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Nutritional composition of four different species of *Azolla*

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ABSTRACT

An attempt has been made to investigate the proximate composition, amino acid and fatty acid content of the four different species of Azolla. Fresh Azolla samples were tested for proximate composition using the AOAC (2000) methodology. Amino acid and fatty acid compositions were determined using High Pressure Liquid Chromatography (ion exchange principle) and Gas chromatography. The proximate composition analysis in four species of Azolla, namely A. microphylla, A. filiculoides, Wrong finger, and a TNAU hybrid are as follows: Moisture (91.77 - 92.25%), crude fat (0.6 - 1.8%), crude protein (3.9 - 5.2%), ash (2%), and carbohydrates (0.2 - 1%). The results of amino acid analysis indicated the presence of all amino acids except for cysteine which was either undetectable or present in small quantities. The range of essential amino acid percentages was 40.53 - 48.75% and that of non-essential was 51.25 - 57.92% in the four species. Total PUFA (%) was high among the total fatty acids detected with 64.12, 62.12, 52.13, and 35.66% in A. microphylla, A. filiculoides, the TNAU hybrid and Wrong finger, respectively while the MUFA (%) was 18.92, 17.50, 20.75, 24.86% and the saturated fatty acid content was 15.32, 18.60, 16.15 and 37.56% for the same species. The ω3: ω6 ratio was 1.46 - 2.34. Based on the results of this investigation, Azolla could be considered as a nutritive aquatic fern.

Keywords: Aquatic fern, Nutritive value, Wrong finger, TNAU Hybrid

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INTRODUCTION

There is always an ever ending search for the availability of nutritionally rich and cheap food resources in the developing countries. Aquatic plants are gaining much interest in food and biomedical research, owing to its broad range of uses such as human food, animal feed and bio-fertilizers [1]. In the modern world, due to the changes in the life style, nature of work and food habits, the incidents of serious diseases like Coronary heart diseases, Obesity, Diabetes are more frequent in the younger generation. This situation apparently demands for the search for medicinally active as well as nutritionally rich non-conventional food sources. *Azolla* is one genus of interest which grows in a symbiotic association with the blue-green alga, *Anabaena azollae*. It may be considered favorably for human consumption [2], because of its nutritive value and ease of cultivation [3-4]. It is traditionally used as bio-fertilizer [5-6], live-stock feed [7] and nutritional supplement [8]. Globally, the food research mainly emphasis on the production of high quality foods and feeds of plant origin, as the green plants are recognized as excellent sources of proteins, fats and pharmacologically active secondary metabolites. Recent study reveals that the aquatic plants are good sources of primary and secondary metabolites. Although much of the research investigations in *Azolla* were done to explore its bio-fertilizing activities; very fewer studies were carried out to investigate its nutritional profiling.

Hence this study aimed to evaluate the proximate, amino acid, and fatty acid composition of fresh *Azolla* species namely *Azolla microphylla* (AM), *Azolla filiculoides* (AF), Wrong finger (WF; hybrid of *A. microphylla* + *A. filiculoides*), and a TNAU hybrid (TH; hybrid of *A. microphylla* + *A. pinnata*) grown under homogenous conditions.

MATERIALS AND METHODS

Chemicals

Concentrated sulphuric acid, copper sulphate, potassium sulphate, Tashiro's indicator, tri sodium citrate, boric acid, distilled ethanol, *o*-phthalaldehyde, sodium carbonate, 2-mercaptoethanol, Brij35(polyoxy ethylene lauryl ether), sodium hypochlorite, petroleum ether, chloroform (Excelar grade), methanol (AR grade), anhydrous sodium sulphate, fatty acid methyl ester standards, sodium hydroxide, boron trifluoride, amino acid standards were all purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sample collection

The four varieties of fresh *Azolla* namely *Azolla microphylla* (AM), *Azolla filiculoides* (AF), Wrong finger (WF) and a TNAU hybrid (TH) were collected from the Agricultural Microbiology Department of Tamil Nadu Agricultural University (TNAU) and further propagated in four separate tanks under homogenous conditions (natural day light, with humid temperature, and no additional nutrients added other than soil and water available on campus) at the Central Institute of Fisheries Technology, Cochin. Before beginning the analysis, samples were washed thoroughly with tap water and rinsed with distilled water.

