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Research Article

Comparative Study on Fatty Acids Composition of *Lemna minor* (Duckweed) Cultured in Indoor Plastic Tanks and Outdoor Earthen Ponds

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Global interest in using duckweed (*Lemna minor*) as a substitute for fish, livestock, and human diets has spurred research on the mass culture of the species. There is a scarcity of information on the *L. minor* fatty acid composition in different aquaculture settings. A comparative study was carried out to investigate the fatty acid composition of *L. minor* cultured in indoor plastic tanks and outdoor earthen ponds for 30 days. During the culture period, culture facilities were fertilized using livestock manure. Fatty acid characterization was done using gas chromatography method. Fifteen fatty acids: five saturated (SAFAs), five monounsaturated (MUFAS), and five polyunsaturated (PUFAs) were identified. Fatty acid compositions varied between indoor and outdoor settings. Percentage composition of *L. minor* PUFAs cultured outdoor (37.13) was higher than that of indoor (21.96) settings. *L. minor* SAFAs percentage composition was higher in the indoor culture at 41.63% while that of outdoor was 33.75%. The composition of *L. minor* MUFAs in indoor tanks was higher (36.32%) than in outdoor earthen ponds (29.10%). This study indicated the presence of docosahexaenoic, eicosapentaenoic, linoelaidic, and eicosanoic acids in *L. minor* seldom reported in past studies. Paired Students' *t*-test indicated that the means of the fatty acid composition were significantly different ($p < 0.05$) in both settings, with docosahexaenoic showing the highest paired mean difference.

1. Introduction

Fatty acids (FAs) serve essential physiological roles in vertebrates, including fish and humans and are available in food webs through trophic interactions [1]. They are classified as saturated (SAFAs), monounsaturated (MUFAs), and polyunsaturated (PUFAs) based on the presence or absence and the number of double bonds between carbon atoms. PUFAs are classified into short- and long-chain (SC PUFA and LC PUFAs). SC PUFAs include linolenic and linoleic acids, while Omega 3 and 6 LC PUFAs include arachidonic (ARA), eicosapentaenoic (EPA), docosapetaenoic (DPA), and docosahexaenoic (DHA) [2, 3]. Sources of LC PUFAs are plants, seeds, aquatic algae, and macrophytes.

LC PUFAs are essential nutrients for metabolism in vertebrate heterotrophs [2, 4, 5]. Marine fish constitute the major sources of almost all Omega-3 LC PUFAs. Although most freshwater fish are sources of Omega-3 and -6 LC PUFAs, they are not able to synthesize them but obtain them from the diet, such as macrophytes and algae. Presence of linolenic and linoleic acid precursors in fish diets enables them to biosynthesize Omega-3 and -6 LC PUFAs. Freshwater fish can convert

linolenic and linoleic acid (SC PUFA) to Omega-3 and -6 LC PUFA due to the presence of desaturation enzymes, unlike marine fish [3, 6]. Humans obtain Omega-3 LC PUFAS through consumption of fish [3]. LC PUFA plays a vital role in regulating cell membrane properties that control the movement of metabolites in and out of the cell. They also act as precursors in the biosynthesis of hormones that regulate the reproductive cycle [6, 7]. Some of the health benefits for humans include: neural development in infants, improvement of immune and inflammatory responses, prevention of cancer, mental, cardiovascular, and chronic diseases [2, 4]

In aquaculture, LC PUFAs are essential for fillets' quality of harvested fish, feed conversion efficiency, and reproduction, all of which are important determinants of production [8, 9]. Most of the LC PUFAs used in aquafeeds are obtained from fish meal and animal oils [3], which are expensive and are increasingly under competition. The development of aquaculture from subsistence to semi with intensive production requires alternative feed ingredients to substitute expensive fish meals and animal oils. There is an urgent need to embrace cheap sources of PUFA ingredients for fish feed. Research efforts are directed at identifying cheap sources of FAs to be used in fish feed, including macrophytes such as duckweed (*L. minor*), aquatic fern (*Azolla* spp.), and water spinach (*Ipomoea Aquatica* var. reptans) [8, 10, 11].

L. minor are aquatic floating angiosperms distributed in tropical and subtropical regions [12]. They exhibit high growth rates that cover ponds or lakes in a few days under favorable environmental conditions [11–14]. Despite the documentation of FAs profiles from wild *L. minor* populations [9, 14–16], there is a paucity of information on the quantitative variation of *L. minor* FAs composition in indoor and outdoor culture settings. Thus, the primary objective of this study was to identify and compare the FAs composition in *L. minor* cultured in indoor plastic tanks and outdoor fish ponds.

