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## **Buoyancy Characteristics of Non-Extruded BSF Maggot-Based Fish Feed Produced Via *Rhizopus* sp. Fermentation Using Cassava Residue**

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### **Abstract**

The metabolic activity of *Rhizopus* sp. during solid-state fermentation can alter the physical properties of feed substrates, particularly density and structural integrity, thereby enhancing buoyancy. Cassava residue (onggok), a byproduct of the tapioca industry rich in starch, may serve as an effective carbohydrate source to support fungal growth and improve pellet flotation without the use of extrusion. This study evaluated the effect of fermentation time and cassava residue addition on the buoyancy of black soldier fly (BSF) maggot-based fish feed fermented using *Rhizopus* sp. An experimental design was applied with three fermentation durations (36, 48, and 60 h) and a commercial sinking pellet as a negative control, each treatment conducted in five replications. Fermented pellets containing

cassava residue were compared with pellets formulated without cassava. Buoyancy was assessed for up to 60 minutes of immersion in water. The results showed that pellets containing cassava residue exhibited significantly higher buoyancy, which increased with fermentation time. The highest buoyancy was observed at 60 h of fermentation, with 70% of pellets remaining afloat after 60 minutes, whereas pellets without cassava showed buoyancy below 20% at the same time point. These findings indicate that fermentation using *Rhizopus* sp. in combination with cassava residue has strong potential as an economical and environmentally friendly biological approach for producing floating fish feed without extrusion.

**Keywords:** Fish Feed Buoyancy, Black Soldier Fly Maggot, *Rhizopus* sp, Cassava Residue (Onggok), Solid-State Fermentation

### **Introduction**

The growing interest in consuming fish as a source of protein has become a public need, making the fisheries sector one of the reasons why many individuals and institutions are engaging in fish farming. However, there are many components to consider when carrying out fish farming. One of these is feed, which is a crucial aspect of fish farming as it provides a source of material and energy that can support the survival and growth of fish (Vita, 2017) <sup>[15]</sup>. The profitability of the livestock sector is highly dependent on the availability of high-quality feed. The use of feed components has not been fully addressed, meaning competition between food and feed, particularly for protein sources, continues, creating challenges for livestock farmers. The protein requirements of different livestock species vary (WDM *et al.*, 2023) <sup>[17]</sup>. However, on the other hand, feed requirements constitute the largest component (50-70%) of production costs. The increasing price of fish feed without a corresponding increase in the selling price of farmed fish is a problem that every fish farmer must face. Therefore, efforts to find alternative feeds, namely affordable and easily accessible natural feeds, are ongoing to reduce production costs. Many factors influence the high price of raw fish feed ingredients, one of which is the use of commercial feed. High feed prices cause the production costs of fish farming to increase (Purnamasari *et al.*, 2023) <sup>[12]</sup>. Some fish farmers still rely on manufactured feed, even though prices remain high on the market. This creates an imbalance between the income earned by fish farmers and their production costs (Amin *et al.*, 2020) <sup>[20]</sup>.

One of the natural protein raw materials in fish feed is black soldier fly maggot larvae (*Hermetia illucens*). According to the Council NR Nutrient Requirements of Swine (2013), BSF maggots contain three essential amino acids that are high compared

to soybean meal, even the valine content is higher than fish meal, namely leucine acid (average 44.6 g/kg, from 27.8 g/kg to 78.3 g/kg), lysine (average 38.8 g/kg, from 23.0 g/kg to 68.2 g/kg) and valine (average 40.1 g/kg, ranging from 28.2 g/kg to 67.9 g/kg). Meanwhile, according to Wang, *et al.*, 2021, the protein content of BSF maggots is 62.44%. Research on BSF maggot diets for Juvenile Tench (*Tinca tinca*) showed that 45% administration allowed for the best growth performance (Carral & Royuela, 2022) [4]. According to Al Rizki (2021) [1], maggot percentages of 50% and 40% increased the length growth of Sangkuriang catfish (*Clarias* sp).

