Ethiop. J. Biol. Sci. **20(2): 143–154, 2021**
 ISSN: 1819-8678

RESEARCH ARTICLE

THE EFFECT OF *ASPERGILLUS NIGER* ON THE NUTRITIVE VALUE OF WATER HYACINTH AS FISH FEED USING SOLID STATE FERMENTATION

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ABSTRACT: Water hyacinth typically has high moisture content, low protein profile and high crude fiber content. Its value as a feed resource can be improved through solid-state fermentation with the fungi species, *Aspergillus niger*. This study was conducted with the aim of producing environmentally friendly and nutrient rich fish feed at low cost from water hyacinth. Water hyacinth leaf and whole plant were collected, dried, milled, sterilized and subjected to incubation with *Aspergillus niger* for a period of eight weeks. Fermentation resulted in an increase ($p<0.05$) in crude protein and ash contents, a decrease ($p<0.05$) in dry matter, crude fiber, nitrogen free extract and fat contents of fermented water hyacinth leaf and whole plant. The results suggested that water hyacinth with six weeks fermentation period contained the highest level of crude protein of both leaf and whole plant. Hence, biological treatment of water hyacinth using *Aspergillus niger* improved its nutritive value, and this finding might be used to produce fish feed and to reduce environmental pollution.

Key words/phrases: Ash content, Crude protein, Fiber, Fish feed.

INTRODUCTION

The price of fish feed covers from 50 to 60% of the total operation cost of aquaculture production (FAO, 2012). Lack of cheap feed ingredients is one of the factors that contribute to the main increase the price of aqua-feed (Fapohunda and Fagbenro, 2006). This necessitates the search for alternative sources of protein-rich feed from locally available, non-conventional, low-cost plant feed ingredients for small and medium scale fish farmers (Makkar *et al*., 1997). However, plant materials used for feeds are not necessarily effective due to a number of factors including; poor palatability, poor digestibility, anti-nutritional factors, high fiber content and low or poor protein (Ogunji and Wirth, 2004).

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Water hyacinth (*Eichhornia crassipes*) is a free floating perennial herb of fresh water ecosystems native to the Amazon basin in tropical and sub-tropical South America (Adeyemi and Osubor, 2016). It is also widely distributed in water bodies of Ethiopia and creates socio-economic and environmental problems affecting agriculture, fisheries, biodiversity and livelihoods of people in the country (Dereje Tewabe, 2015; Erkie Asmare, 2017; Ayenew Gezie *et al*., 2018; Minychl Dersseh *et al*., 2019).

There are several chemical, physical and biological methods that are used to address the aforementioned problems and challenges (Yan *et al*., 2017). It is also established that this herb has potential attribute to remove chromium from tannery wastewater plant (Daniel Woldemichael *et al*., 2016) and a new source of raw material for handicrafts in Africa (Rakotoarisoa *et al*., 2016) indicating the option of using this invasive weed from its menace status into an asset of national value.

Several studies have shown that water hyacinth can serve as supplementary plant feed ingredients in animal feed including fish (Abdel-Sabour, 2010; Aderolu and Akinremi, 2009). However, water hyacinth incorporation into fish feed can be limited by its relatively low protein content and high fiber profile which may limit its effective utilization by fish as feed ingredient (Konyeme *et al*., 2006). The proximate analysis of water hyacinth leaf on a dry matter (DM) basis showed that it contains 11.44% crude protein (CP), 26.61% crude fiber (CF), 2.83% crude lipid (CL), 16.12% ash and 48.18% Nitrogen free extract (NFE) (Mangisah *et al*., 2010). The authors also showed that the feed quality of the weed can be improved through fermentation by using effective microorganisms such as *Aspergillus niger*.

Aspergillus niger is an ubiquitous mold fungus which is widely studied for its ability to convert non-conventional, locally available feed resources into nutrient rich alternative feedstuffs for fishes. Through the process of fermentation, molds produce several extracellular enzymes such as amylase, amyloglucos idase, pectinase, cellulase, catalase and glucosidase and urease (Wahyuni *et al*., 2018). The fungus is characterized by high digestibility of carbon rich materials and increase in protein content through fermentation technologies (Singhania *et al*., 2009).

