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Research Article

Fatty Acid Profiling of *Moina* sp. Preserved in Cryoprotective Agents at Low Temperature

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Abstract

Moina sp. is an important feed for larval and post-larval rearing of aquaculture species. Preservation of *Moina* sp. using various preservation agents and techniques is known to be less time-consuming procedure in maintaining large number of feed in hatchery management. Hence, this study was carried out to determine the changes of fatty acid (FA) composition of *Moina* sp. preserved at 5, 10 and 20% in glycerol (GLY), ethylene glycol (EG), and dimethyl sulfoxide (DMSO) for 1 to 3 months (M1-M3). *Moina* sp. without cryoprotectant agents stored at -40°C as controls. Saturated fatty acid (SFA), polyunsaturated fatty acid (PUFA), ∑ ω6 and ∑ ω3 of *Moina* sp. preserved with 5, 10 and 20% GLY decreased with prolonging storage. FA of *Moina* sp. preserved with 5, 10 and 20% EG showed a significant reduction only after M3. Monounsaturated fatty acid (MUFA) and PUFA of *Moina* sp. preserved with 5, 10 and 20% DMSO increased significantly (p<0.05) after M3. *Moina* sp. preserved in 5% DMSO maintain the docosahexaenoic acid (DHA) level, which could be a potential method for long-term preservation.

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1. Introduction

Live feed plays a vital role in the aquaculture systems and is generally divided into two classes; phytoplankton (organism includes all microscopic aquatic plants) and zooplankton (microscopic aquatic animals). Live feed constitutes the most valuable resource, and has been extensively used as high-quality natural food to ensure high growth rates and survival of early-stage fish and shellfish (Das *et al*., 2012). The larval stage is considered critical in the fish and shellfish life cycle. The success of larval rearing is largely dependent on the availability of suitable live feed to consume, digestion efficiency, and rich nutrients provided for their growth, breeding, and survival (Das *et al*., 2012; Akbary *et al*., 2011). Zooplankton mainly comprises protozoans, rotifers, and planktonic forms of crustaceans (copepods and *Artemia* sp.), cladoceran (*Moina* sp., *Daphnia* sp. and *Ceriodaphnia* sp.), ostracoda (*Cypris, Stenocypris,* and *Euypris*), and their larvae.

The dietary requirements for stage 1 of larvae is mainly SFA (14:0 and 16:0) and MUFA (16:1 and 18:1) and linoleic acid (18:2n-6) as source of energy before feeding. Larvae are capable of converting linoleic acid (18:2n-6) to arachidonic acid (20:4n-6) via metabolic activity. In addition, docosahexaenoic acid, DHA (22:6n-3) is the major energy source during the early stage of larval development (Cavali *et al*., 1999). HUFA plays vital role in reproductive performance and offspring quality (Cavali *et al*., 1999). Juvenile has minimal ability to convert C18 to C ≥ 20 fatty acids in the n−3 (linolenic) and n−6 (linoleic) families (D'Abramo and Sheen, 1993). Fish cannot synthesize the n-3 and n-6 essential fatty acid from saturates or monocytes but must obtain them through dietary food (Kangpanish, 2016). Higher the n-3 HUFA effectively leads to weight gain and increased the specific growth rate (Bordignon *et al*., 2020).

Cladocerans, the genus *Moina* sp. is progressively important in aquaculture. *Moina* sp. is a zooplankton that is widely used as live feed for larval and post-larval rearing of crustaceans and fish. They are the secondary producers in the aquatic trophic hierarchy and can be found in a natural abundance in shallow swamps and rivers (Yan, 2011). In intensive or semi-intensive aquaculture systems, fish fry gets important nutrients from this zooplankton because they are rich in proteins (essential amino acids), essential polyunsaturated fatty acids (PUFA) and vitamins. *Moina* sp. is abundant at a high concentration of organic and inorganic materials such as ammonium, phosphorous, and nitrate in waters. *Moina* sp. tolerates low dissolved oxygen (DO) levels and high ammonia concentration, reproduces and grows rapidly on various food sources (Loh *et al*., 2012). In addition, a single adult *Moina* sp. can easily reproduce up to 25 times throughout its life (Mohammad and Mustafa, 2012).

Moina sp. mass cultivation depends on the culture medium and technique, while they are limited due to their low commercial availability and inconsistent production in nature (Mubarak *et al*., 2017). The development of *Moina* sp. is a constraint to several environmental factors such as the level of resources, thermal conditions, population density, and crowding. In addition, the cultures of *Moina* sp. is also needed in maintaining healthy stock and effective diagnosis of disease-causing organisms. Furthermore, the production of the culture depends on the natural or induced blooms, the impossibility of mass cultivation at all locations and the difficulties of transportations (Srivastava, 2012). Thus, a continuous supply of live feed for cultured species is uncertain throughout the year.

A combination of frozen storage and cryoprotective agent for *Moina* sp. preservation is now recognized as the best technique for the maintaining many cultures for long-term storage period. The cryoprotective agent is a chemical compound that prevents cell or tissue from damages due to the frozen conditions. Cryoprotectants lowers the melting point of the preserving solution and thus tissue can freeze within its surroundings but not within its cells (Bhattacharya, 2018). Slow freezing is preferable for cell preservation where cells are frozen slowly below their freezing point. In addition, at the last stage of slow freezing, the remaining unfrozen fraction is transformed to amorphous solids without ice crystal (Bhattacharya, 2018). Slow freezing with cryoprotectant causes fewer damages (Bhattacharya, 2018).

An extensive study on the fatty acid profiling and nutritional quality of *Moina* sp. preserved at -40°C after coating with cryoprotective agents; glycerol, ethylene glycol, and dimethyl sulfoxide for 1-3 months had been investigated to establish the suitable method for long-term preservation.

2. Materials and Methods

2.1 Sample Preparation

Moina sp. was obtained from Fisheries Research Institute (FRI) Glami Lemi, Negeri Sembilan, Malaysia. *Moina* sp. was mass cultivated in a hygienic culturing medium with approximately 1.0 to 1.5 kg in 2 tons tanks capacity. The water temperature was maintained at 23°C

and the photoperiod was set at L:D=12:12 using triphosphor fluorescent lights with a light intensity of >2000 lux. Water quality parameters were monitored and measured using YSI meter (Professional Plus 13M100665, USA). The samples were collected using scoop net of mesh size 120 µm and immediately transferred into zip lock bag. Samples were subjected to 5, 10, and 15% of the cryoprotective agent; glycerol, ethylene glycol, and dimethyl sulfoxide before being stored at -40°C (Panasonic MDF-U5412 Biomedical Plasma Freezer, Malaysia) for 1-3 months. All samples were transported to Universiti Malaysia Terengganu for further analysis.

