A Preliminary Study of Nutritional Enhancement of Duckweed (*Lemna minor*) through Fermentation with *Saccharomyces cerevisiae* and *Lactobacillus* sp.

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Abstract

Duckweed is generally only used as an alternative feed for animals and is less optimized as a source of additional food for humans. The application of duckweed, Lemna minor, is still limiting. To optimize the utilization of L. minor, increasing nutrient content of L. minor through a fermentation process is necessary. The objective of this research was to investigate the effect of fermentation on nutrient content of the duckweed. L. minor was fermented using the combination of local isolate: Saccharomyces cerevisiae and Lactobacillus sp. Variations on the length of the fermentation time are using ranges of 0, 3 and 7 days for fermentation time. The fermented samples were then subjected to proximate, pH, color analysis. Amino acid analysis was performed on the best treatment.

The results showed that the fermentation process using the consortium of S. cerevisiae and Lactobacillus sp. increased the nutritional quality of L. minor which includes an increase in protein content. The results of amino acid analysis also showed good results for fermented L. minor. Hence, fermentation represents an innovative approach to enhance the protein content and overall nutritional quality of duckweed.

Keywords: Amino acid, Consortium, Fermentation, *Lemna minor*.

Introduction

The incorporation of alternative sources of fishmeal in fish feed is of utmost significance in contemporary aquaculture methods. The utilization of alternative sources such as plant proteins, cereals and other sustainable resources as alternatives for fishmeal enhances the long-term sustainability of aquaculture[14]. These alternatives serve as environmentally benign and economically viable equivalents while simultaneously ensuring the essential nutritional requirements for the growth of fish[17]. By implementing strategies to decrease dependence on fishmeal and advocating for the adoption of other sources, the aquaculture sector has the potential to enhance its sustainability and environmental stewardship.

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Investigating possible and alternative flour substitutes in fish feed is a crucial area for augmenting the sustainability of aquaculture. Fishmeal, a commonly used component in fish feed, encounters obstacles as a result of its dependence on the extraction of fish from natural habitats and the subsequent environmental consequences it entails[11]. In response to these issues, scholars and professionals in the field of aquaculture are progressively exploring alternate sources of flour including soybean meal, maize gluten meal and proteins derived from insects[3,13]. These efforts aim to establish a more sustainable and economically feasible future for the aquaculture sector while simultaneously addressing the nutritional requirements of farmed fish.

Duckweed, *L. minor* exhibits considerable promise as a viable protein alternative for incorporation into animal feed, specifically within the domains of aquaculture and livestock production. The aforementioned aquatic plant exhibits rapid growth, possesses a high nutritional value and may be cultivated with low resource allocation[16]. The product exhibits a protein composition that is similar to that of soybeans, rendering it a desirable source of protein. Nevertheless, a critical element in maximizing the utilization of duckweed in feed compositions is the fermentation[5]. This approach allows for the efficient utilization of this abundant and sustainable resource, hence promoting ecologically conscious and economically feasible agricultural methods. The objective of this study is to assess the influence of fermentation on the proximate content and amino acid composition.

Material and Methods

Materials: *L. minor* used in this study was obtained directly from Sumber Gentong pond, Malang. *L. minor* was then washed thoroughly. After washing, it was dried in the sun for 3-4 days. After completing the drying process, the sample was pulverized using a grinder to a size of 60 mesh[10]. *Lactobacillus* sp. is obtained from stock bacteria stored using glycerol which is then propagated on MRSA media. *Lactobacillus* sp. is a pure culture from Lab. Food Microbiology, Faculty of Fisheries and Marine Science, Brawijaya University. *Saccharomyces cerevisiae* was obtained from instant yeast which was then cultured on PDA (Potato Dextrose Agar) media.