2. Materials and Methods

2.1. Culturing of L. minor. Samples of *L. minor* inocula were collected from the wild at the Ahero irrigation scheme canal in January 2022. They were taken to the Kenya Marine and Fisheries Research Institute (KMFRI), Kegati Aquaculture Centre at latitude 00°42″S; 034°47″E and altitude 1,700 m above sea level, for culturing in nine 3 m^2 circular indoor plastic tanks in a hatchery and three outdoor earthen ponds measuring 150 m^2 with a depth of 0.5 m The *L. minor* culture in indoor plastic tanks was conducted under hatchery shade conditions (Figures 1(A) and 1(B)) and under normal 24 hr day–night cycle light conditions in the outdoor earthen ponds (Figure 1(C)), culture facilities (Figures 1(A) and 1(C)) were cleaned and disinfected using 0.1% potassium permanganate solution and water maintained at 60 cm depth throughout the culture duration. Dry poultry manure was thoroughly mixed with water and left to soak for 72 hr to decompose in 50-l buckets. Fertilization of outdoor earthen ponds and indoor plastic tanks was done at 1.052 kg/m^3, followed by successive refertilization at 0.263 kg/m^3 every week. *L. minor* inoculants were cleaned and disinfected for 30 min using 5 g potassium permanganate crystals dissolved in 10 l of water before culturing.

Physicochemical parameters, namely temperature, dissolved oxygen and pH were monitored using the YSI multiparameter meter model H19828 (Hanna Instruments Ltd., Chicago, USA). Ammonia was monitored using standard methods mentioned in Alpha 2017. Harvesting using a sieve in indoor plastic tanks (Figure 1(D)) and a scoop net in outdoor earthen ponds (Figure 1(E)) was done every 12 days and weights were taken using a Shimadzu digital electronic balance model TX 4202 L. Harvested *L. minor* were dried on a rack under shade (Figures 1(G) and 1(H)) for 1 week. Dry samples were then milled using a manual grinder into fine powder and packed in plastic containers (Figure 1(I)).

2.2. Relative Growth Rate (RGR). The relative growth rate of *L. minor* (RGR) in gram per day was calculated as follows:

$$\mathrm{RGR} = \mathrm{Ln}\left(\frac{W_h}{W_0}\right)/T, \quad (1)$$

where:

Ln = natural logarithm,
W_h = fresh weight of *L. minor* during harvesting (h),
W_0 = *L. minor* weight during inoculation, and
T = time in days.

2.3. Extraction of Oil from L. minor Samples. Extraction of oil from dry matter of *L. minor* was conducted using the maceration method [17]. One thousand six hundred thirty-six grams of ground *L. minor* were soaked and submerged in 95% hexane in a 5-l stoppered conical flask at room temperature for 72 hr. The mixture was stirred every 3 hr using a mechanical shaker to enhance the extraction speed, after which the residual plant material was decanted into 1-l beakers. Simple filtration was performed using a Buchner funnel attached to a side arm flask using a vacuum pump resulting in a supernatant of *L. minor* oil floating on hexane. The two were separated using a vacuum rotary evaporator at 79 revolutions per minute at 40°C to obtain a crude *L. minor* oil residue. This was then thoroughly dried in a desiccator over KOH pellets.

2.4. Characterization of L. minor Oil. Triplicate 2 mg *L. minor* lipid extracts were methylated and refluxed in flasks containing 2 ml 95% methanol-HCl for 1 hr. This was followed by extraction of 1 ml methyl esters in 95% hexane, which were then washed in 3 ml of distilled water. This resulted in the formation of two layers, with hexane *L. minor* oil extract on the top. The hexane layer was dried in a vacuum rotary evaporator, and the *L. minor* oil residue redissolved in 6 μl of hexane. One microliter of the solution was injected into Shimadzu Gas Chromatograph Model GC14B, with a Supelco Omega wax^{TM} silica capillary column (CBPI-S25-050) with dimensions 0.50 μm × 25 m × 0.32 mm ID and nitrogen carrier gas. The respective injection and detection temperatures were for programed elution of the column at 170° C for 3 min and at 230 for 45 total run time under a flame ionization detector. Identification of FA methyl esters was by comparison of

FIGURE 1: Photographs showing culture facilities, harvesting, drying, and *L. minor* powder: (A, B) indoor culture facilities comprising *L. minor* grown in plastic tanks in a hatchery; (C) outdoor earthen pond with *L. minor*; (D, E) harvesting *L. minor* from an indoor plastic tank and outdoor earthen pond respectively; (F) harvested *L. minor*; (G, H) drying of *L. minor*; and (I) packing of powdered *L. minor* in a plastic bag.