2.2 Extraction of Total Lipid

Total lipids were extracted from the samples according to Airina and Jamaludin (2012) with a slight modification. Approximately 2.0±0.1 g of *Moina* sp. were added into the chloroform-methanol solvent (2:1 v/v) with ratio weight: solvent of 1:20. The samples were homogenized three times for 5 min at 7000–8000 rpm (IKA T18 Digital Ultra Turrax, Germany). All samples were cooled at 4°C for 1 hour before being added with 4 mL of 0.034% $MgCl_2$ per g of samples. All samples were incubated overnight to separate the organic (containing the extracts of total lipids) and aqueous layers. The upper layer (aqueous) were discarded meanwhile the lower layer (organic) were rinsed with chloroform-methanol solvent (2:1 v/v). Evaporation of the lower phase demonstrated the total lipid fraction. The solvent was removed using a rotary evaporator under a vacuum at 40°C. These extracts, representing the total lipids, were weighed and dissolved once again in chloroform-methanol solvent (2:1 v/v). The total lipid was expressed as g 100 mg^{-1}.

2.3 Fatty Acid Analysis

Fatty acid analysis was performed by using two consecutive steps, (1) preparation of fatty acid methyl ester (FAME) and (2) chromatographic analysis.

2.3.1 Fatty acid methyl ester (FAME) synthesis

Fatty acid methyl ester (FAME) was synthesised with the extraction-transesterification method described by Majid *et al*. (2003). Approximately 20±0.1 mg of samples were added into the solution mixture of methanol, sulphuric acid, and chloroform (17:3:20 v/v/v). All samples were placed in water bath at 90°C for 30 min. Samples were left to cool at room temperature. The samples weight were recorded before being added with 1 ml of distilled water. Samples were thoroughly homogenized with vortex until an obvious formation of two phases were seen. The lower phase containing FAME was transferred into a clean bottle and dried with anhydrous sodium sulfate Na_2SO_4.

2.3.2 Fatty acid methyl ester (FAME) analysis by Gas Chromatography Mass Spectrometer (GCMS)

Fatty acid compositions of lipid extracts were determined using gas chromatography to corresponding methyl esters. FAME was obtained by transesterification lipid extracts. The gas chromatograph analysis of methylated fatty acids was performed on a Shimadzu GC-2010 Plus Shimadzu equipped with a BPX70 capillary column with a diameter of 0.22 mm and a length of 60 m. The carrier gas is helium with velocity of 25 cm sec^{-1}. The initial temperature was set at 120°C and increased at 3°C per min to 250°C. The injection and detector temperature was set at 250°C. The mass spectrum from the chromatograph was compared with NBS and NIST Mass Spectra Library. FAME was also identified with their retention time to commercial FAME standard (GC18-91).

2.4 Statistical Analysis

Statistical analysis was conducted in SPSS Ver 21 (IBM Corp, Armonk, New York). Fatty acid, and proximate analysis data were analysed by one-way ANOVA. All data analysis was checked for normality distribution through shapiro-wilk test, only data that fulfil normality requirement proceed with One-way ANOVA followed by Sceffe's post-hoc test. If the data analysed did not fulfil the distribution requirement, non-parametric analysis was performed with Kruskal Wallis test. Any difference was considered significant when $p<0.05$.

3. Results and Discussion

3.1 Results

3.1.1 Fatty acid profiling in frozen Moina sp.

The fatty acid content of frozen *Moina* sp. decreased with prolonged storage duration (Table 1). The total saturated fatty acid (SFA) content of *Moina* sp. stored for 1 month (M1) was recorded at 22.27±0.72 mg g^{-1} (Table 1). A decreasing trend was observed for *Moina* sp. frozen at -40°C after M2 (14.60±1.69 mg g^{-1}) and M3 (14.77±5.37 mg g^{-1}). A similar trend was found in MUFA contents that were decreased with prolong storage duration; from M1 (16.06±0.83 mg g^{-1}) to M2 (4.39±0.65 mg g^{-1}) till M3 (9.96±5.77 mg g^{-1}) but not significant ($p>0.05$). Total PUFA content was decreased but not significant from value of 13.69±0.55 mg g^{-1} at M1 to 8.66±3.52 mg g^{-1} after M3 of storage period (Table 1).

The storage duration showed no significant effects on DHA, ∑PUFA/SFA and ∑ PUFA+ MUFA/SFA ratio

Table 1. Fatty acid profiling of frozen *Moina* sp. during storage at -40°C for 1, 2, and 3 months

Fatty Acids	Month 1	Month 2	Month 3
SFA			
C 14: 0	10.87±0.03	0.39±0.12	0.48±0.33
C 15: 0	0.62±0.03	0.29±0.06	0.29±0.29
C 16: 0	15.23±0.45	8.85±1.08	8.97±3.79
C 17: 0	0.97±0.05	0.38±0.08	0.88±0.31
C 18: 0	4.32±0.03	4.52±0.65	3.94±0.87
C 20: 0	0.19±0.04	0.11±0.06	0.20±0.03
C 22: 0	0.07±0.07	0.06±0.06	0.00±0.01
Subtotal	22.27±0.72[a]	14.60±1.69[a]	14.77±5.37[a]
MUFA			
C 14:1	0.49±0.08	0.17±0.09	0.50±0.36
C 16:1	2.76±0.20	0.58±0.10	1.78±1.27
C 18:1n9t	0.10±0.10	0.19±0.10	0.00±0.01
C 18:1n9c	4.82±0.11	1.65±0.26	2.23±0.97
C 20:1	7.80±0.36	1.62±0.27	5.21±3.17
C 22:1n9	0.09±0.01	0.17±0.01	0.24±0.07
Subtotal	16.06±0.83[a]	4.39±0.65[a]	9.96±5.77[a]
PUFA			
C 18:2n6t	0.09±0.09	0.17±0.09	0.00±0.01
C 18:2n6	7.67±0.32	1.82±0.14	5.69±2.96
C 18:3n6	0.27±0.06	0.40±0.11	0.20±0.20
C 20:2	2.30±0.01	0.66±0.17	0.99±0.20
C 20:3n6	0.31±0.04	0.30±0.15	0.02±0.01
C 20:3n3	2.66±0.11	0.66±0.14	1.20±0.47
C 20:4n6	0.07±0.07	0.12±0.12	0.00±0.01
C 20:5n3	-	0.12±0.06	0.06±0.06
C 22:2	-	0.63±0.19	0.10±0.05
C 22:6n3	0.32±0.07	0.11±0.11	0.39±0.24
Subtotal	13.69±0.55[a]	4.98±0.61[a]	8.66±3.52[a]
EPA	-	0.12±0.06[a]	0.06±0.06[a]
DHA	0.32±0.07[a]	0.11±0.11[a]	0.39±0.24[a]
∑ PUFA/SFA ratio	0.61±0.01[a]	0.36±0.07[a]	0.59±0.14[a]
∑ PUFA/MUFA ratio	0.85±0.01[a]	1.19±0.25[a]	1.10±0.20[a]
∑ PUFA + MUFA/SFA ratio	1.34±0.02[a]	0.66±0.10[a]	1.19±0.31[a]
∑ ω6	8.41±0.38[a]	2.80±0.19[a]	5.91±3.15[a]
∑ ω3	2.99±0.18[a]	0.89±0.29[b]	1.66±0.40[a]

PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; SFA: Saturated fatty acids. Results are reported as mean ± standard error of mean unit of mg g^{-1}. Difference superscript of letters indicates significant differences among different lipid diet frequencies at One-way ANOVA (p<0.05). The absence of superscript indicates insignificant differences.