Preparation of the starters: *S. cerevisiae* culture at a density of $1.5x10^8$ spores and *Lactobacillus* sp. at $1x10^8$ CFU/ml at 2 ml were used for each treatment, S and L,

respectively. Mixed inoculum was obtained by mixing *S. cerevisiae* culture with *Lactobacillus* sp. in a ratio of 1:1 for each inoculum. 0.5 ml was mixed with 0.5 ml *Lactobacillus* sp. which has a density of $1x10^8$. The mixture treatment (SL) refers to the modified method of Pinandoyo et al[18].

Fermentation: The fermentation process of L. minor was carried out following the method of Mayangsari[12]. Duckweed was sterilized first. Next, 30 g each was weighed and put into 4 different beaker glasses. Four different treatments were applied to this study, namely control treatment, treatment with the addition of *S. cerevisiae* (S), treatment with the addition of *Lactobacillus* sp. (L) and treatment with the mixing of both (*S. cerevisiae* + *Lactobacillus* sp.) (SL). The proportion of inoculum and molasses was done by mixing the solution that already contained the solution and molasses with a ratio of 1:1. Now put into a standing pouch with pH 4.5 - 5 and store in a steroform with room temperature of 30 C with different lengths of time, namely 0, 3, 7 days. Samples were taken on days 0, 3 and 7. Then to stop the fermentation process, the inactivation process is carried out.

Proximate analysis: Standard analytical procedures were used for the proximate analysis which involved dividing the samples into six groups based on their chemical properties: moisture content, crude protein (kjedahl protein), crude lipids.

Amino acid analysis: The sample was weighed to a mass of 0.1 g and placed in a test tube that was sealed. Subsequently, 5 ml of 6 N hydrochloric acid (HCl) was added to the test tube followed by vortexing. The sample is purged with nitrogen gas. Subsequently, the tube housing the specimen was introduced into a laboratory oven set at a temperature of 110˚C and left undisturbed for a duration of 22 hours. After the process of cooling, the substance was afterwards put into a volumetric flask with a capacity of 50 ml. dH_2O was then poured to the flask until it reached the designated limit mark. The material underwent filtration using a filter membrane with a pore size of 0.45 µm. A volume of 500 µl of the filtrate was pipetted, followed by the addition of 40 µl of AABA and 460 µl of dH_2O. A volume of 10 µl of solution was carefully transferred using a pipette, followed by the addition of 70 µl of AccQ-Fluor borate. The resulting mixture was then subjected to vortexing. Subsequently, a volume of 20 µl of fluorine reagent A was introduced to the mixture and agitated using a vortex mixer.

The resulting solution was then allowed to incubate for a duration of 1 minute. The sample should be subjected to incubation at a temperature of 550°C for a duration of 10 minutes. The HPLC column received an injection of 5 µl of sample solution as reported by Istiqamah[6] and Rutherfurd and Dunn[19].

Statistical analysis: The data were expressed as means ± standard deviation (SD) and all experiments were run at least three times. The statistical software, SPSS 17.0, was used to perform the analyses. Duncan's test found a significant difference at $P < 0.05$.

Results and Discussion

The results of proximate tests (protein, lipid and moisture) in this study are presented in table 1. Protein continues to increase during the fermentation process. The highest protein content was obtained in the L treatment with a fermentation time of seven days with a value of 15.91%. This protein increase also occurred in all treatment. The same results were also obtained in lipid content. The highest lipid content was obtained from the L treatment. At the end of fermentation, the lipid content increased from 1.74% to 2.16 % on the seventh day. This indicates that the fermentation process will significantly increase the lipid content of the material.

Meanwhile, the moisture indicator produces the opposite value. The longer is the fermentation process, the lower is the moisture content in the material. In addition to the control treatment, the lowest decrease in moisture content was found in Duckweed samples with L fermentation treatment. This shows that the longer is the fermentation process, the smaller will the moisture.