Determination of Proximate composition

Pre-weighed fresh *Azolla* whole plants were taken in a Petridish and kept in an oven maintained at approximately 105ºC for 24H. The difference in weight was used to calculate the moisture content. The crude protein content was determined by estimating the total nitrogen content using Kjeldahl method and multiplied by a factor of 6.25. For the determination of crude fat, the sample was placed in a cotton-plugged thimble and placed in a Soxhlet apparatus and extracted with petroleum ether for 16 hrs. The ether was removed by evaporation and the flask with fat was dried at 80 - 100 ºC, cooled in a desiccator and weighed. The ash content was determined by incineration in a muffle furnace at 600ºC for 8 hours. The carbohydrate content was determined by 100 - (total moisture + crude protein + crude fat + ash). All these analyses were conducted according to the AOAC methodology [9].

Analysis of Amino acids

Fresh samples of *Azolla* species were finely minced in mortar and pestle with 6N HCl and transferred the contents to a test tube which was sealed latter. It was then kept in an oven at 120°C for 24 hrs. These contents were filtered, flash evaporated and made up to volume with 0.05N HCl. This solution was filtered through a 0.45 µm pore size filter (Whatman, UK) and injected into an HPLC (Shimadzu-LC 10 AS). The amino acids were separated in a sodium ion exchange column (Shimadzu-CTO-6A) fitted with an oven maintained at 60°C. The post column-derivatized amino acids were detected using a Shimadzu- FLD-6A-type fluorescence detector [10]. Tryptophan was determined separately by a spectrophotometric method [11], because it is labile to the hydrolysis conditions followed above for HPLC.

Analysis of fatty acids

The lipid content of the fresh *Azolla* plants were estimated using chloroform/methanol as a solvent in the ratio of 2:1 [12]. Fatty acid methyl esters (FAME) were analyzed using the standard methods [13]. FAMEs from animal and plant origin were formed by heating fatty acid with BF_3-methanol and methanolic sodium hydroxide. The methyl esters formed were separated and detected by a Trace Gas Chromatography Ultra (GC-Varian CP 3800 USA). Nitrogen was used as the carrier gas at a flow rate of 0.8ml/min, with an FID (Flame Ionisation Detector). The temperature profiles were as follows: Initial temperature = 40°C; heating rate = 2.7°C/min; final temperature = 110°C; injector temperature = 260°C; detector temperature = 275°C. Fatty acids separated were identified by comparing RT (retention time) with those obtained by a mixture of standard fatty acids and quantified using Thermochrom card software (Thermo corporation). Individual fatty acids were expressed as percentage of total fatty acids detected.

Statistical analysis

Experimental values were mean ± SD from three separate experiments except for total carbohydrates whose values were calculated as mentioned in the methodology section. The mean and SD values were calculated using Microsoft Excel-2007 software.

RESULTS AND DISCUSSION

Proximate composition of Azolla

The proximate composition of *Azolla* in the four different species is shown in (Table 1). A high percentage of water content ranging from 91.4 in WF to 92.25 in AM was observed. There observed a considerable amount of variation in the crude protein content of *Azolla* species: 3.9% in WF, 4.15% in TH and AM, 4.65% in AF. The nitrogen content of *Azolla* varied from 0.63% in WF to 0.74% in AF (wet weight). This high protein content could be due to the high nitrogen content fixed by the endosymbiotic nitrogen-fixing bacterium, *Anabaena azolla*e. The variation in the heterocyst frequency of the endosymbiont and also the maturity of leaf affects the nitrogen-fixing ability of *Azolla* [3]. This may explain the variation in nitrogen content among the four species. The crude protein content determined was considerably high when compared with crude protein content of water hyacinth (*Eicchornia crassipes*) and *Azolla Mexicana* [14-15] The percentage of crude fat ranged from 0.59 - 1.8%. The hybrids (WF and TH) have a relatively higher amount of crude fat than the non-hybrid AM and AF, possibly as a result of hybridization. The total ash content was similar in all species and the total carbohydrates ranged from 0.59 - 1%. Generally, floating macrophytes have lower fiber levels. The lower carbohydrate content indicates that *Azolla* has lower fiber content.