(Table 1). Similarly, ∑PUFA/MUFA ratio of frozen samples showed an increasing trends but not significant (p>0.05) from 0.85±0.01 mg g^{-1} (at M1) to 1.19±0.25 mg g^{-1} (at M2) then decreased to 1.10±0.20 mg g^{-1} at M3 of storage period. The ∑ ω6 content of frozen samples decreased but not significantly (p>0.05) from 8.41±0.38 mg g^{-1} (at M1) to 2.80±0.19 mg g^{-1} (at M2) then increased to 5.91±3.15 mg g^{-1} after the M3 storage period. Only ∑ω3 content showed significant (p<0.05) decrease from 2.99±0.18 mg g^{-1} (at M1) to 0.89±0.29 mg g^{-1} (at M2) but did not significantly (p>0.05) increase to 1.66±0.40 mg g^{-1} after M3 storage period (Table 1).

3.1.2 Fatty acid profiling in Moina sp. *preserved with glycerol (GLY)*

Moina sp. preserved with 5, 10, and 20% GLY showed a decreasing trend on SFA, PUFA, ∑ω6, and ∑ω3 with prolonged storage duration (Table 2). In contrast, MUFA showed an increasing trend with prolonged storage duration (Table 2). The total SFA for *Moina* sp. preserved with 5% GLY was significantly decreased (p<0.05) from 8.38±0.31 mg g^{-1} at M1 to 2.28±0.07 mg g^{-1} at M3. Similarly, SFA of *Moina* sp. preserved with 10% GLY was significantly decreased (p<0.05) from 5.71±0.37 mg g^{-1} at M1 to give an amount of 2.51±0.22 mg g^{-1} at M3. However, the total SFA of *Moina* sp. preserved with 20% GLY showed a slight decrease with prolonged storage duration (p>0.05) (Table 2). The total MUFA in *Moina* sp. preserved with 5% GLY decreased significantly from 1.45±0.15 mg g^{-1} at M1 to 0.52±0.08 mg g^{-1} after three months (M3). On the contrary, MUFA content of *Moina* sp. preserved with 10% GLY showed a significant increase from 0.77±0.03 mg g^{-1} at M1 to give an amount of 3.62±0.11 mg g^{-1} at the end of storage period (M3). A similar trend was found in *Moina* sp. preserved with 20% GLY (Table 2). Total PUFA in *Moina* sp. preserved with 5% GLY was significantly decreased (p<0.05) from 3.79±0.43 mg g^{-1} at M1 to 0.15±0.07 mg g^{-1} at M3. Total PUFA at M2 were recorded at 4.74±1.25 mg g^{-1}. Meanwhile, *Moina* sp. preserved with 10% GLY were slightly decreased from 3.11±0.36 mg g^{-1} at M1 to 2.08±0.39 mg g^{-1} at M2 but significantly increased to 4.00±0.41 mg g^{-1} at the end of the storage period (M3). PUFA in *Moina* sp. preserved with 20% GLY decreased significantly from 1.65±0.29 mg g^{-1} at M1 to give an amount of 0.22±0.02 mg g^{-1} at M3.

Specifically, EPA in *Moina* sp. preserved with 5% GLY was recorded at 0.02±0.01 mg g^{-1} and 0.01±0.01 mg g^{-1} at M1 and M2, respectively (Table 2). Similar results were recorded of EPA in *Moina* sp. with 10% GLY. However, *Moina* sp. preserved with 20% GLY was significantly increased from 0.02±0.01 mg g^{-1} at M1 to 0.04±0.02 mg g^{-1} at M2. EPA was not detected at M3 in *Moina* sp. preserved with GLY. In addition, DHA was not detected in *Moina* sp. preserved with GLY. ∑ω6 in *Moina* sp. preserved with 5% GLY showed a slight increase but not significant from 3.77±0.43 mg g^{-1} at M1 to 4.73±1.25 mg g^{-1} at M2. However, after M3, ∑ ω6 in *Moina* sp. preserved with 5% GLY showed a significantly lower (p<0.05) amount. Furthermore, *Moina* sp. preserved with 10% GLY, the storage duration showed a fluctuation effect to the ∑ω6, while the storage duration showed a significant decreasing trend of ∑ω6.

At 1st month of preservation, the SFA showed a significant reduction with an increasing GLY concentration. Similar trends were found at M2 and M3 of preservation duration. Similarly, MUFA content at M1 showed a significant reduction with an increasing GLY concentration. However, GLY concentration showed no significant effects on MUFA content at M3. PUFA content at M1 showed a significantly low amount in *Moina* sp. preserved with 20% GLY. However, the PUFA content were significantly decreased with increasing GLY concentration at M2, while PUFA content was not significantly influenced by the percentage (20% GLY) at M3.

3.1.3 Fatty acid profiling in Moina sp. *preserved with ethylene glycol (EG)*

The total SFA of *Moina* sp. preserved with ethylene glycol (EG) showed a significantly decreasing (p<0.05) trend with a prolonged storage duration (M1 to M3) of all EG treatments (5, 10 and 20% EG). MUFA of *Moina* sp. preserved with EG (at both the 5 and 20%) showed a significant (p<0.05) decrease only at M3. Similar trends were found in regards to total PUFA. The total SFA of *Moina* sp. preserved with 5% EG was significantly decreased (p<0.05) over storage duration from 3.13±0.29 mg g^{-1} at M1 to 2.39±0.05 mg g^{-1} at M2 and gives an amount of 0.22±0.05 mg g^{-1} at M3. The total MUFA content of *Moina* sp. preserved with 5% EG showed a significantly (p<0.05) decreasing amount from 1.29±0.07 mg g^{-1} at M1 to 0.64±0.15 mg g^{-1} at M3. Similar trends were found in regards to *Moina* sp. preserved with 20% EG. It has been recorded that *Moina* sp. preserved with 20% EG was significantly (p<0.05) decreased from 0.72±0.05 mg g^{-1} at M1 to 0.20±0.07 mg g^{-1} at M3. Interestingly, MUFA of *Moina* sp. preserved with 10% EG showed a significantly (p<0.05) increasing effect at M3; 0.94±0.25 mg g^{-1} (M1) to 2.26±0.40 mg g^{-1} (M3). The total PUFA of *Moina* sp. preserved with 5% EG showed a significantly (p<0.05) decreased amount from 1.86±0.12 mg g^{-1} at M1 to 0.11±0.03 mg g^{-1} at M3. Similar trends were found in *Moina* sp. preserved with 20% EG,