Table 1: Showing water quality parameters in indoor tanks and outdoor ponds for culture of *L. minor*.

Parameter	Indoor plastic tanks		Outdoor earthen ponds	
	Mean	SE	Mean	SE
Temperature (°C)	27.5	0.04	24.58	0.68
Dissolve oxygen (mg/l)	2.19	0.63	3.01	0.32
pH	6.53	0.27	6.44	0.27
Ammonia (mg/l)	7.43	7.19	0.78	—

retention times and peak areas (Figure S1 and Table S1) with known commercial FAME standards (Table S3) obtained from Kobian chemicals. The FAs were expressed as percentages of total methyl esters (Figure S2 and Table S2).

3. Data Analysis

FAs composition data from each of the two experiments were considered nested factors and subjected to factorial analysis of variance (ANOVA). Tukey's HSD test was used to identify significant differences between FAs obtained from *L. minor* cultured in indoor tanks and outdoor earthen ponds ($p < 0.05$). Paired Students' *t*-test was used to compare means of FA composition at $p < 0.05$. Correlation analysis was conducted on the FAs to ascertain the strengths and magnitudes of association between individual FAs.

4. Results

4.1. L. minor Biomass and Relative Growth Rate. An average of 106.6 kg of wet *L. minor* was harvested per one outdoor earthen fish pond of 150 m^2 in 30 days. The production per unit pond surface area was 0.69 kg/m^2 wet weight. The growth rate of the biomass was estimated at 0.08 kg/m^2/day. For the indoor plastic tanks, the average yield per 3 m^2 plastic tank was 0.41 kg wet weight in 30 days while production per unit tank surface area was 0.14 kg/m^2 wet weight. The relative growth rate in the tanks was 0.04 kg/m^2/day. Hence, the *L. minor* production per unit surface area was higher in the outdoor earthen pond than in the indoor plastic tanks.

4.2. Water Quality Parameters. Data for four water quality parameters monitored are presented in Table 1. In outdoor earthen ponds, temperatures ranged from 24.23 to 26.50°C with a mean of 24.58 ± 0.68°C while that of indoor plastic tanks ranged from 26.50 to 27.83°C with a mean of 27.25 ± 0.04°C. pH ranged from 6.01 to 7.23 with an average of 6.44 ± 0.27 in outdoor plastic tanks and 6.09 to 7.30 in indoor plastic tanks. Outdoor earthen ponds had dissolved oxygen levels, ranging from 2.04 to 3.40 (mg/l) with a mean of 3.01 ± 0.32 (mg/l) while that of indoor plastic tanks varied from 1.32 to 3.84 (mg/l) with a mean of 2.19 ± 0.63 (mg/l). Ammonia levels in outdoor earthen ponds ranged from 5.06 to 8.84 with a mean of 7.19 ± 0.78 (mg/l) while that of indoor tanks ranged from 5.34 to 9.84 (mg/l) with a mean of 7.43 ± 0.96 (mg/l).

4.3. Characterization of L. minor Oil. A total of 15 FAs were detected in *L. minor* cultured in indoor plastic tanks and outdoor earthen ponds. Results of factorial ANOVA analysis indicated that there were 10 FAs whose percentage abundance (composition) was significantly higher in *L. minor* cultured in outdoor earthen ponds than those of indoor plastic tanks at $p < 0.05$ (Table 2). On the other hand, there were five FAs, namely, palmitic, stearic, oleic, elaidic, and linoleic, whose percentage of composition was significantly higher in *L. minor* cultured in indoor plastic tanks than in outdoor earthen ponds. There were also significant differences in individual FAs compositions within *L. minor* cultured in indoor plastic tanks and outdoor earthen ponds. The percentage composition of the five major FAs in indoor plastic tanks varied significantly from each other at $p < 0.05$ (Table 2). Furthermore, the percentage composition of individual FAs within *L. minor* cultured in outdoor earthen ponds indicated that the composition of oleic acid and DHA, EPA, linoelaidic, linoleic, and stearic acids were not significantly different (Table 2). Palmitic acid showed the highest percentage composition in indoor and outdoor culture facilities. Nervonic and pentadecanoic acids had the lowest percentage FA compositions in indoor plastic tanks and outdoor earthen ponds, respectively (Table 2).