Fish feed with quality content is expected to be more reliable for fish farmers if the final form is in the form of pellets that have good qualifications, including having long buoyancy and low water absorption. The manufacture of fish pellets that can float generally uses adhesives and fillers to prevent sinking pellets, including tapioca flour. These additional ingredients can sometimes change the ideal composition of fish feed because they will increase the carbohydrate content in the feed. In addition, the cost of making pellets will increase because the composition of this adhesive is generally around 10%. Therefore, another medium that has a lower cost is needed, namely the *Rhizopus* fungus, known as tempeh fungus. The hyphae and mycelium of this fungus will fill the voids in the cavity of the pellet and lighten the specific gravity of the pellet so that it is easy to float. Research by Sriherwanto *et al.* (2017) [13] shows that both fermented floating fish feed and commercial floating fish feed have a buoyancy of  $\geq 95\%$  for 60 minutes. According to Leiskayanti (2017) [10], fish feed using fermented *Rhizopus* has better stability, absorption, and buoyancy in water compared to commercial sinking feed. However, when compared to commercial floating feed, it is still lower.

Cassava is a byproduct of the tapioca industry, made from cassava. The starch content in cassava is used as a carbohydrate source in fish feed formulations. The addition of cassava as a feed ingredient is known to affect the buoyancy of feed compared to feed that does not contain cassava. Research on the buoyancy of cassava-based fish feed conducted by Zaman *et al.* (2018) [14] showed that fermentation of a mixed substrate consisting of commercial sinking feed, cassava, and duckweed using the mold *Rhizopus oryzae* was able to produce a fermented product with buoyancy properties.

Binders and extruders are required to produce fish feed with high water stability, preventing disintegration and feed waste. This technique is complex and expensive, so the use of live microorganisms as binders without high-temperature extrusion has been developed. Fungal granules formed from complex mycelium contribute to increased biomass control and stability in the pellet fermentation process (El Enshasy, 2022) [6].

## Materials and methods

This study used an experimental method conducted at the Biology Laboratory of Universitas Muhammadiyah Prof. Dr. Hamka, East Jakarta. There were four different fermentation time treatments, namely 36 hours, 48 hours and 60 hours and one negative control in the form of commercial sinking pellets with 5 replications. This study compared mixed pellets of maggots given cassava waste with pellets without cassava waste. The entire fermentation

process was carried out in non-sterile conditions and without sterilization steps. 50 grams of feed substrate consisting of wet maggots (*Hermentia illucens*), bran, cassava waste, soybean flour, corn flour, tapioca flour was put into a 250 ml beaker and then added with 50 ml of water. After that, the wet substrate was mixed with 1 gram of *Rhizopus* sp. tempeh yeast inoculum. This mixture was stirred thoroughly and left to soak for different times (four treatments), namely 0, 36, 48 and 60 hours in a petri dish. Next, the fermented feed was formed into 6-7 mm balls, then dried in an oven at 100°C for 1 hour. This fermented feed was then tested to assess its buoyancy by placing the pellets into a 500 ml beaker containing 400 ml of water. Buoyancy was recorded at 10, 20, 30, 40, 50, and 60 minutes. The formula used to calculate the buoyancy value of each fish feed is:

$$\text{Buoyancy (\%)} = \frac{\text{Number of pellets still floating} \times 100\%}{\text{Initial number of pellets}}$$

## Results and Discussion

The fermentation results with time differences of 0, 36, 48 and 60 hours in the petri dish were completely covered with grayish white mycelia and the substrate hardened with a distinctive tempeh smell (Fig 1).



Fig 1: Fermentation of pellet material

Both the corn-ongowa pellets and the corn-ongowa pellets were coated with mycelium of relatively equal thickness. The fermented feed media was then removed from the petri dish and manually ground into 6-7 mm pellets. The formed pellets were then placed in an oven and cooled before being tested for buoyancy (Fig 2).



Fig 2: Pellets that have been heated in the oven

Buoyancy testing was conducted by placing each treatment and its replicates into a 500 ml beaker. There was a variation in buoyancy in the cassava-based pellets depending on the

length of the previous fermentation. Pellets with the longest fermentation time tended to have longer buoyancy (Fig 3), but pellets made from non-cassava-based pellets showed a non-linear variation with fermentation time.