Table 1. Proximate composition (g/100g of wet *Azolla*)

Plants	Moisture	Protein	Ash	Crude fat	Carbohydrate	Total nitrogen
AF	91.81 ± 0.30	4.65 ± 0.2	2 ± 0.1	0.72 ± 0.02	0.82	0.74 ± 0.03
TH	91.77 ± 1.04	4.15 ± 0.38	2 ± 0.13	1.49 ± 0.14	0.59	0.66 ± 0.06
AM	92.25 ± 1.56	4.15 ± 0.19	2 ± 0.16	0.59 ± 0.09	1.00	0.66 ± 0.03
WF	91.4 ± 0.87	3.9 ± 0.31	2 ± 0.05	1.8±0.05	0.90	0.63 ± 0.05
AF: Azolla filiculoides, TH: TNAU Hybrid, AM: Azolla microphylla, AF: Azolla filiculoides						

Table 2. Amino acids composition (g/100g protein) of *Azolla*

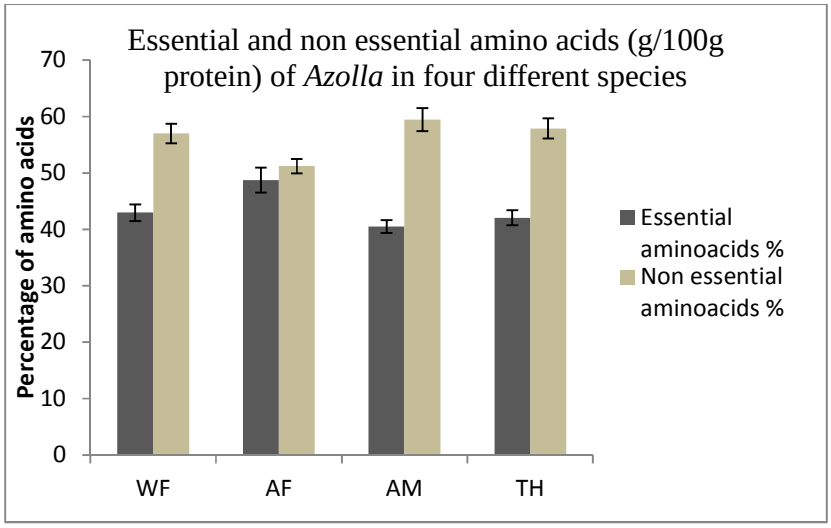
Amino acids	AM	WF	TH	AF
Threonine	7.61 ± 0.12	8 ± 0.20	7.69 ± 0.26	6.56 ± 0.20
Valine	7.67 ± 0.30	8.67 ± 0.25	7.37 ± 0.53	6.93 ± 0.35
Leucine	10.6 ± 0.32	10.26 ± 0.52	10.56 ± 0.19	9.24 ± 0.8
Isoleucine	5.75 ± 0.19	6.65 ± 0.33	5.53 ± 0.11	6.02 ± 0.09
Phenyl alanine	5.1 ± 0.12	5.54 ± 0.05	5.12 ± 0.13	4.66 ± 0.29
Lysine	2.34 ± 0.06	2.06 ± 0.03	3 ± 0.04	12.82 ± 0.39
Methionine	0.46 ± 0.12	0.43 ± 0.12	0.51 ± 0.12	0.52 ± 0.12
Tyrosine	0.92 ± 0.03	1.22 ± 0.04	0.93 ± 0.02	0.34 ± 0.01
Aspartic acid	11.58 ± 0.53	10.77 ± 0.27	10.97 ± 0.35	10.46 ± 0.19
Glutamic acid	14.2 ± 0.25	11.7 ± 0.39	11.68 ± 0.45	11.28 ± 0.19
Serine	9.36 ± 0.26	9.54 ± 0.36	9.58 ± 0.18	7.83 ± 0.31
Proline	1.62 ± 0.03	1.6 ± 0.01	1.53 ± 0.03	1.43 ± 0.06
Glycine	8.59 ± 0.39	8.87 ± 0.27	9.05 ± 0.15	7.57 ± 0.26
Alanine	7.86 ± 0.39	7.63 ± 0.25	8.91 ± 0.33	6.81 ± 0.11
Cysteine	ND	ND	ND	ND
Histidine	3.3 ± 0.09	3.68 ± 0.05	3.17 ± 0.10	3.21 ± 0.11
Arginine	2.04 ± 0.05	2.01 ± 0.09	2.4 ± 0.15	2.32 ± 0.02
Tryptophan	1 ± 0.02	1.37 ± 0.07	2.3 ± 0.09	2 ± 0.06
Essential amino acids %	40.53 ± 1.14	42.98 ± 1.47	42.08 ± 1.36	48.75 ± 2.2
Non essential amino acids %	59.47 ± 2.02	57.02 ± 1.72	57.92 ± 1.76	51.25 ± 1.26
AM: Azolla microphylla, WF: Wrong finger, TH: TNAU Hybrid, AF: Azolla filiculoides, %: percentage, ND: Not Detected				