Solid-state fermentation is one of the most important technologies employed to produce quality products using abundant and cheap agro-industrial substrates with relatively less risk of contamination (Osma *et al*., 2007). According to Do Santos *et al*. (2015), a fermentation process using *Aspergillus niger* and *Rhyzopus* sp on a cactus pear (*Nopalea cochenillifera*

(L.) Salm Dyck) increased protein content and decreased crude fiber content after 192 hours. Similarly, solid-state fermentation using *Aspergillus niger* on vegetable wastes increased protein content with the reduction of crude fiber contents after 9 days of fermentation (Joseph *et al*., 2010). Doughan and Dzogbefia (2018) showed the same pattern of protein increase and a decrease in crude fiber content of yam peels with *Aspergillus niger* and *Pleurotus ostreatus* fermentation for a period of eight weeks.

Shamim *et al*. (2017) reported an increase in crude protein and ash content with a decrease in crude fiber, fat and carbohydrate contents of water hyacinth after solid state fermentation with *Pleurotus sajor-caju* for about eight weeks. Research conducted by Velasquez *et al*. (2011) found an increase in protein, amino acid, ash and a decrease in fiber contents of whole water hyacinth after fermentation with lactic acid bacteria.

Despite the abundance and easily availability of water hyacinth in Ethiopia, it has been poorly investigated as alternative or supplement feed for cultured fish in the form of fermented product. This may be due to lack of information on its processing, nutritional characteristics and fermentation properties. Therefore, the objective of this study was to characterize the effects of fermentation on nutritional content of both water hyacinth leaf and whole plant using *Aspergillus niger.*

MATERIALS AND METHODS

Water hyacinth collection and processing for fermentation

Leaves and other parts of the water hyacinth (*Eichornia crassipes*) samples were collected from Koka Reservoir and washed thoroughly with tap water to remove adhering mud and debris. Approximately, 250 g of fresh sample from each site was taken and oven-dried at 60°C for 48 hours until a constant weight to measure dry matter content. Then, the samples were ground and passed through a two-millimetre meshed sieve to ensure homogeneity. Finally, water hyacinth powder samples were used for fermentation experiment.

Fermentation processes

Fermentation of water hyacinth leaves and whole plant was undertaken using *Aspergillus niger* for about eight weeks.

Culture preparation

Aspergillus niger was isolated from soil and cultured on PDA (potato dextros agar) medium at 28°C for seven days, according to agar plate

technique (Raimbault and Alazard, 1980). Spores were harvested and counted in a Neubauer chamber under a microscope to prepare the appropriate inoculum size for inoculation.

Substrate preparation

The water hyacinth leaf and whole plant powder were mixed with distilled water at the ratio of 100 ml 300 g^{-1} in heat resistant bags) and inoculated with *Aspergillus niger* inoculum at 10^8 spores g^{-1}. The bags were kept at 28°C for about eight weeks at Ecology and Ecophysiology Laboratory, Addis Ababa University. Samples were taken in triplicates at weekly interval and oven-dried at 60°C for 48 hours.

Biochemical analysis of fermented and unfermented water hyacinth samples

The product samples were dried at 80 ± 1°C to a constant moisture level. They were finely ground for biochemical analysis for crude protein (CP), crude fiber (CF), nitrogen free extract (NFE), and crude lipid and ash contents following AOAC (2000). Crude protein was determined by micro-Kjeldahl digestion (N x 6.25) and distillation after acid digestion using a Kjeltec 1026. Ash was determined by incineration at 550°C for 12 h in a Muffle furnace to constant weight. Crude lipid was determined by Soxhlet extraction with diethyl ether at 40–60°C for 7–8 h, while crude fiber content was determined as a loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH. All measurements were conducted three times taking the average for each sample.

Statistical analysis

The data on nutritional attributes of the unfermented and fermented water hyacinth leaf and whole plant at various fermentation periods were analyzed by SPSS (ver.21).