Table 2. Fatty acid profiling of *Moina* sp. preserved with glycerol (GLY) during storage at -40°C for 1, 2, and 3 months

Fatty acid	5%			10%			20%		
	Month 1	**Month 2**	**Month 3**	**Month 1**	**Month 2**	**Month 3**	**Month 1**	**Month 2**	**Month 3**
SFA									
C 14: 0	0.16±0.04	0.11±0.05	0.32±0.05	0.08±0.01	0.06±0.02	0.31±0.06	0.27±0.05	0.15±0.05	0.23±0.01
C 15: 0	0.11±0.08	0.16±0.05	0.35±0.05	0.23±0.01	0.04±0.01	0.30±0.01	0.19±0.02	0.13±0.04	0.09±0.09
C 16: 0	6.24±0.73	3.82±0.72	1.53±0.06	4.43±0.39	1.31±0.28	1.67±0.18	1.82±0.19	2.15±0.21	1.30±0.06
C 17: 0	0.04±0.00	0.11±0.05	-	0.04±0.01	0.05±<0.01	-	0.08±0.01	0.07±0.01	-
C 18: 0	1.09±0.20	2.47±0.41	0.08±0.01	0.82±0.14	0.87±0.14	0.23±0.04	0.02±0.01	0.02±0.01	0.22±0.02
C 20: 0	0.65±0.20	0.03±0.00	-	0.09±0.03	0.01±<0.01	-	0.01±0.01	0.02±0.00	-
C 22: 0	0.09±0.03	0.02±0.01	-	0.02±<0.01	0.02±0.01	-	0.02±0.00	0.02±0.00	-
Subtotal	$8.38±0.31^{A,a}$	$6.72±1.15^{A,a}$	$2.28±0.07B^{,ab}$	$5.71±0.37^{A,b}$	$2.35±0.44^{B,b}$	$2.51±0.22^{B,a}$	$2.39±0.15^{A,c}$	$2.56±0.29^{AB,b}$	$1.83±0.11^{A,b}$
MUFA									
C 14:1	-	-	-	-	-	0.21±0.01	-	-	-
C 16:1	-	-	-	-	-	0.43±0.02	-	-	0.40±0.02
C 18:1n9t	1.14±0.15	2.39±0.42	0.52±0.08	0.60±0.03	0.71±0.09	0.81±0.02	0.12±0.06	-	-
C 18:1n9c	0.21±0.03	0.19±0.04	-	0.14±0.01	0.18±0.05	2.16±0.12	0.07±0.04	0.08±0.02	-
C 20:1	-	-	-	-	-	-	-	-	-
C 22:1n9	0.10±0.02	0.08±0.07	-	0.04±0.02	0.01±<0.01	-	0.01±0.00	0.05±0.01	-
Subtotal	$1.45±0.15^{A,a}$	$2.66±0.49^{B,a}$	$0.52±0.08^{A,a}$	$0.77±0.03^{A,b}$	$0.90±0.12^{A,b}$	$3.62±0.11^{B,b}$	$0.20±0.05^{A,c}$	$0.14±0.02^{A,c}$	$0.40±0.02^{B,a}$
PUFA									
C 18:2n6t	1.80±0.16	1.18±0.26	-	1.45±0.15	1.25±0.13	0.00±0.00	0.13±0.03	0.10±0.04	0.00±0.00
C 18:2n6	0.03±0.00	0.04±0.02	-	0.03±0.01	0.01±0.01	3.79±0.38	0.16±0.02	0.02±0.02	0.00±0.00
C 18:3n6	1.92±0.27	3.48±1.00	-	1.61±0.21	0.80±0.29	-	1.33±0.26	0.50±0.19	0.00±0.00
C 20:2	-	-	-	-	-	-	-	-	-
C 20:3n6	0.01±0.01	-	-	-	-	-	-	-	-
C 20:3n3	-	-	-	-	-	-	-	-	-
C 20:4n6	0.01±0.01	0.03±0.01	0.15±0.07	0.01±<0.01	0.01±<0.01	0.22±0.03	0.01±<0.01	0.01±0.00	0.22±0.02
C 20:5n3	0.02±0.00	0.01±0.00	-	0.02±<0.01	0.01±0.01	-	0.02±0.01	0.04±0.02	-
C 22:2	-	-	-	-	-	-	-	-	-
C 22:6n3	-	-	-	-	-	-	-	-	-
Subtotal	$3.79±0.43^{A,a}$	$4.74±1.25^{A,a}$	$0.15±0.07^{B,a}$	$3.11±0.36A^{B,a}$	$2.08±0.39^{A,b}$	$4.00±0.41^{B,b}$	$1.65±0.29^{A,b}$	$0.66±0.21^{B,b}$	$0.22±0.02^{B,a}$
EPA	$0.02±0.00^{A,a}$	$0.01±0.00^{B,a}$	-	$0.02±<0.01^{A,a}$	$0.01±0.01^{AB,a}$	-	$0.02±0.01^{Aa}$	$0.04±0.02^{A,a}$	-
DHA	-	-	-	-	-	-	-	-	-
∑ PUFA/SFA ratio	$0.45±0.04^{A,a}$	$0.68±0.08^{B,a}$	$0.07±0.03^{C,a}$	$0.55±0.09^{A,a}$	$0.89±0.04^{A,a}$	$1.64±0.28^{B,b}$	$0.70±0.13^{A,a}$	$0.25±0.05^{B,b}$	$0.12±0.01^{B,a}$
∑ PUFA/ MUFA ratio	$2.71±0.49^{A,a}$	$1.72±0.19^{A,a}$	$0.28±0.10^{B,a}$	$4.02±0.56^{A,a}$	$2.53±0.86^{AB,a}$	$1.10±0.08^{B,b}$	$9.92±4.12^{A,a}$	$4.78±1.28^{AB,a}$	$0.54±0.07^{B,a}$
∑ PUFA + MUFA/SFA ratio	$0.62±0.02^{A,a}$	$1.07±0.10^{B,a}$	$0.30±0.06^{C,a}$	$0.69±0.09^{A,a}$	$1.31±0.13^{A,a}$	$3.10±0.43^{B,b}$	$0.78±0.12^{A,a}$	$0.30±0.05^{B,b}$	$0.34±0.01^{B,a}$
∑ ω6	$3.77±0.43^{A,a}$	$4.73±1.25^{A,a}$	$0.15±0.07^{B,a}$	$3.10±0.36^{AB,a}$	$2.08±0.39^{A,ab}$	$4.00±0.41^{AC,b}$	$1.63±0.29^{A,b}$	$0.62±0.22^{B,b}$	$0.22±0.02^{B,a}$
∑ ω3	$0.02±<0.01^{A,a}$	$0.01±<0.01^{B,a}$	-	$0.02±<0.01^{A,a}$	$0.01±<0.0^{A,a}$	-	$0.02±0.01^{A,a}$	$0.04±0.02^{A,a}$	-

PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; SFA: Saturated fatty acids; M: Month. Results are reported as mean ± standard error of mean unit of mg g^{-1}. Difference superscript of letters indicates significant differences among the percentages in month at One-way ANOVA (p<0.05). The absence of superscripts indicates insignificant differences. Superscript in capital letter (A, B, C) indicates significant difference between the months in same percentages, while superscript in small letter (a, b, c) indicates significant differences between percentages in the same month.