Amino acid composition of Azolla

The protein quality is a function of amino acids present. Individual amino acid content among the four species of *Azolla* is shown in (Table 2). All the essential amino acids were present ranging from 40.53% in AM to 48.75% in AF. Among the essential amino acids, WF contained the maximum percent of threonine (8%), valine (8.67%), isoleucine (6.65%) and phenylalanine (5.54%). Lysine and methionine were present maximum in AF with 12.82%

and 0.52%, respectively. AM contained high proportion of leucine (10.6%) and TH had high tryptophan content (2.3%). The essential amino acids that can only be provided in the diet must be adequate for normal protein turnover in the body. The amino acid content of the analyzed plants compared favorable and well exceeded with the WHO "ideal pattern of adults" (% of amino acids) except for phenylalanine and tryptophan [16]. The essential amino acids content was also higher than the essential amino acids content present in *Azolla mexicana* except for methionine [15]. This could be due to the differences in the geographical conditions and water environment. The tryptophan content in all the four species was higher than the tryptophan content of *Spirulina* which had only 0.93g/100g of protein, USDA standard reference [17]. A higher proportion of non-essential amino acids were also illustrated by the four species in the range of 51.25 - 59.47% out of the total amino acids obtained. The aspartate and glutamate were the predominant non-essential amino acids detected in the range of 10.4 - 11.6% and 11.28 - 14.2%. The values of aspartate and glutamate are slightly higher than the values reported by Carranco *et al* in AM and Leterme P *et al* in AF [15, 18]. In all the species, cysteine was either undetectable or present in very minute quantities, which is in concordance with earlier studies [8]. The levels of essential and non-essential amino acids in the four species of *Azolla* are shown in (Figure 1).

Figure 1. Levels of essential and non essential amino acids content in four species of *Azolla*