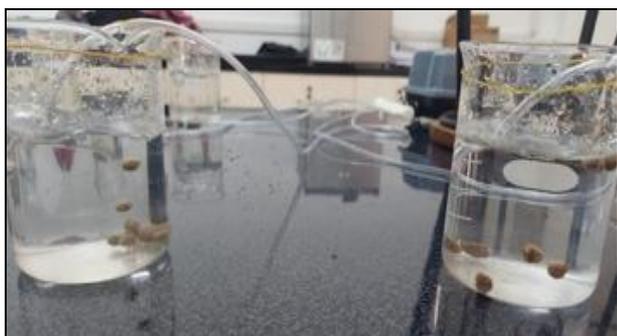


Fig 3: Pellet floatation test

The results of observations of pellet grains using a stereo microscope showed differences in structure between floating pellets and sinking pellets. Floating pellets have a solid surface and almost no air cavities because they are covered by black mycelia after being heated in an oven. In contrast to sinking pellets which have many cavities although some are covered by *Rhizopus* mold mycelia. Brouvier & Brisset (2006) revealed the presence of a layer of *Rhizopus* sp. mycelia that covers fermented floating feed, as one of the reasons for the increased buoyancy of this mold-fermented fish pellet.

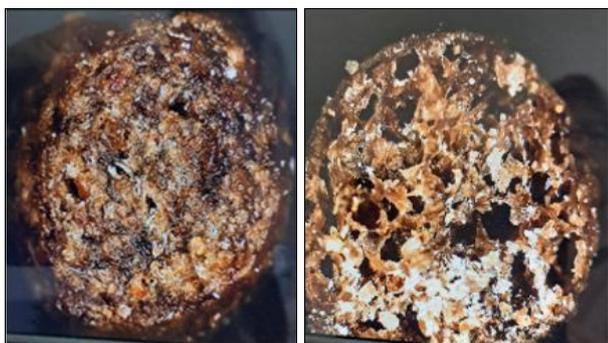


Fig 4: Floating and sinking pellet structure

The following are the results of buoyancy tests on pellets made from cassava and non-cassava fermented using *Rhizopus* sp. (Figures 5 and 6). There were four treatments with different fermentation times: 0 hour, 36 hours, 48 hours, and P3 (60 hours).

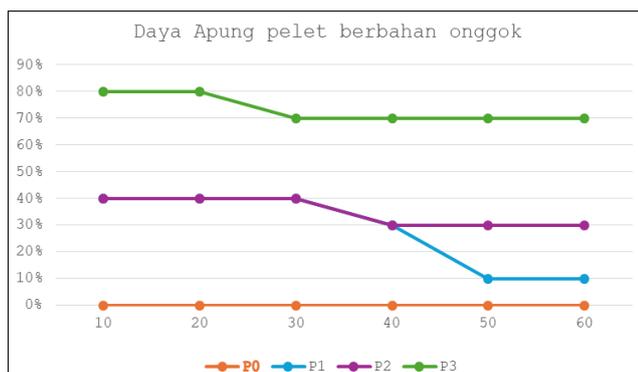


Fig 5: Buoyancy of corn pellets & cassava waste with fermentation time P0 (0 hours), P1 (36 hours), P2 (48 hours) and P3 (60 hours)

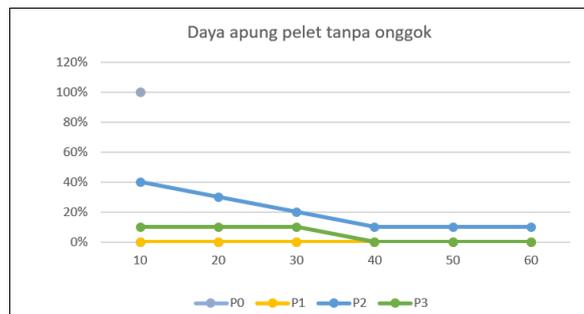


Fig 6: Buoyancy of pellets made from non-cassava waste with fermentation times P0 (0 hours), P1 (36 hours), P2 (48 hours) and P3 (60 hours).