Multiple studies have demonstrated the efficacy of fermentation in augmenting the protein content of duckweed. For instance, research conducted by Shen et al[22] showed that fermentation with beneficial microorganisms significantly increased the protein content of duckweed. During fermentation, these microorganisms break down complex compounds in duckweed such as cellulose and starch, making the protein more bioavailable and easier for the body to digest[15]. This not only ensures a higher absorption of protein but also reduces the presence of anti-nutritional factors that can hinder nutrient absorption[8]. These findings emphasize the potential of fermentation as a sustainable and efficient means to enhance the protein content and overall nutritional value.

Color analysis: The highest lightness value was obtained in the early days of fermentation. At the beginning of the fermentation process, the lightness value ranged from 37.39 to 39.72. But after the fermentation process on day seven, the lightness value ranged from 37.10 to 40.64. The decrease in lightness value was observed on the S and SL treatments. While for the control treatment, there is a tendency to increase although not significant. The results of the analysis are shown in table 2.

Fermentation can alter the color of fermented duckweed due to various biochemical and enzymatic processes that occur during this microbial transformation[1]. The color changes observed in fermented duckweed are often attributed to factors such as the breakdown of pigments, formation of new compounds and microbial activities. During fermentation, enzymatic reactions can lead to the degradation of pigments

present in duckweed[7,20]. For example, chlorophyll, the green pigment responsible for photosynthesis in plants, can undergo degradation into colorless or differently colored compounds under the influence of enzymes produced by fermenting microorganisms[4].

Amino acid analysis: Amino acid analysis was only performed on duckweed samples that showed the highest protein change. Samples fermented with *Lactobacillus* sp. (L) were then subjected to amino acid analysis. The results of the analysis are shown in table 3.

The amount of essential chained amino acids has a high value. Essential amino acids such as L-lysine, L-alanine, l-leucine and L-isoleucine have a value of 3413.345±4.46, 4618.96±10.17, 6035.015±2.11 and 3661.81±11.17 respectively. Fermentation can lead to the synthesis and additional amino acid profile.

Furthermore, fermentation process can reduce the levels of anti-nutrients like phytic acid which can inhibit the absorption of key minerals[2,21]. This not only makes fermented duckweed a more robust source of protein but also a more nutritionally complete food source[9,22].

Table 1
The results of proximate, protein, lipid, moisture (%) on the different duration of fermentation.

Treatment	0 day			3 days			7 days		
	protein	lipid	moisture	protein	lipid	moisture	protein	lipid	moisture
C	12.93	1.59	57.19	14.52	1.76	53.56	15.67	2.02	47.74
S	14.28	1.84	54.05	15.46	1.53	51.45	15.52	1.89	50.89
L	13.11	1.74	58.92	15.35	2.00	52.17	15.91	2.16	48.03
SL	13.87	1.84	58.51	16.14	2.22	50.03	15.62	1.99	56.23

Table 2
Color analysis result of fermented Duckweed (*Lemna minor*).

Duration of Fermentation	Color indicator	Treatment			
		C	S	L	SL
0 day	L*	39.72	39.02	37.39	39.20
	a*	-3.48	-2.67	-4.77	-3.56
	b	9.93	8.28	7.25	6.44
3 days	L*	38.22	40.10	38.40	38.57
	a*	-4.59	-5.14	-4.97	-4.49
	b	9.15	7.13	7.81	7.94
7 days	L*	40.64	37.28	38.34	37.10
	a*	-5.38	-2.65	-4.44	-3.67
	b	7.04	7.15	6.70	6.96

Table 3
The amino acid profile of *Lemna minor*.

Amino acid	Content (mg/kg)
L-Serine	3426.26±21.84
L-Glutamic acid	7746.195±5.35
L-Phenylalanine	4190.185±23.93
L-Isoleucine	3661.81±11.17
L-Valine	4338.21±13.56
L-Alanine	4618.96±10.17
L-Arginine	3646.565±31.05
Glycine	4602.34±11.37
L-Lysine	3413.345±4.46
L-Aspartic acid	5892.57±1.31
L-Leucine	6035.015±2.11
L-Tyrosine	1839.935±3.76
L-Proline	2972.84±2.67
L-Threonine	4044.11±5.21
L-Histidine	1312.18±3.65