Fatty acid composition of Azolla

In general, the nutrient content of the aquatic macrophytes could vary depending on the season, the place, the water and the morphology of the plants [19]. The percentage composition of PUFAs among the four species is shown in (Table3). The following *Azolla* species namely AF, AM and TH had the highest proportion of PUFAs (62 - 64%) and AM, TH, WF contained maximum content of MUFAs that accounted for (17 - 24%), out of the total fatty acids detected. Essential fatty acids are fatty acids that cannot be synthesized within an organism from other components by any known chemical pathways, and therefore must be obtained from the diet. The alterations in lipid membrane [20], salinity levels [21] and geomagnetic field [22] can significantly affect the fatty acid compositions of a plant. Among the ω-3 and ω-6 family of fatty acids, linolenic acid was the predominant fatty acid present in the range of 19.8 - 37.95% followed by linoleic acid (5.11 - 15.38%). The linolenic acid, linoleic acid and arachidonic acid are few important members of the ω-3 and ω-6 series of PUFAs which were present in good proportion in *Azolla*. The values of linolenic acid were higher than the content, present in some of the edible oils [23]. The ratio of ω-3 to ω-6 was 1.4 - 2.3 which is shown in (Table 4). In the past 100 years, changes in the food habits have caused the ω3/ω6 fatty acids ratio to fall below 0.1 [24]. There is a need of balance in our consumption of ω-3 and ω-6 fatty acids in order to stay healthy. In the other hand, expert suggests a 1:1 ratio and never more than 2:1 as a healthy index [25]. This ratio in all the species of *Azolla* compared well within or slightly higher than the limits prescribed in various reports. Arachidonic acid helps to protect the brain from oxidative stress and maintain hippocampal cell membrane fluidity [26]. Eicosapentanoic acid (EPA) content was detected at minimum levels in all the species except TH which contained maximum of 4.9%. A lesser quantity of fatty acids like γ - linolenic acid, docosohexaenoic acid, cis-13,16-Docosadienoic acid, cis-11,14,17-Eicosatrienoic acid, cis-8,11,14- Eicosatrienoic acid were also present. Among omega-3 fatty acids, EPA is thought to possess healing mental disorders, such as schizophrenia [27-28].

EPA value meets with the EPA content in some of the fishes like herring, catfish which contained 6 and 1% respectively [29].

Table 3. Polyunsaturated fatty acids percentage (in terms of total fatty acid) in *Azolla*

Carbon length	Fatty acids	WF	AF	AM	TH
C18:2(n6)	Linoleic acid	10.32 ± 0.26	15.26 ± 0.15	15.38 ± 0.30	5.11 ± 0.12
C18:3(n6)	γ - Linolenic acid	0.37 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	1.03 ± 0.02
C18:3(n3)	Linolenic acid	19.80 ± 0.75	37.52 ± 0.55	37.95 ± 0.30	30.64 ± 0.33
C20:2(n6)	cis- 11, 14- Eicosadienoic acid	0.09 ± 0.01	0.15 ± 0.05	0.05 ± 0.01	0.42 ± 0.09
C20:3(n6)	cis-8, 11, 14- Eicosatrienoic acid	0.59 ± 0.04	0.90 ± 0.04	0.88 ± 0.05	2.56 ± 0.09
C20:4(n6)	Arachidonic acid	2.85 ± 0.02	5.41 ± 0.3	5.84 ± 0.33	6.41 ± 0.21
C20:3(n3)	cis-11, 14, 17-Eicosatrienoic acid	0.24 ± 0.02	0.19 ± 0.07	0.26 ± 0.01	0.57 ± 0.04
C20:5(n3)	cis-5, 8, 11, 14, 17-Eicosapentanoic acid	0.89 ± 0.05	2.36 ± 0.05	3.00 ± 0.01	4.90 ± 0.25
C22:2(n6)	cis-13, 16-Docosadienoic acid	0.29 ± 0.02	0.11 ± 0.01	0.10 ± NA	0.10 ± NA
C22:6(n3)	cis-4, 7, 10, 13, 16, 19-Docosahexaenoic acid	0.23 ± NA	0.12 ± NA	0.54 ± NA	0.43 ± NA
Total percent		35.66 ± 1.17	62.13 ± 1.22	64.13 ± 1.03	52.13 ± 1.15
WF: Wrong finger, AF: Azolla filiculoides, AM: Azolla microphylla, TH: TNAU Hybrid, NA: Not applicable					

Table 4. Ratio of omega 3/omega 6 fatty acid in *Azolla*

Fatty acids	WF	AF	AM	TH
% of ω3	21.17	40.19	41.76	36.53
% of ω6	14.50	21.94	22.37	15.61
Ratio of ω3/ ω6	1.46	1.83	1.87	2.34