In both experiments, there were three SC PUFAs, namely, linolenic, linoleic, and linoelaidic acids, and two LC PUFAs, namely, EPA and DHA. Of the three SC PUFAs in indoor plastic tanks, linoelaidic acid had the lowest percentage composition (Table 2). Similarly, linoleic acid had the lowest composition in *L. minor* cultured in outdoor earthen ponds. ARA and DPA FAs, of which linoleic acid is a precursor, were not detected in *L. minor* cultured in both set-ups. In contrast, EPA and DHA, for which linolenic acid is a precursor, were present in both culture facilities. Among the PUFAs, DHA presented the highest percentage composition in outdoor earthen ponds (Table 2). Therefore, this study demonstrates that *L. minor* cultured in indoor and outdoor settings has LC PUFAs whose biosynthesis is mediated by linolenic acid.

Out of the 15 identified FAs, five were SAFAs, namely, myristic, palmitic, stearic, arachidic, and eicosanoic, while five were MUFAs: pentadecanoic, palmitoleic, oleic, elaidic, and nervonic. The five PUFAs comprised of two long chains (EPA and DHA) and three short chains: linoleic, linoelaidic, and linolenic acid (Table 2). Four FAs that have not been found in *L. minor* from the same geographical region were detected in the species from both culture facilities. These included: linoelaidic, eicosanoic, EPA, and DHA (Table 2). Other researchers who analyzed *L. minor* from other regions did not detect the four FAs [15, 16, 18].

Paired *t*-test for FAs percentage composition of *L. minor* cultured in indoor plastic tanks and outdoor earthen ponds established significant differences in the two set-ups at $p \leq 0.05$ (Table 3). Percentage composition of DHA in *L. minor* cultured in the two culture facilities showed the highest paired mean difference of 10.47 ± 1.25 at $t = 14.49$, $df = 2$, $p = 0.005$ while palmitic acid indicated the least at −8.32 ± 0.37, $t = -0$ 39.44, $df = 2$, $p = 0.001$. Other FAs with high

Table 2: Fatty acid composition of *L. minor* cultured in indoor plastic tanks and outdoor earthen ponds compared to the reported values from past studies.

Fatty acid type	Fatty acids	*N*	Mean[a] (%)		(%) *L. minor*^{RC}	Nile Tilapia fed on 20% *L. minor* [9]	Reference *L. minor*^{RC}
			Indoor plastic tanks	Outdoor earthen ponds			
SAFAs	Myristic	3	1.14 ± 0.01^{h}	2.95 ± 0.24^{f}	1.30, 0.90, 0.55	2.00	[9, 16, 18]
	Palmitic	3	27.38 ± 0.34^{a}	18.98 ± 0.11^{a}	27.10, 20.66, 24.10, 21.74	24.40	[9, 16, 18]
	Stearic	3	9.27 ± 0.05^{c}	4.24 ± 0.12^{de}	4.80, 1.33, 2.20, 2.10	9.70	[9, 15, 16, 18]
	Arachidic	3	2.93 ± 0.03^{g}	4.93 ± 0.22^{d}	0.60, 0.39, 0.33	—	[9, 15, 18]
	Eicosanoic	3	0.99 ± 0.02^{h}	2.65 ± 0.14^{f}	—	—	nr
	Total		41.71	33.75	—	—	—
MUFAs	Oleic	3	24.03 ± 0.12^{b}	16.26 ± 0.17^{b}	6.00, 1.43	22.40	[9, 15]
	Elaidic	3	7.15 ± 0.08^{d}	3.41 ± 0.12^{ef}	1.58	—	[16]
	Pentadecanoic	3	0.84 ± 0.01^{h}	1.55 ± 0.09^{g}	0.50, 0.81	—	[9, 16]
	Palmitoleic	3	3.59 ± 0.19^{f}	4.48 ± 0.27^{d}	1.70, 4.79	4.50	[9, 15]
	Nervonic	3	0.71 ± 0.09^{h}	3.4 ± 0.08^{ef}	0.88	3.70	[15]
	Total		36.32	29.10	—	—	—
PUFAs	Linoleic	3	9.46 ± 0.69^{c}	4.34 ± 0.21^{de}	17.20, 17.40	16.30	[9, 15]
	Linoelaidic	3	0.82 ± 0.02^{h}	4.40 ± 0.09^{de}	—	—	nr
	Linolenic	3	5.23 ± 0.24^{e}	8.78 ± 0.26^{c}	29.00, 46.20, 54.00	1.70	[9, 15, 18]
	EPA	3	1.32 ± 0.06^{h}	4.02 ± 0.17^{de}	—	5.10	nr
	DHA	3	5.14 ± 0.06^{e}	15.61 ± 1.24^{b}	—	4.90	nr
	Total		21.97	37.15	—	—	—

