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Duckweed Quality Improvement Through Fermentation Using *Trichoderma harzianum* and *Saccharomyces cerevisiae* on Dry Matter, Ash and Crude Fat

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Abstract. Duckweed has been used as animal feed which contains high nutrients, ie proteins and amino acids, but the water content is high. This is a limitation in its use Therefore, research was conducted to improve dry matter, mineral content and lower duckweed crude fat content. Duckweed fermentation is divided into two stages, firstly, *Trichoderma harzianum* (Th) ($3x10^7$ spores/100 grams substrate) with added $ZnCO_3$ (186 ppm) and dl-methionine (286 ppm), secondly, *Saccharomyces cerevisiae* (Sc) ($3x10^7$ spores /100 grams of a substrate). Completed randomized design (CRD) was used in experimental design with 20 experimental units, the treatments consist of P1 (Fermentation using Th for 1day continued with Sc for 9 days), P2 (Th for 3 days continued with Sc for 7 days), P3 (Th 5 days continued with Sc 5 days), P4 (Th 7 days continued with Sc 3 days), P5 (Th 9 days continued with Sc 1 day) with four replications. Analysis of variance was conducted to know the treatment effect, then followed by the Duncan test to know the difference between treatments. Fermentation using Thand Sc has a significant effect ($P<0.05$) on dry matter, ash, and crude fat in the duckweed. The best duration of fermentation is Th for 3 days and Sc for 7 days (P2) that increasing dry matter (92.95%) and ash (21.35%), and lower crude fat (2.37%). Duckweed fermentation with the combination of Th for 3 days and continued Sc for 7 days with added dl-methionine and Zn yield the highest dry matter and ash content, and lowest crude fat contents.

1. Introduction

Duckweeds are small floating aquatic plants [1, 2]. The productivity of *Lemna sp*. planted in an effective planting system reach to 12−38 tons of dry weight/ha/year [3]. *Lemna sp.* has high protein content (38%), however it also rich in crude fiber content. Duckweed has a 6-7% lower ash content. Duckweed has been used as animal feed. The use of duckweed in poultry nutrition is limited to 5% level due to high water content, low ash and fat content.

Fermentation is considered as one way to solve the limited use of duckweed. This is because fermentation can increase nutrient availability and reduce anti-nutrition [4]. Fermentation process raised the content of minerals. Fermentation can raise the content of crude fat in fermented Lemna meal increased up to 5.76% after fermentation [5].

The use of *Trichoderma harzianum* for fermentation has been reported because of its positive effect on the increase of dry matter and ash due to the decrease in crude fiber. The duckweed crude fiber content decreased by 76.06% after fermentation by *Trichoderma harzianum* for 6 - 9 days [6].

Saccharomyces cerevisiae is a yeast that can produce heterologous proteins. The fermentation

using *Saccharomyces cerevisiae* yeast can enrich the amino acid dl-methionine and inorganic Zinc (Zn) mineral because processing of vegetable-based feed usually results in a limiting amount of amino acids and essential minerals, in turn such a process will increase the content of dry matter and duckweed ash.

Duckweed quality can be improved by fermentation process using *Trichoderma harzianum* and *Saccharomyces cerevisiae*. The secondary metabolic pathway of *Trichoderma harzianum* and *Saccharomyces cerevisiae* are synergistic. *Trichoderma harzianum* can provide nitrogen for the growth of *Saccharomyces cerevisiae* and subsequently used as a raw material for the formation of metallic bonds and amino acids. The activity of both microbes produces complementary enzymes that will result in better results. That way can be used to imitate a natural microbial growth place that is coexisting in a complex microbial community. Duckweed fermentation process using two microbes (co-culture) is expected to produce functional complementary enzymes. This is due to the mutual expression of the metabolic pathways in the substrate utilization. However, the research on the quality improvement of duckweed through fermentation using *Trichoderma harzianum* and *Saccharomyces cerevisiae* has not been carried out. Therefore, the purpose of this research is to determine the best nutrient content of duckweed from the fermentation process based on two types of microbes.

2. Materials and Methods

2.1. Materials and Research Tools

The research materials consisted of duckweed, potato dextrose, PDA agar, Aquadest, $ZnCO_3$, dl-mehionin, rice, tripton, cotton, *Trichoderma harzianum*, and *Saccharomyces cerevisiae.* The equipment consisted of analytical scales, technical scales, measuring flask 1000 ml, Erlenmeyer 1000 ml, stirrer mugs, reaction tubes, Oase needles, spirtus lights, Erlenmeyer glasses, sterilisator, beaker glass, etiquette paper, electric oven, refrigerator, autoclave, and fermentor.