The percentage composition of MUFAs in *Azolla* is shown in (Table 5). The major MUFAs observed were oleic acid (OA) and palmitoleic acid (PA), which was present maximum in TH and WF. Oleic acid content was nearly two times higher in TH (11.83%) than in WF, AF and AM, respectively. On the other hand, WF had the highest palmitoleic acid (15.38%) while TH had the lowest. It has been reported that the new occurrence of cardiovascular abnormalities could be prevented by the dietary intake of 13 % OA in the total caloric value, but an increase to 20 % of the same fatty acid could limit this beneficial potential by increasing the LDL levels in the blood [24]. New research suggests that methyl and ethyl esters of palmitoleic acid are shown to possess strong antimicrobial activity against some of the oral pathogens like *Streptococcus mutans*, *Candida albicans* and few others [30]. Other MUFAs like erucic and nervonic acid were present in trace amounts.

Table 5. Monounsaturated fatty acids percentage (in terms of total fatty acid) in *Azolla*

Carbon length	Fatty acids	WF	AF	AM	TH
C14:1	Myristoleic acid	0.01 ± 0.01	0.55 ± 0.04	0.21 ± 0.01	1.47 ± 0.05
C15:1	cis-10-Penta decenoic acid	0.23 ± 0.01	0.19 ± 0.03	0.14 ± 0.01	0.32 ± 0.02
C16:1	Palmitoleic acid	15.38 ± 0.9	8.95 ± 0.5	11.12 ± 0.5	0.44 ± 0.02
C17:1	cis-10 Heptadecenoic acid	0.64 ± 0.01	0.12 ± 0.01	0.12 ± 0.02	0.35 ± 0.03
C18:1(n9)	Oleic acid	6.99 ± 0.12	6.50 ± 0.4	6.09 ± 0.3	11.83 ± 0.8
C20:1	cis-11-Eicosenoic acid	0.85 ± 0.06	0.88 ± 0.02	0.91 ± 0.01	5.80 ± 0.16
C22:1(n9)	Erucic acid	0.32 ± 0.01	0.1 ± NA	0.18 ± NA	0.04 ± 0.02
C24:1(n9)	Nervonic acid	0.36 ± 0.01	0.21 ± 0.01	0.14 ± 0.02	0.51 ± 0.01
Total percent		24.86 ± 1.13	17.50 ± 1.01	18.92 ± 0.87	20.76 ± 1.09
WF: Wrong finger, AF: Azolla filiculoides, AM: Azolla microphylla, TH: TNAU Hybrid, NA: Not applicable					

The percentage of saturated fatty acids is shown in (Table 6)**.** There observed the presence of saturated fatty acids in the range of (15 - 37%) and also a small amount of saturated fatty acids with odd number of carbon chain. Palmitate content was illustrated high (12.3%) in WF. TH had the highest myristate content (4.24%) while WF had the lowest, lignoceric acid in WF was nearly double the amount recorded in AM, while TH registered the least. Among the saturated fatty acids a good proportion of palmitic acid was observed, which is higher than the green leaves palmitate content reported by Vidrin *et al* [31]. Myristic acid is a very important fatty acid, which the body utilizes to stabilize many different proteins, including proteins used in the immune system and to fight tumors. Thus, the loss of myristic acid from the diet can have unfortunate consequences, including cancer and immune system dysfunction. Lignin is present in all vascular plants and that may be considered as a reason for its high amounts. Other saturated fatty acids were also present in minor quantities. The saturated fatty acids with odd number of carbon atoms like undecanoic acid, tridecylic acid, pentadecylic acid and margaric acid were observed in negligible amounts. The odd chain fatty acids are found predominantly in many bacterial species [32]. The presence of endosymbiotic nitrogen