Factorial ANOVA followed by Tukey post hoc. Means within a column followed by different letters (a, b, c, d, e, f, g, and h) are significantly different ($p < 0.05$) with respect to the type of FAs, which result in %. [a]Mean = mean ± std. dev, *L. minor*^{RC} = Reported percentage composition in *L. minor*, Nile tilapia^{RC} = reported percentage composition in Nile tilapia fed with 20% *L. minor*. nr: not reported.

Table 3: Paired *t*-test examining mean differences (95% confidence interval) in FAs compositions of *L. minor* cultured in indoor plastic tanks and outdoor earthen ponds.

Number	Fatty acids	Paired differences			*t*	df	*p*-Value
		(%) Mean[a]	Lower	Upper			
Pair 1	Myristic	1.81 ± 0.23	1.23	2.38	13.560	2	0.005
Pair 2	Pentadecanoic	0.72 ± 0.09	0.49	0.95	13.311	2	0.006
Pair 3	Palmitic	−8.32 ± 0.37	−9.22	−7.41	−39.444	2	0.001
Pair 4	Palmitoleic	0.90 + 0.13	0.57	1.22	11.795	2	0.007
Pair 5	Stearic	−5.03 ± 0.15	−5.39	−4.66	−59.501	2	0.000
Pair 6	Oleic	−7.77 ± 0.28	−8.47	−7.08	−47.914	2	0.000
Pair 7	Elaidic	−3.74 ± 0.05	−3.87	−3.61	−123.594	2	0.000
Pair 8	Linoleic	−5.12 ± 0.70	−6.85	−3.39	−12.749	2	0.006
Pair 9	Linoelaidic	3.58 ± 0.08	3.38	3.78	76.583	2	0.000
Pair 10	Linolenic	3.55 ± 0.28	2.86	4.24	22.227	2	0.002
Pair 11	Arachidic	2.01 ± 0.21	1.50	2.52	16.940	2	0.003
Pair 12	Eicosanoic	1.66 ± 0.13	1.33	1.98	21.990	2	0.002
Pair 13	EPA	2.71 ± 0.21	2.17	3.24	21.812	2	0.002
Pair 14	DHA	10.47 ± 1.25	7.36	13.58	14.488	2	0.005
Pair 15	Nervonic	2.69 ± 0.17	2.27	3.11	27.456	2	0.001

(%) Mean[a] = mean ± std. dev. and are significantly different at the level of $p \leq 0.05$.