2.2. Research Procedure

Substrate consisted of a mixture of duckweed and selective medium. The mixtures were boiled in the water for 60 minutes at a temperature of 115°C and a pressure of 1.1 atmospheres. *Trichoderma harzianum* microbes were incubated in each treatment of 3 x 10^7 spores / 100 grams of the substrate for 1, 3, 5, 7, and 9 days at room temperature. After first stage fermentation, $ZnCO_3$ (186 ppm) and dl-methionine (286 ppm) were added. Next, each substrate was fermented using *Saccharomyces cerevisiae* at 3 x 10^7 spores / 100 grams substrate for 9, 7, 5, 3, and 1 day. After fermentation, the product was dried in the oven.

2.3. Experimental Design

The experimental design used Completely Randomized Design (CRD) for five treatments with a total of 20 experimental units. The treatments consisted of P1 (Fermentation using *Trichoderma harzianum* for 1 day continued with *Saccharomyces cerevisiae* for 9 days), P2 (*Trichoderma harzianum* for 3 days continued with *Saccharomyces cerevisiae* for 7 days), P3 (*Trichoderma harzianum* for 5 days continued with *Saccharomyces cerevisiae* for 5 days), P4 (*Trichoderma harzianum* for 7 days continued with *Saccharomyces cerevisiae* for 3 days), P5 (*Trichoderma harzianum* for 9 days continued with *Saccharomyces cerevisiae* for 1 day) with four replications.

2.4. Measurement Variables

2.4.1. Dry Matter

Dry matter refers to material remaining after removal of water. Dry matter content was determined by Wendee method. Proximate analysis was done by drying method of the material at temperature 105^0C.

2.4.2. Ash Content

The analytical method used to determine ash content is the proximate analysis of the Wendee method. The sample is added to the completely dry crucible and lid and together they are weighed to determine the mass of the sample by difference. The sample is placed in the hot furnace long enough so that complete combustion of the sample occurs. The crucible, lid and ash then are re-weighed.

2.4.3. Fat Content

Methods currently used for quantitating total fat and fatty acid composition in feedstuffs require solvent extraction, purification, and esterification followed by gas Chromatographic analysis.

2.5. Statistical Analysis

Data collected were subjected to one-way analysis of variance (ANOVA) as per Steel and Torrie. If there are mean differences, they were compared using Duncan's multiple range test with 5% significant level.

3. Results and Discussion

3.1. Fermented Duckweed Dry Matter

The fermentation using *Trichoderma harzianum* followed by *Saccharomyces cerevisiae* has a significant effect ($P<0.05$) to increase the duckweed dry Matter content. The rank of treatments effect are (higher to lower) P2 (92.71%), P3 (92.55%), P1 (92.50 %), P4 (92.49), and P5 (91.53 %) (Table 1).

Table 1. Dry Matter, Ash Content, Fat Content of Duckweed fermented with *Trichoderma harzianum* followed by *Saccharomyces cerevisiae*

Parameters	Treatments				
	P1	P2	P3	P4	P5
Dry Matter (%)	92.50 ± 0.57^{a}	92.71 ± 0.36^{b}	92.55 ± 0.38^{a}	92.49 ± 0.46^{a}	91.53 ± 0.45^{ab}
Ash Content (%)	21.73 ± 0.41^{c}	21.35 ± 0.38^{c}	18.42 ± 0.38^{a}	19.65 ± 0.73^{b}	22.76 ± 0.37^{d}
Fat Content (%)	2.23 ± 0.13^{a}	2.57 ± 0.37^{c}	2.37 ± 0.24^{b}	1.82 ± 0.15^{ab}	1.66 ± 0.19^{ab}

Mean in the same column with different superscript differs significantly ($P<0.05$),
P1: Fermentation using *Trichoderma harzianum* (Th) for 1 day continued with *Saccharomyces cerevisiae* (Sc) for 9 days, P2: Th 3 days continued with Sc 7 days, P3: Th 5 days continued with Sc 5 days, P4: Th 7 days continued with Sc 3 days, P5: Th 9 days continued with Sc 1 day.

The results of the present study are in-line with other studies. In the present fermentation process, dry matter weight loss was highly correlated with the biomass [7]

The Duncan's multiple-range test showed that the fermentation using *Trichoderma harzianum* for one day followed by *Saccharomyces cerevisiae* for nine days (P1) on dry matter content was not significantly different from fermented duckweed using *Trichoderma harzianum* for five days followed by *Saccharomyces cerevisiae* for five days (P3). Similarly, the dry matter content of fermented duckweed using *Trichoderma harzianum* for seven days followed by *Saccharomyces cerevisiae* for three days (P4) was not significantly different.