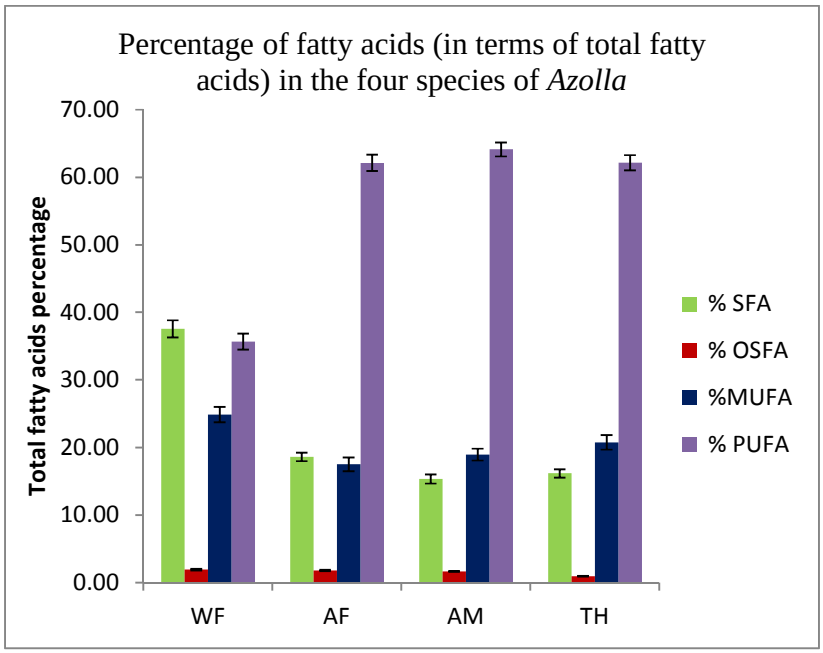
fixing bacterium *Anabaena azolla*e, may have contributed to the detection of the trace amounts of saturated fatty acids with odd carbon number in *Azolla*. The levels of total fatty acids detected in the four species are shown in (Figure 2).

Table 6. Saturated fatty acids percentage (in terms of total fatty acid) in *Azolla*

Carbon length	Fatty acids	WF	AF	AM	TH
C10	Capryic acid	0.15 ± 0.01	0.52 ± 0.03	0.18 ± 0.01	0.42 ± 0.01
C12	Lauric acid	0.93 ± 0.04	1.96 ± 0.08	0.78 ± 0.01	2.21 ± 0.1
C14	Myristic acid	2.59 ± 0.05	3.02 ± 0.19	3.12 ± 0.12	4.24 ± 0.25
C16	Palmitic acid	12.63 ± 1.05	0.12 ± 0.01	0.38 ± 0.03	0.11 ± NA
C18	Stearic acid	2.96 ± 0.05	2.76 ± 0.01	2.69 ± 0.16	5.76 ± 0.08
C20	Arachidonic acid	0.38 ± 0.01	0.33 ± NA	0.29 ± NA	0.88 ± 0.06
C22	Behenic acid	0.86 ± 0.01	0.69 ± 0.03	0.60 ± NA	2.28 ± 0.06
C24	Lignoceric acid	17.06 ± 0.66	9.21 ± 0.21	7.29 ± 0.33	0.25 ± 0.05
Total percent		37.56 ± 1.27	18.60 ± 0.62	15.32 ± 0.66	16.15 ± 0.61

WF: Wrong finger, AF: Azolla filiculoides, AM: Azolla microphylla, TH: TNAU Hybrid, NA: Not applicable

Figure 2. Levels of PUFA, MUFA, SFA and saturated fatty acid with odd carbon chain (OSFA) in four species of *Azolla*



CONCLUSION

Based on the results of the study it is concluded that, *Azolla* could be used as an unconventional food source having a potential to include in diets. The results re-enforce the growing awareness that aquatic plants can contribute essential nutrients like amino acids and fatty acids. Although this study has revealed much about the amino acid and fatty acid compositions, additional knowledge remains to be secured regarding the metabolism of fatty acids in individual and hybrid varieties of *Azolla*. However further studies have to emphasis on clinical trials to prove its *in vivo* bioavailability and nutritive value efficiently.

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REFERENCES

[1] Balaji K, Jalaludeen A, Richard Churchill R, Peethambaran PA, Senthil KS, *Indian J Poult Sci*, **2009**, 44, 195.

