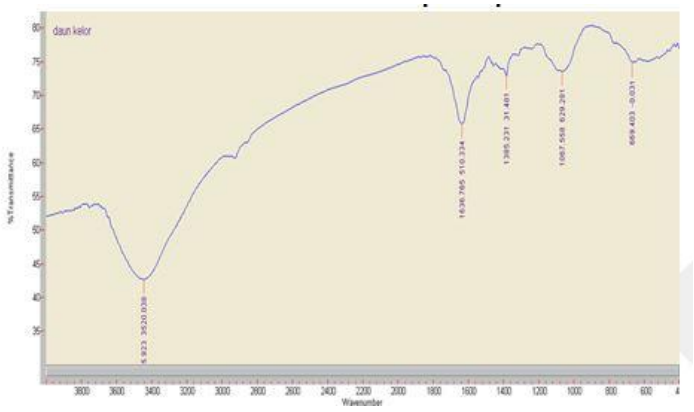


Figure 4. *Results of FTIR Fermented M. oleiera leaves day 6*

Based on Fourier Transform Infra Red Spectrophotometer (FTIR), the fermented M. oleifera leaves showing 5 regions of frequency indicating the type of bond in a compound with the following details.

Table 3. *FTIR Frequency Characteristics Fermented M. oleifera leaves day 6*

Area Frequency (cm^{-1})	Functional Groups
3520,038	Alcohol/phenol (H- bonded) Primary amine
1636,765	Secondary amine
1385,231	Alcohol/phenol (H- bonded) Carboxylic acid
1067,558	Alcohol, Ether
669,403	Esters, Amide

4. Conclusion

Fermentation of M. oleifera leaves using R. oligosporus resulted the best treatment was on the day 6 (for physical score, total phenol, protein content, and crude fiber).

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