Table 3. Fatty acid profiling of *Moina* sp. preserved with ethylene glycol (EG) during storage at -40°C for 1, 2 and 3 months

Carbon	5%			10%			20%		
	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
SFA									
C 14: 0	0.17±0.03	0.25±0.03	0.01±<0.01	0.20±0.04	0.14±0.05	-	61.95±6.51	22.98±6.25	0.30±0.06
C 15: 0	0.97±0.08	0.57±0.09	0.16±0.05	0.27±0.08	0.15±<0.01	-	0.71±0.01	-	-
C 16: 0	1.15±0.21	0.55±0.08	0.02±0.01	0.37±0.10	0.21±0.03	0.01±0.01	0.76±0.12	0.70±0.08	0.52±0.19
C 17: 0	-	-	-	0.15±0.01	0.09±0.01	0.17±0.01	80.50±6.20	2.62±1.94	-
C 18:0	-	-	-	1.12±0.14	1.04±0.10	0.17±0.14	1.03±0.51	-	0.01±<0.01
C 20: 0	0.23±0.02	0.36±0.09	-	0.36±0.03	0.06±0.01	-	0.25±0.03	0.49±0.11	0.32±0.10
C 22: 0	0.62±0.11	0.66±0.07	0.03±0.01	0.44±0.07	0.19±0.01	-	17.85±1.30	0.15±0.06	0.19±0.07
Subtotal	$3.13±0.29^{A,a}$	$2.39±0.05^{B,a}$	$0.22±0.05^{C,a}$	$2.91±0.2^{A,a}$	$1.89±0.20^{B,a}$	$0.35±0.14^{C,a}$	$163.06±13.47^{A,b}$	$26.94±6.39^{B,b}$	$1.28±0.33^{B,b}$
MUFA									
C 14:1	0.19±0.02	0.20±0.03	0.02±<0.01	-	-	-	-	-	-
C 16:1	0.47±0.03	0.51±0.04	0.01±<0.01	0.29±0.02	0.48±<0.01	0.05±0.03	0.30±0.03	0.02±0.01	-
C 18:1n9t	-	-	-	-	-	0.67±0.12	-	-	-
C 18:1n9c	0.29±0.03	0.27±0.07	0.31±0.07	0.05±0.08	-	0.73±0.08	-	0.20±0.10	0.08±0.06
C 20:1	0.32±0.03	0.41±0.08	0.30±0.07	0.46±0.22	0.08±<0.01	0.65±0.02	0.39±0.06	0.28±0.09	0.15±0.08
C 22:1n9	-	0.10±0.01	0.01±<0.01	0.14±0.02	0.23±0.01	0.16±0.15	0.04±0.03	-	-
Subtotal	$1.29±0.07^{A,a}$	$1.49±0.08^{A,a}$	$0.64±0.15^{B,a}$	$0.94±0.25^{A,ab}$	$0.79±0.02^{A,b}$	$2.26±0.40^{B,b}$	$0.72±0.05^{A,b}$	$0.49±0.18A^{B,b}$	$0.20±0.07^{B,a}$
PUFA									
C 18:2n6t	0.31±0.07	0.32±0.05	0.01±<0.01	0.23±0.13	-	0.14±0.13	0.31±0.01	0.00	0.00
C 18:2n6	0.17±0.04	0.24±0.06	0.07±0.02	0.11±0.11	-	0.22±0.04	0.00	0.16±0.08	0.00
C 18:3n6	0.23±0.02	0.25±0.04	0.02±<0.01	0.16±0.08	0.25±0.07	0.02±0.02	0.29±0.05	0.15±0.10	0.55±0.06
C 20:2	-	-	-	-	-	-	-	-	-
C 20:3n6	0.39±0.07	0.28±0.12	0.00±<0.01	0.25±0.04	0.45±0.09	-	0.44±0.11	-	-
C 20:3n3	-	-	-	-	-	0.01±0.01	-	-	-
C 20:4n6	0.28±0.04	0.29±0.06	0.00±<0.01	-	-	0.02±0.02	-	0.32±0.06	-
C 20:5n3	0.20±<0.01	0.12±0.04	-	0.04±0.04	-	-	-	0.07±0.03	-
C 22:2	-	-	-	-	-	0.11±0.09	-	-	-
C 22:6n3	0.26±0.04	0.31±0.07	0.00±<0.01	0.25±0.03	0.43±0.06	-	0.22±0.02	0.08±0.02	0.16±0.03
Subtotal	$1.86±0.12^{A,a}$	$1.81±0.12^{A,a}$	$0.11±0.03^{B,a}$	$1.04±0.31^{A,b}$	$1.12±0.31^{A,b}$	$0.52±0.25^{A,ab}$	$1.26±0.18^{A,ab}$	$0.77±0.15^{AB,b}$	$0.72±0.09^{B,b}$
EPA	$0.20±<0.01^{A,a}$	$0.12±0.04^{B,a}$	-	$0.04±0.04^{A,b}$	-	-	-	0.07±0.03	-
DHA	$0.26±0.04^{A,a}$	$0.31±0.07^{A,a}$	$0.00±<0.01^{B,a}$	$0.25±0.03^{A,a}$	$0.43±0.06^{A,a}$	-	$0.22±0.02^{A,a}$	$0.08±0.02^{B,a}$	$0.16±0.03^{AB,b}$
∑ PUFA/SFA ratio	$0.60±0.03^{A,a}$	$0.76±0.06^{A,a}$	$0.61±0.22^{A,a}$	$0.37±0.14^{A,a}$	$0.59±0.10^{A,a}$	$1.45±0.14^{B,a}$	$0.01±<0.01^{A,b}$	$0.03±<0.01^{A,b}$	$0.72±0.31^{B,a}$
∑ PUFA/ MUFA ratio	$1.48±0.08^{A,ab}$	$1.21±0.02^{B,a}$	$0.18±0.01^{C,a}$	$1.09±0.04^{A,ab}$	$1.43±0.43^{A,a}$	$0.22±0.07^{B,a}$	$1.76±0.25^{A,b}$	$3.06±2.06^{A,a}$	$4.92±2.20^{A,a}$
∑ PUFA + MUFA/SFA ratio	$1.01±0.05^{A,a}$	$1.38±0.09^{AB,a}$	$4.10±1.43^{B,a}$	$0.71±0.25^{A,a}$	$1.01±0.05^{A,b}$	$8.72±1.66^{B,b}$	$0.01±<0.01^{A,b}$	$0.05±0.01^{A,c}$	$0.91±0.38^{B,a}$
∑ ω6	$1.40±0.10^{A,a}$	$1.38±0.10^{A,a}$	$0.11±0.03^{B,a}$	$0.74±0.30^{A,a}$	$0.69±0.22^{A,b}$	$0.40±0.16^{A,ab}$	$1.03±0.17^{A,a}$	$0.63±0.20^{A,b}$	$0.55±0.06^{A,b}$
∑ ω3	$0.46±0.05^{A,a}$	$0.43±0.03^{A,a}$	$0.00±<0.01^{B,a}$	$0.30±0.01^{A,b}$	$0.43±0.09^{B,a}$	$0.01±0.01^{C,a}$	$0.22±0.02^{A,b}$	$0.15±0.06^{A,b}$	$0.16±0.03^{A,b}$

PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; SFA: Saturated fatty acids; M: Month. Results are reported as mean ± standard error of mean unit of mg g^{-1}. Difference superscript of letters indicates significant differences among the percentages in month at One-way ANOVA (p<0.05). The absence of superscripts indicates insignificant differences. Superscript in capital letter (A, B, C) indicates significant difference between the months in same percentages, while superscript in small letter (a, b, c) indicates significant differences between percentages in the same month.

where the total PUFA was significantly decreased (p<0.05) from 1.26±0.18 mg g^{-1} at M1 to 0.72±0.09 mg g^{-1} at M3. Interestingly, the PUFA contents of *Moina* sp. preserved with 10% EG were not significantly affected by the storage duration.

The EPA contents of *Moina* sp. preserved with 5% EG showed a significant (p<0.05) decrease after M2. DHA content of *Moina* sp. preserved with 5% EG showed a significant (p<0.05) decrease only at M3. Meanwhile the DHA content of *Moina* sp. preserved with 10% EG showed no significant (p>0.05) changes after M2. On the other hand, the DHA contents of *Moina* sp. preserved with 20% EG decreased from 0.22±0.02 mg g^{-1} at M1 to 0.16±0.03 mg g^{-1} at M3 but not significant (p>0.05). The ∑PUFA/SFA ratio showed no significant difference during 3 months of storage for *Moina* sp. preserved with 5% EG. Interestingly, the ∑PUFA/SFA ratio of *Moina* sp. preserved with 10 and 20% EG showed a significant (p<0.05) increase at M3. The ∑PUFA/MUFA ratio of *Moina* sp. preserved with 5% EG showed a significant decrease (p<0.05) from 1.48±0.08 mg g^{-1} at M1 to 1.21±0.02 mg g^{-1} at M2 and 0.18±0.01 mg g^{-1} at M3. The ∑PUFA+ MUFA/SFA ratio showed a significantly (p<0.05) increasing amount at M3 in all EG treatments (5, 10 and 20% EG). Both the ∑ω6 and ∑ω3 in *Moina* sp. preserved in 5% EG showed a significant decrease at M3. Meanwhile the ∑ω6 and ∑ω3 in *Moina* sp. preserved in 10 and 20% EG were not significantly (p<0.05) affected by the storage duration.

At 1st month of preservation, SFA of *Moina* sp. showed a significantly (p<0.05) higher amount at 20% EG compared to 5 and 10% EG. Similar trends were found for M2 and M3. MUFA contents, for M1 and M2, were significantly (p<0.05) decreased with increasing EG concentration. The PUFA contents were significantly (p<0.05) decreased with increasing EG concentration at M1. PUFA content was not significantly (p>0.05) influenced by the EG concentration (10 and 20% EG) at 3M (Table 3).

3.1.4 Fatty acid profiling in Moina sp. *preserved with dimethyl sulfoxide (DMSO)*

The total SFA amount of *Moina* sp. preserved with dimethyl sulfoxide (DMSO) showed a significant (p<0.05) decrease with a prolonged storage duration (M1 to M3) of all DMSO treatments (5, 10 and 20%). MUFA of *Moina* sp. preserved with DMSO (at both the 10 and 20%) showed a significant (p<0.05) increase only at M3. Similarly, the PUFA content of *Moina* sp. preserved with 5, 10, and 20% DMSO showed significantly (p<0.05) higher value at M3. *Moina* sp. preserved with 5% DMSO at M1 had significantly (p<0.05) higher SFA contents (159.44±11.00 mg g^{-1}) compared to SFA contents preserved at M2 (1.16±0.09 mg g^{-1}) and M3 (40.81±2.87 mg g^{-1}). Similar trends were found in *Moina* sp. preserved with 10% DMSO. The highest SFA contents were valued at 169.73±24.20 mg g^{-1} at M1, and lower amount at M2 and M3 (1.98±0.19 and 38.30±5.06 mg g^{-1}, respectively). *Moina* sp. preserved with 20% DMSO, decreased significantly (p<0.05) from 140.17±6.15 mg g^{-1} at M1 to 3.03±0.58 mg g^{-1} at M2 and 29.90±1.77 mg g^{-1} at M3. In contrast, MUFA content showed significantly (p<0.05) increased amount at M3. MUFA content of *Moina* sp. preserved with 5% DMSO decreased (p<0.05) significantly from 0.60±0.06 mg g^{-1} at M1 to 0.13±0.01 mg g^{-1} at M2. Yet, the MUFA content increased significantly (p<0.05) to 3.93±0.09 mg g^{-1} at M3. Similarly, the MUFA content of *Moina* sp. preserved with 10% DMSO only showed significantly (p<0.05) increased amount at M3. MUFA content of *Moina* sp. preserved with 20% DMSO showed significantly (p<0.05) increased amount from 0.37±0.11 mg g^{-1} at M1 to 0.10±0.02 mg g^{-1} and 3.05±0.18 mg g^{-1} at M2 and M3, respectively. PUFA content of *Moina* sp. preserved with 5, 10, and 20% DMSO had a significantly (p<0.05) increasing amount only at M3. PUFA content of *Moina* sp. preserved with 5% DMSO increased significantly (p<0.05) from 1.71±0.21 and 0.14±0.01 mg g^{-1} (at M1 and M2, respectively) to 34.33±4.35 mg g^{-1} at M3. Similar trends were found in regards to 10 and 20% DMSO.