RESULTS AND DISCUSSION

Crude protein, crude lipid and dry matter content of water hyacinth

Solid state fermentation with *Aspergillus niger* induced significant variation ($p<0.05$) of the product in crude protein content (CP), crude lipid (CL) and dry matter (DM) of leaves and whole parts of water hyacinth compared to the unfermented control plants (Table 1).

Table 1. Dry matter, crude protein and crude lipid contents of fermented and unfermented water hyacinth leaf and whole (on dry weight basis).

	Nutrient composition*Plant part					
Time/ Weeks	**DM. Leaf**	**DM. Whole**	**CP. Leaf**	**CP. Whole**	**CL. Leaf**	**CL. Whole**
0	92.78 ± 0.10	93.89 ± 0.02	9.49 ± 0.01	5.92 ± 0.03	4.76 ± 0.20	4.92 ± 0.01
1	91.5 ± 0.76	92.6 ± 0.10	14.63 ± 0.03	12.24 ± 0.01	4.67 ± 0.12	4.68 ± 0.02
2	89.02 ± 0.01	90.63 ± 0.15	16.06 ± 0.02	14.90 ± 0.02	4.54 ± 0.03	4.64 ± 0.01
3	86.047 ± 0.03	88.16 ± 0.02	19.56 ± 0.01	17.30 ± 0.02	4.45 ± 04	4.60 ± 0.10
4	84.95 ± 0.06	85.07 ± 0.02	23.88 ± 0.01	20.11 ± 0.01	4.11 ± 0.11	4.20 ± 0.10
5	83.56 ± 0.49	84.02 ± 0.02	25.89 ± 0.02	23.80 ± 0.01	3.94 ± 0.01	3.91 ± 0.04
6	81.24 ± 0.37	82.04 ± 0.05	29.45 ± 0.02	26.61 ± 0.02	3.03 ± 0.22	3.78 ± 0.04
7	79.31 ± 0.50	80.98 ± 0.01	25.66 ± 0.01	21.24 ± 0.01	2.75 ± 0.01	2.91 ± 0.02
8	77.4 ± 0.10	81.98 ± 0.01	22.38 ± 0.01	17.53 ± 0.02	1.94 ± 0.01	1.03 ± 0.02

Note: DM = Dry matter; CP = Crude protein; CL = Crude lipid

There were also significant variations on protein content of both water hyacinth leaf and whole plant among different fermentation periods. Accordingly, protein content increased along with fermentation periods (Fig. 1) and reached the maximum at sixth week (29.45 ± 0.02 and 26.61 ± 0.02%) for leaf and whole plant, respectively.

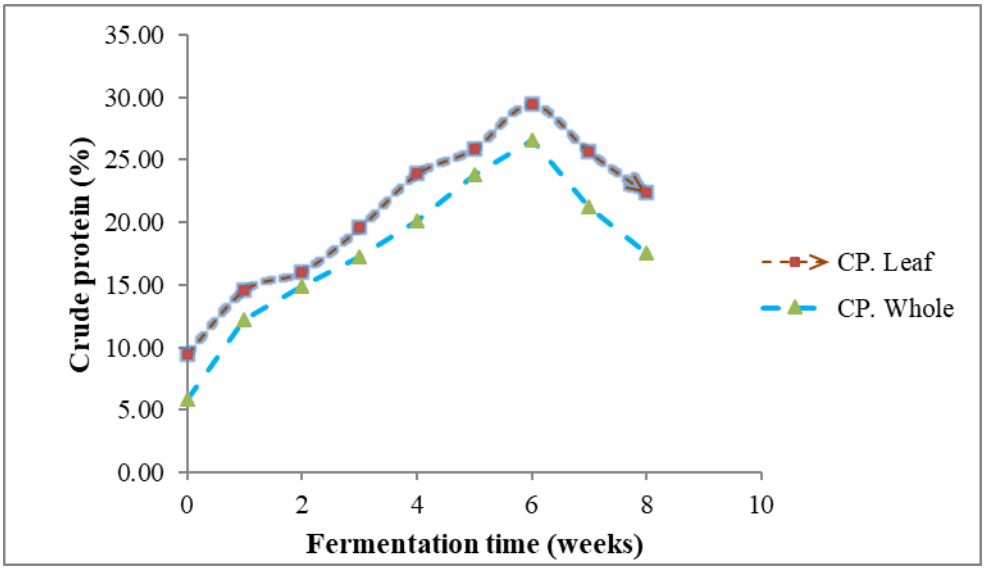

Fig. 1. Effect of fermentation on crude protein content (%) of water hyacinth leaf and whole plant.