Figures 5 and 6 show a comparison of the buoyancy of pellets made from cassava and those without cassava. Testing the buoyancy of fish pellets made from corn and cassava showed that the buoyancy increased with the length of fermentation. The highest results were found in treatment P3 (60 hours of fermentation) with a buoyancy of 70% at the 60th minute (Fig 5). Meanwhile, fish pellets made from corn without cassava showed a lower buoyancy of less than 20% at the 60th minute.

Fungal granules, known as pellets, play a role in improving biomass control and stability during the fermentation process. Fungal pellets are a unique ultrastructural configuration characterized by tightly interwoven hyphae, forming compact biomass aggregates with a spherical or elliptical morphology (Enhasi, 2022).

Giving *Rhizopus* sp. mold to fish feed has a buoyancy effect. The buoyancy of fermented substrates occurs when *Rhizopus* sp. mold is able to grow optimally on the substrate. This condition is characterized by the formation of dense, cotton-like mycelium, which plays a role in uniting the substrate granules and filling the spaces between the granules. The strength and density of the *Rhizopus* sp. mycelial network contribute to the formation of a compact and lightweight substrate structure, thus giving rise to floating characteristics (Sriherwanto *et al.* (2017) [13]).

The non-extrusion flotation method using *Rhizopus* sp. mold used in this study demonstrated better buoyancy in materials containing cassava residue compared to those without cassava residue. Previous research on feed flotation without the use of an extruder has been conducted by Haryono *et al.* (2021) showed that pellets fermented with *Rhizopus* sp. mold produced better nutritional value and physical characteristics. Fermented fish feed produced a protein content of 29.72%; fiber 12.13%; fat 26.57%; and ash 7.83%. Meanwhile, the average buoyancy value was 83.33% and water stability was 92.38%. The chemical and physical parameters of fermented fish feed had higher values compared to the control without fermentation. Research on the buoyancy of fish feed using cassava by Zaman *et al.* (2018) [14] showed that Fermentation of a mixed substrate consisting of commercial sinking feed, cassava waste, and duckweed using the fungus *Rhizopus oryzae* produced a fermented product with buoyancy. Compared to commercial floating feed, the fermented floating feed showed a relatively similar stability curve pattern in water during immersion for up to 20 hours. The fermented floating feed absorbed water up to almost four times its initial dry weight, while the commercial floating feed absorbed water up to approximately two times its initial dry weight. During the

three-hour buoyancy test, both types of feed remained completely buoyant with no pellets sinking.

The floating nature of this fish food is physically related to a decrease in the material's density caused by a reduction in the dry weight of the substrate due to the metabolic activity of the mold *Rhizopus* sp. The process that occurs is the conversion of some components of the solid substrate, such as carbohydrates (e.g., glucose), into simpler and more volatile compounds, such as carbon dioxide (Maas *et al.* 2008) [11].

Onggok is a byproduct of the tapioca industry, made from cassava. During the starch extraction process, a filter is used to prevent impurities from entering the starch solution. These impurities are retained on the filter media and then carried away with the starch. Onggok. The starch content in onggok is used as a source of carbohydrates in fish feed.

The provision of cassava as a component of feed ingredients has an effect on the buoyancy of the feed, compared to feed without cassava. The hydrolysis process in this cassava material decomposes its constituent molecules. Starch hydrolysis breaks down starch into dextrin, isomaltose, maltose and glucose (Rindit *et al.*, 1998). The specific gravity of cassava with carbohydrase hydrolysis reduces its specific gravity. Btype, particle size, pile density, pile compaction density, total solubility of the pile without carbohydrase hydrolysis is higher compared to the pile hydrolyzed by carbohydrase (Gusthamad *et al.*, 2023).

### Conclusion

The provision of cassava as a feed ingredient has an effect on the buoyancy of the feed. The buoyancy of fish pellets made from cassava and non-cassava showed different buoyancy capabilities. The results of the test of pellets made from cassava increased in line with the length of fermentation. The highest results were in treatment P3 (60 hours of fermentation) with a buoyancy time at the 60th minute of 70%. (Meanwhile, fish pellets made from corn without cassava showed a lower buoyancy of less than 20% at the 60th minute. Fermentation using *Rhizopus* sp. and cassava has the potential to be developed as a biological buoyancy agent in the production of floating feed that is more economical and environmentally friendly.

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