Fermentation can enrich duckweed with essential vitamins such as B vitamins and can reduce anti-nutrients like phytic acid which can inhibit the absorption of key minerals. This not only makes fermented duckweed a richer source of protein but also a more nutritionally comprehensive food source[10]. Fermented duckweed stands as a promising solution that aligns with the growing interest in alternative protein sources[10,23]. Furthermore, the benefits of fermentation extend beyond protein content enhancement. Fermentation can contribute to an overall improvement in duckweed's nutritional quality by increasing the bioavailability of essential nutrients like vitamins, minerals and amino acid.

Conclusion

Fermentation increases the protein content of the fermented duckweed (L. minor). Furthermore, the favorable amino acid profile was also obtained. Hence, fermentation represents an innovative approach to enhance the protein content and overall nutritional quality of duckweed.

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References

1. Degrain A., Manhivi V., Remize F., Garcia C. and Sivakumar D., Effect of Lactic Acid Fermentation on Color, Phenol ic Compounds and Antioxidant Activity in African Nightshade, *Microorganisms*, **8**, 1324 **(2020)**

2. Dinika I., Nurhadi B., Masruchin N., Balia R.L. and Utama G.L., The roles of Candida Tropicalis Toward Peptide and Amino Acid Changes in Cheese Whey Fermentation, *IJ Tech.*, **10(8)**, 1533-1540 **(2019)**

3. Gałęcki R., Zielonka Ł., Zasępa M., Gołębiowska J. and Bakuła T., Potential Utilization of Edible Insects as an Alternative Source of Protein In Animal Diets In Poland, *Front. Sustain. Food Syst.*, **5**, 675796, DOI: 10.3389/fsufs.2021.675796 **(2021)**

4. Goto T., Horita H. and Saito K., Chlorophyll Metabolism in Higher Plants VII. Chlorophyll Degradation in Senescing Tobacco Leaves: Phenolic-Dependent Peroxidative Degradation, *Can. J. Bot.*, **53(4)**, 315-321 **(2015)**

5. Herawati V.E., Pinandoyo, Darmanto Y.S., Rismaningsih N., Widarto S. and Radjasa O.K., The effect of fermented duckweed (*Lemna minor*) in feed on growth and nutritional quality of tilapia (*Oreochromis niloticus*), *Biodiversitas*, **21**, 3350-3358 **(2020)**

6. Istiqamah A., Lioe H.N. and Adawiyah D.R., Umami Compounds Present in Low Molecular Umami Fractions of Asam Sunti–A Fermented Fruit of *Averrhoa bilimbi* L., *Food Chem*., **270**, 338-343 **(2019)**

7. Janiszewska-Turak E., Tracz K., Bielińska P., Rybak K., Pobiega K., Gniewosz M., Woźniak Ł. and Michałowska A., The Impact of the Fermentation Method on the Pigment Content in Pickled Beetroot and Red Bell Pepper Juices and Freeze-Dried Powders, *App. Sci.*, **12**, 5766 **(2022)**

8. Knez E., Kadac-Czapska K. and Grembecka M., Effect of Fermentation on the Nutritional Quality of the Selected Vegetables and Legumes and Their Health Effects, *Life*, **13**, 655 **(2023)**

9. Lam E., Appenroth K.J, Michael T., Mori K. and Fakhoorian T., Duckweed in Bloom: The 2nd International Conference on Duckweed Research and Applications Heralds the Return of a Plant Model for Plant Biology, *Plant Mol. Biol.*, **84(6)**, 737–742, DOI: https://doi.org/10.1007/s11103-013-0162-9 **(2014)**

10. Li S., Wang Z., Zhi X., Yang C., Zhang Y. and Wang Z., Effects of Fermentation on the Nutritional Properties and Anti-Nutritional Factors of Duckweed (*Lemna minor* L.), *Food Chem*., **336**, 127717 **(2020)**