The increase in protein content of water hyacinth after fermentation with *Aspergillus niger* might be due to the production of urease enzymes that break urea into amino acids and CO_2 (Wahyuni *et al*., 2018). Suthar and Singh (2008) also reported that secretion of extracellular enzymes like

xylanases and cellulases into the fermentation media decrease the carbohydrate contents; whereas proliferation of the fungus increase protein in the form of single cell protein (SCP) of fermented products.

Mangisah *et al*. (2010) also indicated that the nutritive value of diet from fermented WHL with *Aspergillus niger* significantly increased CP digestibility, true metabolizable energy (TME) and nitrogen retention (NR) of water hyacinth. The importance of *Aspergillus niger* to enhance protein content in fermentation process was also reported on cocoa bean shells (Bentil, 2015), sour cherry kernel (Güngör *et al*., 2017) and pineapple waste (Omwango *et al*., 2013). The present study is also in agreement with the findings of Shamim *et al*. (2017), who showed the protein enrichment of whole water hyacinth after solid state fermentation by *Pleurotus sajor-caju* for use as animal feed.

Apart from increasing the protein content, fermentation caused a significant decrease ($p<0.05$) in dry matter content of both fermented water hyacinth leaf and whole plant compared to the unfermented ones (Table 1). Bhatnagar (2004) also reported significant ($p<0.05$) reduction in dry matter content throughout the fermentation period for wheat bran using *Aspergillus niger*, suggesting utilization of nutrients present in the substrate by fungi for its growth and metabolic activities. It is also indicated that the reduction in dry matter content is due to the intake of fibrous components by *Aspergillus niger* (Do Santos *et al*., 2015).

The present study showed a decrease in crude lipid content of both fermented water hyacinth leaf and whole plant (Fig. 2). Shamim *et al*. (2017) also observed a decrease in crude lipid contents of water hyacinth after solid state fermentation technology. A study by Abu *et al*. (2000) also shows a similar trend in lipid content of sweet potatoes after solid state fermentation using *Aspergillus niger*. It is suggested that fermentation by microbes enhances assimilation of lipid from substrates for biomass production and cellular activities leading to a general reduction of the overall lipid content (Iluyemi *et al*., 2006; Lateef *et al*., 2008).