One day fermentation using *Trichoderma harzianum* was expected to increase the availability of dry matter. However, it could not break crude fiber of duckweed. Therefore, many complex cellulose bonding could be found resulting in low dry matter content in P1 (92.50%). On the other hand, the dry matter content has increased to 92.55% (P3) because five days fermentation were sufficient to form a dry matter.

The dry matter content in P4 (92.49%) were influenced by the fermentation duration of *Saccharomyces cerevisiae* (3 days). In P5, nine days fermentation of *Trichoderma harzianum* and

one days of *Saccharomyces cerevisiae* are not balanced causing declines dry matter content at 91.53%. This is in line with the study by [7] found that the loss of dry matter weight was caused by chemical composition changes (in fibres, starch and free-sugar content) of solid state fermentation

Saccharomyces cerevisiae for seven days (P2) has significantly ($P<0.05$) increased the dry matter content (92.71%) compared with other treatments (P1, P3, P4, and P5). It showed that the addition of *Saccharomyces cerevisiae* can be used as a duckweed modification agent to increase dry matter content.

3.2. Fermented Duckweed Ash Content

The highest ash content of fermentation to the lowest in sequence is P5 (22.76%), P1 (21.73%), P2 (21.35%), P4 (19.65%), and P3 (18.42%) as shown in Table 1. The crude fiber content in the Fermented duckweed reduced 15.27% as a result of the activity of the cellulose enzyme contained in *Saccharomyces cerevisiae*.

The fermentation by using *Trichoderma harzianum* followed by *Saccharomyces cerevisiae* was significantly ($P<0.05$) decreasing the content ash of duckweed. In terms of the differences of the treatments, the results showed that fermented ash content by using *Trichoderma harzianum* for one day continued by *Saccharomyces cerevisiae* for nine days (P1) was not significantly different from the fermentation using *Trichoderma harzianum* for three days continued by *Saccharomyces cerevisiae* for seven days (P2).

Trichoderma harzianum has higher cellulolytic activity than other *Trichoderma sp* groups. The longer fermentation time of the crude fiber content the higher substrate will be gained. This is due to the decrease in water content on the substrate that result in the more concentrated crude fiber. In addition, increasing the amount of *Trichoderma harzianum* raise the crude fiber from the cell wall. The amount of inoculum (3×10^7 spores/100 grams of substrate) used as the exact dose, and the minimum fermentation time by *Trichoderma harzianum* are the determinant of the crude fiber content of fermented duckweed.

The average duckweed content of fermented ash using *Trichoderma harzianum* for one day followed by *Saccharomyces cerevisiae* for nine days (P1) was significantly higher than other treatments (P2, P3, and P4). Running the fermentation for one day has not successfully broken the crude fiber in the duckweed. Fermentation using *Trichoderma harzianum* for three days followed by *Saccharomyces cerevisiae* for seven days (P2) has significantly decreased ash content originally from 31.36% to 21.35%.

3.3. Fermented Duckweed Fat Content

Duckweed fermented in *Trichoderma harzianum* followed by *Saccharomyces cerevisiae* was significant ($P<0.05$) to increase the content of fat in the duckweed. The highest to the lowest mean scores in sequence are P2 (2.57%), P3 (2.37%), P1 (2.23 %), P4 (1.82%) and P5 (1.66%). Crude fat in fermented lemna minor increased up to 5.76% after fermentation. The increase of fat content was caused by the microorganism's capability to produce lipid / fat during fermentation process. Microorganisms, as other life cell systems, produce lipid or fat [5].

Fermentation by using *Trichoderma harzianum* for three days followed by *Saccharomyces cerevisiae* for seven days (P2) has significantly ($P<0.05$) increased the content of fat in the duckweed (2.57%) compared with the other treatments (P1, P3, P4, and P5). It showed that the addition of *Trichoderma harzianum* and *Saccharomyces cerevisiae* can be used as a duckweed modification agent to increase the content of fat in the duckweed.

4. Conclusion

Fermentation with the combination of *Trichoderma harzianum* for three days and continued with *Saccharomyces cerevisiae* for seven days that are supplemented with amino acid dl-methionine and Zn is the best treatment to improve duckweed quality. It can increase the content of dry matter content (92.71%) and fat (2.57%), and decreased ash content (21.35%).

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