11. Luhur E.S., Deswati R.H., Apriliani T. and Arthatiani F.Y., Driving Factors of Indonesian Import of Fish Meal, IOP Conf. Ser. Earth Environ Sci., **860**, 012057 **(2021)**

12. Mayangsari V. and Abtokhi A., Analysis of the Effect of Temperature and Time Variations in The Hydrolysis Process on Glucose Levels in The Utilization of *Lemna Minor* as Bioethanol, *J. Phyis. And App*., **7(1)**, 16-22 **(2014)**

13. Moura M., Martins B.A., Oliveira G.P. and Takahashi J.A., Alternative Protein Sources of Plant, Algal, Fungal and Insect Origins for Dietary Diversification in Search of Nutrition and Health, *Crit Rev Food Sci Nutr*., **14**, 1-18, DOI: 10.1080/10408398.2022.2085657 **(2022)**

14. Neginah Vijayasingh and Athiappan Murugan, Bio-transforming Syzygium Cumini Kernel into Microbial Metabolites: Towards enhancing Pharmacological Characterization, *Res. J. Biotech.*, **18(5)**, 31-44 **(2023)**

15. Nkhata S.G., Ayua E., Kamau E.H. and Shingiro J.B., Fermentation and Germination Improve Nutritional Value of Cereals and Legumes Through Activation of Endogenous Enzymes, *Crit Rev Food Sci Nutr.*, **6(8)**, 2446-2458 **(2018)**

16. Pagliuso D., Grandis A., Fortirer J.S., Camargo P., Floh E.I.S. and Buckeridge M.S., Duckweeds as Promising Food Feedstocks Globally, *Agronomy*, **12(796)**, DOI: https://doi.org/10.3390/ agronomy12040796 (**2022)**

17. Pérez-Pascual D., Pérez-Cobas A.E., Rigaudeau D., Rochat T., Bernarder J., Cassy S.S., Marchand Y., Duchaud E. and Ghico J., Sustainable Plant-Based Diets Promote Rainbow Trout Gut Microbiota Richness and Do Not Alter Resistance to Bacterial Infection, *Anim Microbiome*, **3(47)**, DOI: https://doi.org/10.1186/ s42523-021-00107-2 **(2021)**

18. Pinandoyo, Hutabarat J., Darmanto Y.S., Radjasa O.K. and Herawati V.E., Growth and Nutrient Value of Tilapia (*Oreochromis niloticus*) Fed with *Lemna Minor* Meal Based on Different Fermentation Time, *AACL Bioflux*., **12(1)**, 191-200 **(2019)**

19. Rutherfurd S.M. and Dunn B.M., Quantitative Amino Acid Analysis, *Curr Protoc Protein Sci*., **63(1)**, 321-326 **(2011)**

20. Santra K., Song A., Petrich J.W. and Rasmussen M.A., The Degradation of Chlorophyll Pigments in Dairy Silage: The Timeline of Anaerobic Fermentation, *J Sci Food Agr*., **101(7)**,

2863-2868 **(2021)**

21. Sharma R., Garg P., Kumar P., Bhatia S.K. and Kulshrestha S., Microbial Fermentation and Its Role in Quality Improvement of Fermented Foods, *Fermentation*, **6**, 106, DOI: https://doi.org/10.3390/fermentation6040106 **(2020)**

22. Shen N.K., Zhang H.Y., Qin Y., Wang Q.Y., Zhu J., Li Y., Jiang M.G. and Huang R.B., Efficient Production of Succinic Acid from Duckweed (*Landoltia punctata*) Hydrolysate by *Actinobacillus succinogenes* GXAS137, *Bioresour. Technol*., **14**, 35–42 **(2018)**

23. Xu J., Zhao Y., Xu K., Wang X., Gao H. and He X., Improvement of Nutritional Value of Duckweed by Fermentation with *Bacillus amyloliquefaciens* W19, *J. Appl. Microbiol.*, **127(3)**, 949-959 **(2019)**.

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