EPA content was negligible detected in *Moina* sp. preserved with DMSO. DHA content of *Moina* sp. preserved with (5, 10, and 20%) DMSO was not significantly influenced by the storage duration. The ∑PUFA/SFA ratio had a significantly (p<0.05) higher amount at M3 for *Moina* sp. preserved with 5 and 20% DMSO. Meanwhile the ∑PUFA/SFA ratio of *Moina* sp. preserved with 10% DMSO was significantly (p<0.05) higher after M2. The ∑PUFA/MUFA ratio of *Moina sp.* preserved with 5 and 20% DMSO showed a fluctuating trend during M3. The ∑ PUFA+ MUFA/SFA ratio of *Moina sp.* preserved with 5 and 20% DMSO recorded a significantly (p<0.05) higher amount at M3. Interestingly, the ∑PUFA/SFA ratio of *Moina* sp. preserved with 10% DMSO showed the highest (p<0.05) amount at M2. In addition, the ∑ω6 and ∑ω3 in *Moina* sp. preserved in 5, 10, and 20 % DMSO showed a significantly (p<0.05) increased effects with prolonging storage duration.

At 1st month of preservation, DMSO concentrations did not significantly (p>0.05) influence the SFA content of *Moina* sp. However, at prolonged storage duration (M2), the SFA content of *Moina* sp. preserved with 20% DMSO gave significantly (p<0.05) higher amount

Table 4. Fatty acid profiling of *Moina* sp. preserved with dimethyl sulfoxide (DMSO) during storage at -40°C for 1, 2 and 3 months

Carbon	5%			10%			20%		
	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
SFA									
C 14: 0	63.37±5.87	0.69±0.06	26.71±4.70	69.27±14.30	1.45±0.20	24.27±5.95	56.97±2.48	0.66±0.08	16.53±1.04
C 15: 0	0.76±0.02	0.18±<0.01	3.99±0.68	0.80±0.01	0.20±<0.01	3.62±0.88	0.71±0.04	0.11±0.03	2.49±0.14
C 16: 0	0.73±0.07	0.01±<0.01	1.34±0.43	0.70±0.06	0.01±<0.01	0.48±0.05	0.64±0.05	-	-
C 17: 0	78.22±5.02	0.27±0.03	7.80±3.12	83.86±10.01	0.32±0.01	6.29±2.68	66.85±2.68	0.27±0.03	8.27±0.45
C 18:0	2.46±0.17	0.04±0.01	-	2.41±0.07	0.05±<0.01	0.00±0.00	1.63±0.10	1.93±0.79	-
C 20: 0	0.22±0.01	0.01±<0.01	0.93±0.24	0.22±0.03	0.01±<0.01	0.87±0.22	0.19±0.03	0.05±0.04	0.61±0.03
C 22: 0	16.14±0.66	0.00<±0.01	0.05±0.01	14.88±0.19	-	2.76±0.59	14.81±0.88	-	1.99±0.13
Subtotal	$159.44±11.00^{A,a}$	$1.16±0.09^{B,a}$	$40.81±2.87^{C,a}$	$169.73±24.20^{A,a}$	$1.98±0.19^{B,a}$	$38.30±5.06^{C,a}$	$140.17±6.15^{A,a}$	$3.03±0.58^{B,b}$	$29.90±1.77^{C,a}$
MUFA									
C 14:1	0.20±0.02	0.01±<0.01	0.77±0.14	0.21±0.04	0.00±0.00	0.83±0.25	-	-	0.62±0.04
C 16:1	-	-	-	-	0.03±<0.01	-	-	-	-
C 18:1n9t	-	-	-	-	-	-	-	-	-
C 18:1n9c	0.12±0.06	0.06±<0.01	2.59±0.21	-	-	1.78±0.54	0.12±0.12	0.05±0.02	2.12±0.14
C 20:1	0.28±0.06	0.06±<0.01	0.56±0.08	-	0.62±0.02	0.45±0.07	0.24±0.01	0.04±0.02	0.31±0.01
C 22:1n9	-	-	-	-	0.01±<0.01	-	-	-	-
Subtotal	$0.60±0.06^{A,a}$	$0.13±<0.01^{B,a}$	$3.93±0.09^{C,a}$	$0.21±0.04^{A,b}$	$0.67±0.02^{A,b}$	$3.06±0.22^{B,b}$	$0.37±0.11^{A,b}$	$0.10±0.02^{A,a}$	$3.05±0.018^{B,b}$
PUFA									
C 18:2n6t	0.20±0.01	0.02±<0.01	0.28±0.01	0.35±0.10	0.02±<0.01	0.00±0.00	0.17±<0.01	0.02±0.01	16.05±0.98
C 18:2n6	-	-	-	-	0.20±0.01	3.92±0.37	-	0.13±0.08	2.93±0.20
C 18:3n6	1.23±0.08	0.08±0.01	32.62±4.65	1.27±0.13	1.11±0.14	28.83±5.09	0.68±0.05	0.04±0.03	3.3.65±1.72
C 20:2	-	-	-	-	-	0.14±0.02	-	-	0.04±0.04
C 20:3n6	-	0.00±<0.01	0.33±0.07	0.20±0.03	0.00±0.00	2.86±0.50	-	-	1.62±0.81
C 20:3n3	-	0.01±<0.01	0.64±0.32	-	0.01±<0.01	-	0.15±0.15	-	0.83±0.03
C 20:4n6	-	-	-	-	-	0.86±0.03	-	-	-
C 20:5n3	-	-	-	-	-	0.48±0.06	-	-	0.37±0.01
C 22:2	-	0.02±<0.01	0.16±0.04	-	0.03±<0.01	0.17±0.04	-	0.02±0.01	0.12±0.01
C 22:6n3	0.28±0.14	0.01±<0.01	0.30±0.03	0.43±0.13	-	0.17±0.03	0.50±0.05	0.04±0.01	-
Subtotal	$1.71±0.21^{A,a}$	$0.14±0.01^{A,a}$	$34.33±4.35^{B,a}$	$2.25±0.19^{A,a}$	$1.37±0.14^{A,b}$	$37.42±5.11^{B,a}$	$1.50±0.16^{A,a}$	$0.27±0.07^{A,a}$	$55.60±2.10^{B,b}$
EPA	-	-	-	-	-	0.48±0.06	-	-	0.37±0.01
DHA	$0.28±0.14^{A,a}$	$0.01±<0.01^{A,a}$	$0.30±0.03^{A,a}$	$0.43±0.13^{A,a}$	-	$0.17±0.03^{A,b}$	$0.50±0.05^{A,a}$	$0.04±0.01^{A,b}$	-
∑ PUFA/SFA ratio	$0.01±<0.01^{A,a}$	$0.12±<0.01^{A,a}$	$0.84±0.16^{B,a}$	$0.01±<0.01^{A,a}$	$0.69±0.03^{B,b}$	$1.04±0.24^{B,a}$	$0.01±<0.01^{A,a}$	$0.25±0.06^{A,a}$	$1.86±0.05^{B,b}$
∑ PUFA/MUFA ratio	$2.97±0.60^{A,a}$	$1.06±0.08^{A,a}$	$8.74±1.17^{B,a}$	$10.70±0.93^{A,b}$	$2.04±0.16^{B,b}$	$12.11±0.90^{A,b}$	$4.66±1.85^{A,a}$	$2.77±0.08^{A,c}$	$18.27±0.46^{B,c}$
∑ PUFA + MUFA/SFA ratio	$0.01±<0.01^{A,a}$	$0.24±0.01^{A,a}$	$0.94±0.16^{B,a}$	$0.01±<0.01^{A,a}$	$1.04±0.05^{B,b}$	$1.12±0.26^{B,a}$	$0.01±<0.01^{A,a}$	$0.34±0.08^{A,c}$	$1.97±0.05^{B,b}$
∑ ω6	$1.43±0.08^{A,a}$	$0.10±0.01^{A,a}$	$35.27±4.60^{B,a}$	$1.82±0.06^{A,b}$	$1.34±0.14^{A,b}$	$36.46±5.26^{B,a}$	$0.85±0.04^{A,c}$	$0.21±0.07^{A,a}$	$54.25±2.06^{B,b}$
∑ ω3	$0.28±0.14^{AB,a}$	$0.02±<0.01^{A,a}$	$0.94±0.34^{B,a}$	$0.43±0.13^{A,a}$	$0.01±<0.01^{B,a}$	$0.65±0.10^{A,a}$	$0.65±0.20^{A,a}$	$0.04±0.1^{B,b}$	$1.20±0.04^{C,a}$

PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; SFA: Saturated fatty acids; M: Month. Results are reported as mean±standard error of mean unit of mg g^{-1}. Difference superscript of letters indicates significant differences among the percentages in month at One-way ANOVA (p<0.05). The absence of superscript indicates insignificant differences.Superscript in capital letter (A, B, C) indicates significant difference between the months in same percentages, while superscript in small letter (a, b, c) indicates significant differences between percentages in same month.

compared to 5 and 10% DMSO. In contrast, the SFA content of *Moina* sp. preserved with 5% DMSO were significantly (p<0.05) higher compared to 10 and 20% DMSO at M3. The MUFA content, at M1 and M3, the amount was decreased with increasing DMSO concentration. PUFA content was not significantly (p<0.05) influenced by the DMSO concentration at M1. In addition at M3 the PUFA content showed significantly (p<0.05) increased value at the highest percentage (20% DMSO) (Table 4).

3.2 Discussion

In this study, the PUFA content in controls were higher than in the preserved samples. However, these dropped almost by 70% from 13.69±0.55 mg g^{-1} at M1 to 4.98±0.61 mg g^{-1} at M2. Interestingly, the cryoprotective agent benefits in maintaining MUFA and PUFA in *Moina* sp. throughout the storage duration of three months. Nevertheless, the concentration of cryoprotective agents that had been used had a significant effect to the FA content in *Moina* sp. As stated earlier, at 5% GLY and EG showed a significantly lower amount of both the MUFA and PUFA compared to the 20% GLY and EG. Remarkably, these are exceptions to the *Moina* sp. preserved in DMSO. The concentration of DMSO did not show any significant effects on the MUFA and PUFA content.

The concentration of cryoprotective agent could either harm or protect the sample cells from denaturing. The uses of glycerol (GLY), ethylene glycol (EG), and dimethyl sulfoxide (DMSO) are excellent preservatives as they have low molecular weight and effectively penetrate into the cell and prevent intracellular ice formation at low temperature (Jang *et al*., 2017). It was stated that glycerol protects the membrane structure and maintain the nature of the internal protein. In addition, glycerol effectively reduces the electrolyte concentration in the residual unfrozen solution in and around the cell at any temperature (Jang *et al*., 2017). DMSO makes the cell membrane porous which protect the cell from damaging during thawing process. DMSO is also known of its ability to solubilize polar and nonpolar substances and transpose hydrophobic barriers, such as the plasma membrane (Costa *et al*., 2017).

A study by Davies *et al.* (2014) emphasized that to protect cells from damage during the cryopreservation process and to maximize cell recovery cryoprotectants are incorporated within the freezing medium. In addition, cryopreservation is ideal in maintaining the cell functional properties and genetic characteristics in long term (Hubel, 1997). However, the type of freezing also possesses significant risks to freeze injury and leads to cell loss. Therefore, a slow freezing method was introduced in this study to prevent intra and extracellular ice formation in *Moina* sp. samples. Furthermore, this study was not using non-sterile liquid nitrogen and thus prevents the potential contamination of pathogenic agents. It is also important for the cell to maintain their viability and functional properties. Previous research stated that DMSO efficiently prevents cell rupture by reducing the water activity within the cell and thus inhibiting the formation of intracellular ice crystals (Berz *et al*., 2007). Earlier, Tuschong *et al.* (2002) stated that 5% DMSO offers less cytotoxic and activates the xenogeneic immune responses. Interestingly, 5% DMSO is able to maintain the cell phenotype and their functional properties (Yong *et al*., 2015). In addition, the cell preserved with 5% DMSO showed a similar proliferation rate at the initial day and after 14 days of storage (Yong *et al*., 2015). Therefore, 5% DMSO is significantly potential for cell storage and long-term preservation practice.

Previous research stated that higher DMSO concentration could lead to decrease in the survivability of cells up to 73% (Chen and Thibeult, 2013). A study by Yong *et al.* (2015) documented that 10% DMSO gave adverse effects and toxic reactions such as respiratory depression and neurotoxicity. Besides, utilization of a high concentration of EG has been shown to cause an initiation or exacerbation of neuronal cell damage (Regulska *et al*., 2010). Usage of high concentration of glycerol is reported to result in the lesion and loss of plasma membrane integrity of the cell (Armitage and Mazur, 1984). Preservation at more than 20% is ineffective in storing the fatty acid composition of *Moina* sp.

4. Conclusion

Moina sp., a high-quality natural food, is essential to feed the larvae, fry, and fingerlings. Therefore a sufficient supply and availability of *Moina* sp. are crucial in the aquaculture industry. This study recommends *Moina* sp. preserved with cryoprotective agents of 5% dimethyl sulfoxide (DMSO) for an optimum nutritional retention. DHA content of *Moina* sp. preserved with 5% DMSO was constant at range of 0.28±0.14 to 0.30±0.03% during three months of storage duration. Thus, offers an optimal dietary requirement for larval stage and suitable sparing proteins for energy usage. Even though other preservation formula offers higher DHA, the storage duration significantly affects their quality.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author as follows, Karim, N.U.; conceptualization, methodology, formal analysis, writing original draft preparation, writing review and editing, supervision, project administration, and funding acquisition. Yusuf, H.; methodology, resources, and funding acquisition. Sofian, M.F.; formal analysis, investigation, data curation, and writing original draft preparation. Abu Hena M.K.; writing review and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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