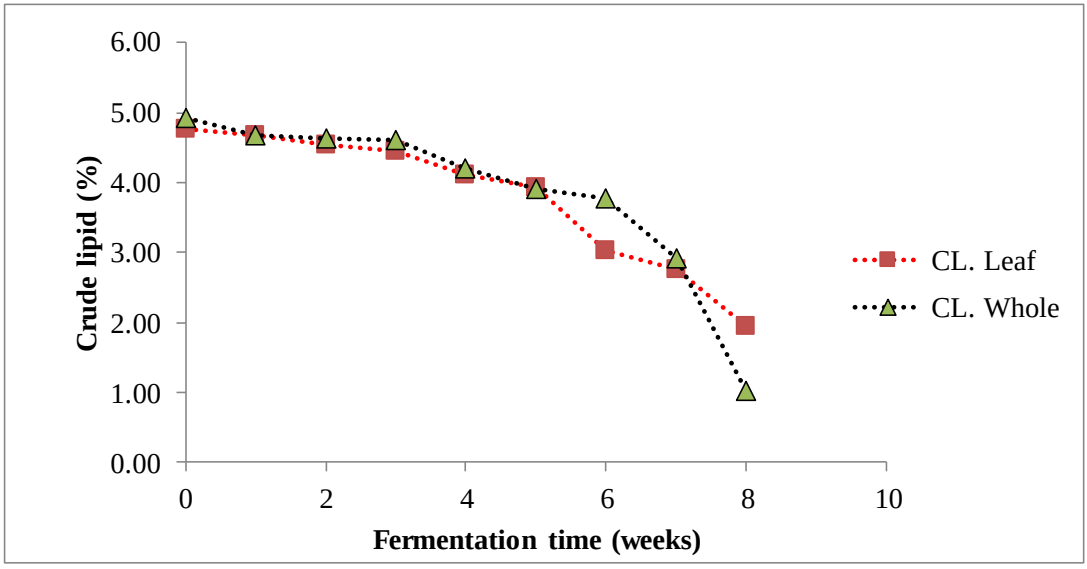

Fig. 2. Effect of fermentation on crude lipid (%) content of water hyacinth leaf and whole plant.

Crude fiber, Nitrogen free extract and ash content of water hyacinth

The fermentation process also affected the crude fiber (CR), carbohydrate content (nitrogen free extracts, NFE) and ash contents of the water hyacinth (Table 2). Thus, the fermentation process significantly decreased CR and NFE. There was a significant decline ($p<0.05$) in the fiber content of water hyacinth observed from the start of fermentation from 30% to 7.92% upon 8th week fermentation period (Fig. 3). There was also a significant decline ($p<0.05$) in the fiber content of whole water hyacinth part from 33% to 15.78% at the beginning and the end of 8th week fermentation period, respectively (Table 2). Microbial enzymes secreted by the mold during the fermentation process degrade the cell wall components and cause a decrease in crude fiber content of the fermented product compared to the unfermented ones (Oboh, 2006). The decrease in fiber content of fermented water hyacinth by *Aspergillus niger* is also in line with the study of Mangisah *et al*. (2010).

Table 2. Crude fiber, nitrogen free extracts and ash contents of fermented and unfermented water hyacinth leaf and whole.

	Nutrient composition*Plant part					
Time/ Weeks	**CF. Leaf**	**CF. Whole**	**NFE. Leaf**	**NFE. Whole**	**Ash. Leaf**	**Ash. Whole**
0	29.78 ± 0.01	32.97 ± 0.24	31.56 ± 0.25	41.37 ± 0.03	19.12 ± 0.01	21.86 ± 0.05
1	27.66 ± 0.02	28.38 ± 0.25	29.78 ± 0.18	39.61 ± 0.09	21.26 ± 0.45	23.03 ± 0.02
2	24.87 ± 0.17	25.57 ± 0.17	28.75 ± 0.03	38.47 ± 0.05	22.78 ± 0.11	24.85 ± 0.13
3	21.11 ± 0.02	23.56 ± 0.04	27.34 ± 0.06	38.23 ± 0.02	22.81 ± 0.12	24.89 ± 0.06
4	18.23 ± 0.15	21.08 ± 0.01	26.63 ± 0.05	37.47 ± 0.15	23.40 ± 0.03	25.56 ± 0.05
5	15.85 ± 0.05	18.22 ± 0.03	26.38 ± 0.01	36.52 ± 0.03	23.89 ± 0.15	26.05 ± 0.18
6	10.08 ± 0.07	16.56 ± 0.01	25.50 ± 0.10	34.48 ± 0.05	24.63 ± 0.02	27.03 ± 0.01
7	8.45 ± 0.02	14.67 ± 0.02	24.05 ± 0.02	32.33 ± 0.01	25.78 ± 0.05	27.89 ± 0.12
8	7.92 ± 0.04	14.03 ± 0.01	23.49 ± 0.12	30.33 ± 0.02	27.71 ± 0.02	30.61 ± 0.15

Note: CF = Crude fiber; NFE = Nitrogen free extract

Doughan and Dzogbefia (2018) also showed a decrease in crude fiber content of yam peels after solid state fermentation by *Aspergillus niger* The decrease in fiber content of fermented water hyacinth is important, as high fiber content is a sign of having less nutritive value of the feed and prediction of total digestible nutrients and net energy .