[2] Waseem R, Preeti R, Suchit AJ, Pramod WR, *Inter J Res Biol Sci*, **2012**, 2, 68.
[3] Waseem R, Preeti R, Suchit AJ, Pramod WR, *Hacettepe J Biol Chem*, **2012**, 40, 1.
[4] Alalade OA, Iyayi EA, *Indian J Poult Sci*, **2006**, 5, 137.
[5] Ladha JK, Dawe D, Ventura TS, Singh U, Ventura AW, Watanabe I, *Soil Sci Soc Am J*, **2000**, 64, 1993.
[6] van Hove C, Lejeune A, *Cyanobacteria in symbiosis*, Kluwer academic publishers, Dordrecht, Netherlands, **2002**, pp 179–193.
[7] Basak B, Pramani K, Rahmnan MS, Taradar SU, Roy BS, *Int J Poult Sci*, **2002**, 1, 29.
[8] Pabby A, Radha Prasanna, Sindh PK, *Proc Indian Natl Sci Acad B Biol Sci*, **2004**, 70, 301.
[9] Association of Official Analytical Chemists, Official methods of analysis, 15th Edn. AOAC International, Washington DC, **2000**.
[10] Ishida Y, Fujita, Arai K, *J Chromatogr*, **1981**, 204, 143.
[11] Sastry CSP, Tammuru MK, *J Food Sci Technol*, **1985**, 22, 146.
[12] Folch J, Less, Stanley HA, *J Biol Chem*, **1957**, 226, 497.
[13] Sankar TV, Mathew S, Anandan R, Asha KK, Mohanty BP, *Handbook on Nutrient Profiling of Fish*, Central Institute of Fisheries Technology, ICAR, **2010**, pp 50–51.
[14] Aboud AAO, Kidunda RS, Osarya J, *Livest Res Rural Dev*, **2005**, 17, 23.
[15] Carranco ME, Castillo RM, Escamilla A, Martinez M, Perez Gil F, Stephan E, *Cuban J Agric Sci*, **2002**, 36, 237.
[16] Glew RH, Kramer JKG, Hernandez M, Pastuszyn A, Ernst J, Ngouya DN, VanderJagt DJ, *Food*, **2010**, 4, 1.
[17] Harriet Volkmann, Ulisses Imianovsky, Jorge LB Oliveira, Ernanis, Sant Anna, *Braz J Microbiol*, **2008**, 39, 98.
[18] Leterme P, Londono Angela M, Munoz JE, Suarez J, Bedoya CA, Souffrant WB, Buldgen A, *Anim Feed Sci Technol*, **2009**, 149, 135.
[19] Kamal HS, Thanaa M El-Komi, Ebrahem M Eid, *Feddes Repert*, **2013**, 123, 37.
[20] Wang SY, Lin HS, *Sci Hortic (Amst)*, **2006**, 108, 35.
[21] Ben Taarit MK, Msaada K, Hosni K, Marzouk B, *Food Chem*, **2010**, 9, 951.
[22] Novitskaya GV, Kocheshkova TK, Novitskii YI, *Russ J Plant Physiol*, **2006**, 53, 638.
[23] Chowdhury K, Banu LA, Khan S, Latif A, *Bangladesh J Sci Ind Res*, **2007**, 42, 311.
[24] Stuchlík M, Žák S, *Biomed Pap (Olomouc)*, **2002**, 146, 3.
[25] Russell Blaylock, *The Blaylock wellness report*, **2005**, 2, 1-5.
[26] Wang Z, Liang C, Li G, Yu C, Yin M, *Chem-Biol Interact*, **2006**, 163, 207.
[27] Peet M, Brind J, Ramchand CN, Shah S, Vankar GK, *Schizophr Res*, **2001**, 49, 243.
[28] Song C, Zhao S, *Expert Opin Investig Drugs*, **2007**, 16, 1627.
[29] Bimbo AP, *Lipid Technol*, **2007**, 19, 176.
[30] Chifu B Huang, Brian George, Jeffery L Ebersole, *Arch Oral Biol*, **2010**, 55, 555.
[31] Vidrin R, Filip S, Hribar J, *Czech J Food Sci*, **2009**, 27, S125.
[32] Tomas Rezanka, Karl Sigler, *Prog Lipid Res*, **2009**, 48, 206.