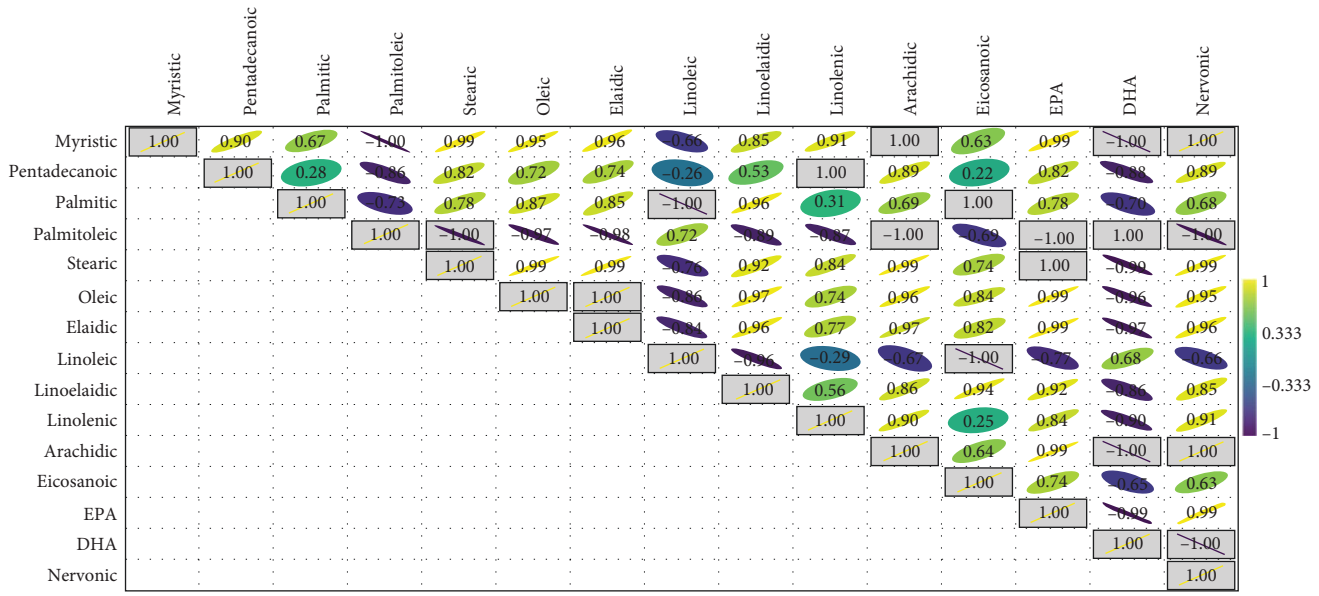

Figure 2: Correlation analyses between the FAs of *L. minor* oil cultured in indoor plastic tanks. Means are significantly different at the$p \leq 0.05$ level and are boxed.

mean differences were stearic, oleic, elaidic, and linoelaidic acids (Table 3).

4.4. Correlations of Fatty Acids Cultured in Indoor Plastic Tanks and Outdoor Earthen Ponds. Correlation coefficients of percentage FAs composition cultured in indoor plastic tanks and outdoor earthen ponds are presented in Figures 2 and 3. These were different, with indoor facilities presenting both strong and negative coefficients while that of outdoor only having negative coefficients (Figures 2 and 3). Percentage FAs composition of *L. minor* cultured in indoor plastic tanks showed strong positive correlations ($p \leq 0.05$) between myristic with arachidic, myristic with nervonic, pentadecanoic with linolenic, eicosanoic with palmitic, palmitoleic with DHA, stearic with EPA, and nervonic with arachidic (Figure 2). Strong negative correlations ($p \leq 0.05$) were established between myristic with DHA, linolenic with palmitic, eicosanoic with linoleic, arachidic, and DHA. The percentage composition of palmitoleic acid showed strong positive correlations with arachidic, myristic, stearic, EPA, and nervonic acids (Figure 2).

Correlation between the FA composition of *L. minor* cultured in outdoor earthen ponds only showed two strong negative correlations between linoelaidic with DHA and linolenic with EPA, (Figure 3) implying inverse proportionality between the FAs.