Carbohydrate content (NFE) of water hyacinth also decreased slowly (Table 2) (Fig. 4). Thus, the decrease was from 32–24% from the leaf part and 41–30% from the whole part of the plant, respectively as a function of fermentation. In addition, when the different parts were compared, the whole water hyacinth contained higher carbohydrate content than the leaf parts (Fig. 4). In general, the decrease in carbohydrates is that the microbes involved in fermentation convert the lignocellulosics into simple sugars for different physiological activities (Akinyele *et al*., 2011). Other researchers also reported a decrease in carbohydrate contents of pearl millet after solid state fermentation (Osman, 2011).

There was an increase in the ash contents of fermented water hyacinth by *Aspergillus niger* (Table 2 and Fig. 5) throughout the various fermentation periods compared to both the unfermented water hyacinth leaf and whole plant. Since ash determination is a measure of mineral level, it can be inferred that solid state fermentation contributed to the elevation of mineral levels in both the fermented water hyacinth leaf and whole plant. Other studies also showed similar improvement of ash content during solid state fermentation (Shamim *et al*., 2017 and O'Toole, 1999).

In general, the increase in the ash content of the fermented products could be attributed to the enrichment of minerals by mycelia of the fungi grown on water hyacinth leaf and whole plant and the loss of organic carbon due to

utilization by the fermenting microbe (Doughan and Dzogbefia, 2018). The present finding on an increment of ash content after solid state fermentation is also in agreement with Victor (2019).

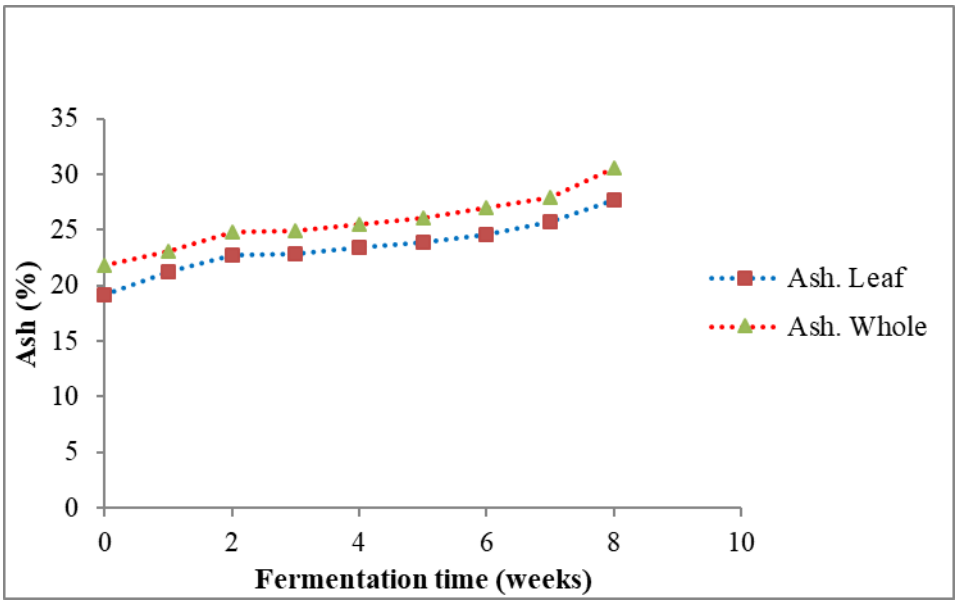

Fig. 5. Effect of fermentation on ash content of water hyacinth leaf and whole plant.

CONCLUSION AND RECOMMENDATIONS

From the present study, it is evident that harvesting of water hyacinth on week six yielded the highest level of crude protein. At these peak levels, the fermented water hyacinth leaf and whole plant also contained an acceptable level of fiber and modest levels of NFE and ash. Although both the leaf and whole water hyacinth improved their nutritive value after fermentation, the fermented leaf exhibited the highest crude protein and the lowest crude fiber content over the whole water hyacinth plant. All of these changes enhance the value of water hyacinth as an animal feed. The agricultural benefit of these changes to the composition of water hyacinth by the fungus needs to be tested in animal feeds, including fish feed.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Addis Ababa Science and Technology University for providing all laboratory facilities used to analyze feed samples proximate composition. Special thanks go to Mr. Afrasa Mulatu for

his support in culturing microorganisms at Mycology Laboratory, Department of Microbial, Cellular and Molecular, Addis Ababa University.

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