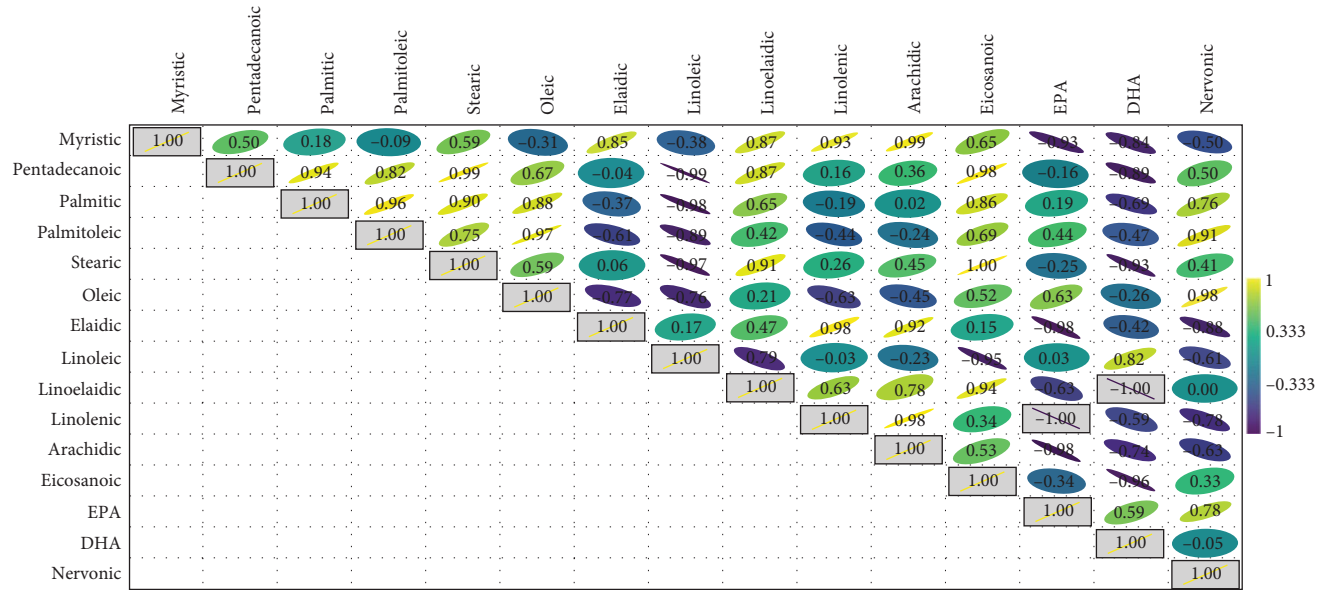

FIGURE 3: Correlation analyses between FAs of *L. minor* cultured in outdoor earthen ponds. Means are significantly different at the $p \leq 0.05$ level and $p \leq 0.05$ boxed.

5. Discussion

5.1. L. minor Biomass Production. There were slight differences in the *L. minor* biomass production between the indoor plastic tanks and outdoor earthen ponds. This was presumed to be due to the differences in dissolved oxygen concentrations and pH which were slightly acidic in indoor plastic tanks at 2.19 mg/l and 6.53 as opposed to 3.01 mg/l in outdoor earthen ponds and 6.44, respectively. The low dissolved oxygen concentrations and acidic pH were attributed to the use of organic manure from livestock droppings which during decomposition could have caused a decrease in dissolved oxygen and made the pH somewhat acidic in both the two culture settings. The difference in biomass production could also be due to temperature differences. For instance [14] compared *L. minor* production under different temperature regimes in ponds and found that there were differences in *L. minor* biomass production which was higher at higher temperatures than lower ones. This study confirms the observations whereby production in ponds was high at higher temperatures.

5.2. Fatty Acids Composition of L. minor Cultured in Indoor Plastic Tanks and Outdoor Earthen Ponds. Understanding the FAs composition of *L. minor* is indispensable for its possible use for human and fish nutrition. Sources of FAs constitute plants and animals, however, those derived from the latter are expensive. Their biosynthesis in plants occurs in photosynthetic cells and thylakoid membranes where the carbohydrate carbon skeleton is converted into FAs through enzymatic reactions [19]. Current efforts are aimed at finding cheap sources of FAs that can be used in the formulation of fish and animal feed. Plant sources of FAs are considered cheaper than the animal ones. One such FA source under investigation is *L. minor*.

In macrophytes, biosynthesis yields straight-chain varieties of SAFAs, with 16–18 carbons [20], whose structural modifications and enzymatic actions produce all other FAs and are therefore regarded as percussors [19]. In the present study, it may be argued that *L. minor* MUFAs and PUFAs were biosynthesized from five SAFAs: myristic, palmitic, stearic, arachidic, and eicosanoic. The observation that palmitic acid had the highest percentage composition in this study is in tandem with the findings by Tang et al. [16] and Yan et al. [18]. In living organisms, palmitic acid is required for numerous enzymatic reactions, such as maintenance of phospholipid balance and physical properties of the cell membrane [21].

The correlation results showed that some FAs in both set-ups were positively and negatively correlated. These could be indicators of some FAs serving as raw materials for the production of the respective counterparts. For instance, the concentrations of two PUFAs: linolenic with DHA and linoleic with linoelaidic, in indoor and outdoor settings, respectively, had strong negative correlation coefficients indicating inverse proportionality. It is interesting to note that linolenic acid is a precursor for the biosynthesis of DHA. LC PUFAs DHA and EPA are the products of $\Delta 5$ and $\Delta 6$ desaturation enzymes, respectively [22]. Although the latter enzymes are found in freshwater fish, the presence of these FAs in *L. minor* signals that the plant has metabolic ability for their production and can be a good source of fish feed preparation. DHA is vitally important in human nutrition since it is already a desaturated PUFA [23, 24]. These observations are in line with the reported presence of the $\Delta 5$ and $\Delta 6$ desaturase genes in *Lemna* species for the conversion of stearic acid into stearidonic acid [25].

The findings of this study concurred with those of a survey on the bioaccumulation of *L. minor* FAs by *Oreochromis niloticus* [9] which indicated that a higher amount of linolenic acid ingested produced an almost similar amount of DHA in fish muscles as that observed in the *L. minor*

analyzed in this study (Table 2). However, the survey utilized *L. minor* from the wild as opposed to the one analyzed in this study cultured using chicken manure in plastic tanks and outdoor earthen ponds. Could it be that the *L. minor* analyzed by Opiyo et al. [9] had not grown to a state, which it could convert linolenic to DHA? In our study, contrary to the survey findings [9], *L. minor* was found to contain moderate amounts of DHA and linolenic acid (Table 2). Other than humans, obtaining DHA from *O. niloticus* fed on feed containing *L. minor*, there appears to be a possibility of them obtaining it directly from the latter as a prepared supplement. Such nutrient supplements have been prepared from algal monocultures such as those of *Spirulina* [26–28].

SC PUFAs, linolenic and linoleic acid extracted from *L. minor* used in the current study had lower percentage composition compared to that analyzed by Opiyo et al. [9], Yosef et al. [15], and Yan et al. [18]. The lower levels in the present study could have probably resulted from physiological bioconversion of linolenic acid by $\Delta 6$ desaturation enzymes, which could be present in *L. minor* plant and therefore made it possible for synthesis EPA and DHA observed in the study. The difference in composition of linolenic and linoleic acids between the current and those of previous studies could also be due to the growth of *L. minor* in different biogeographical areas, the method of extraction or harvesting and storage conditions of oil. Furthermore, FAs are capable of undergoing oxidative rancidity, which might have affected the original FAs amount [29].

Differences in the number of FAs observed in this study with those presented by Opiyo et al. [9] in the same region, could be due to the direct analysis of *L. minor* from the wild by the latter compared to those analyzed which were obtained from samples that were cultured in indoor plastic tanks and outdoor earthen ponds. The absence of DHA, EPA, eicosanoic, and linoelaidic acids in the experiments of Yosef et al. [15], Tang et al. [16], and Yan et al. [18] could be due to differences in climatic and biogeographical areas from where they picked *L. minor* for analysis.

6. Conclusions

Similar *L. minor* oil FAs types were identified in both indoor plastic tanks and outdoor earthen pond setups with palmitic, oleic, stearic, and linoleic acids being among the major FAs and pentadecanoic, myristic and eicosanoic as minor FAs. However, *L. minor* cultured in outdoor earthen ponds, produced significantly higher percentage composition for most FAs compared to that cultured in indoor plastic tanks.

The study demonstrates that in both settings, out of the 15 identified FAs, there are five SAFAs (myristic, palmitic, stearic, arachidic, and eicosanoic), five MUFAs (pentadecanoic, palmitoleic, oleic, elaidic, and nervonic), and five PUFAs comprising two LC PUFAs: EPA and DHA and three SC PUFAs: linoleic, linoelaidic, and linolenic acid. Four FAs (linoelaidic, eicosanoic, EPA, and DHA) were in *L. minor*, seldom found in other studies for the same species within the region, a possible indicator of the existence of $\Delta 5$ and $\Delta 6$ as a desaturation enzyme in *L. minor* for their biosynthesis.

Under normal circumstances, DHÀ (an important Omega-3 FA) is availed to humans through the consumption of fish since they have desaturation enzymes to convert linolenic acid into DHA. The presence of DHA in *L. minor* plants cultured in indoor plastic tanks and outdoor earthen ponds is an exciting rare finding which needs further investigations. LC, PUFAs, ARA, and DPA, of which linoleic acid is a precursor, were not detected in *L. minor* cultured in the current study.

The quantitative variation in FAs percentage composition in the two culture set-ups, especially DHA, forms a strong basis for considering *L. minor* as an alternative source of DHA for direct human consumption.

Data Availability

The data on the percentage composition of fatty acids used in the indoor plastic tanks and outdoor earthen ponds used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they do not have any competing financial interests or personal relationships that could have affected the reported work.

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Supplementary Materials

Supplementary materials for this paper include the standard and representative sample chromatogram along with the qualitative data on the retention times and peak areas (Supplementary files, Figures S1 and S2 and Tables S1 and S2) of the fatty acids present. Details of the fatty acids methyl esters (FAME) standards have also been provided (Supplementary files, Table S3). (*Supplementary Materials*)

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