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INFLUENCE OF CULTURE MEDIA ON THE FATTY ACID COMPOSITION OF MARINE MICROALGA *Tetraselmis gracilis* KYLIN (BUTCHER, 1959) USED IN MOLLUSCAN HATCHERY

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ABSTRACT

The present study was done to analyze the fatty acid composition of *Tetraselmis gracilis* in different culture media viz. f/2, Walne's, Chu and WC. Standard protocol was followed for the culture of *Tetraselmis gracilis* under laboratory conditions which include factors such as, temperature (25°C), pH (7), light intensity (1500 lux), and photoperiod (12 hours light: 12 hours dark). Total lipid was extracted during exponential and stationary growth phases of the alga. Fatty acids were analyzed using gas chromatography. Analysis showed the presence of saturated fatty acids (f/2- 42.93%, Walne's- 48.16%, Chu- 57.24%, WC- 66.27%), mono unsaturated fatty acids (f/2- 32.81%, Walne's- 27.21%, Chu- 30.92%, WC- 32.55%) and polyunsaturated fatty acids (f/2- 24.44%, Walne's- 25.35%, Chu- 11.80%, WC- 1.16%) in the exponential phase. The presence of saturated fatty acids (f/2- 48.50%, Walne's- 45.54%, Chu- 51.91%, WC- 43.81%), mono unsaturated fatty acids (f/2- 40.02%, Walne's- 34.37%, Chu- 35.81%, WC- 50.78%) and polyunsaturated fatty acids (f/2- 11.43%, Walne's- 20.05%, Chu- 12.23%, WC- 5.37%) in stationary phase was also found out. In the exponential phase of growth *T. gracilis* was found to produce maximum saturated fatty acids in WC medium (66.27%), monounsaturated fatty acids in f/2 medium (32.81%) and polyunsaturated fatty acids in Walne's medium (25.35%). In stationary phase of growth *T. gracilis* was found to produce maximum saturated fatty acids in Chu medium (51.91%), monounsaturated fatty acids in WC medium (50.78%) and polyunsaturated fatty acids in Walne's medium (20.05%). In both the phases *T. gracilis* was found to produce more Eicosapentaenoic acid (EPA-17.11%) in Walne's medium. So Walne's medium can be considered as the best medium for providing maximum PUFA to *T. gracilis* for the larval rearing of shell fish.

KEYWORDS: *Tetraselmis gracilis*, Fatty Acids, Culture Medium, Polyunsaturated Fatty Acids, Eicosapentaenoic Acid, Molluscan Hatchery.

The culture of microalgae as food for the commercial rearing of the marine animals is of critical importance in the mariculture industry. Microalgae are directly utilized by all stages of bivalves, by larval stages of some crustacean species, and by very early growth stages of some fish species. Algae are also used to rear mass quantities of zooplankton (rotifers, copepods, and brine shrimp), for food for late- larval and juvenile stages of crustaceans and fish. The value of algae in this zooplankton food chain is also critical, since essential algal nutrients are passed on via the intermediary zooplankton to the cultivated animals (Brown et al., 1989).

Marine fish, mollusks and crustaceans produced via aquaculture generally exhibit poor ability to synthesize certain essential long chain PUFAs in quantities high enough for growth and survival especially EPA (20:5n-3) as well as DHA (22:6n-3) through chain elongation and desaturation (Martin-Creuzburg and Elert, 2004). As a consequence, it is very important that food sources provided to these animals supply all the essential PUFAs which are present or exist abundantly in microalgae in order to satisfy their dietary requirements.

Furthermore, microalgal lipids are greatly influenced by culturing conditions which include nutrient deprivation, light quality, photon flux density (PFD), photoperiod (L/D cycle) as well as temperature both qualitatively and quantitatively (Lourenco et al., 1997: Huerlimann et al., 2010). The effect of variation of these parameters on many algae species has been studied in order to better understand their physiology, as well as to answer specific and relevant questions for mass culture and nutrition of bivalves and others herbivores (Uriarte et al., 1993).

So, this study was aimed to study the fatty acid composition of marine microalga *Tetraselmis gracilis* in different culture media. This study provides basic information on the fatty acid composition of this microalga in different culture media and different growth phases, and possible uses of this knowledge can generate more studies which look deep in to the effect of different culture parameters on the fatty acid composition of different microalgae.

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MATERIALS AND METHODS

Collection of Sample and Culture Conditions

Axenic stock culture of *T. gracilis* was collected from Central Marine Fisheries Research Institute, Cochin. *Tetraselmis gracilis* having a cell density of 10×10^4 cells/ ml was used as inoculum. Ten percent of inoculum having a cell density of 10×10^4 cells/ ml was inoculated in to 4 litre Haffkine's flasks containing sea water of salinity 33‰ and pH was maintained at 7 (Anderson, 2005). The sea water was enriched with the four media viz. f/2 (Guillard, 1975), Walne's (Walne, 1966), Chu (Chu, 1942) and WC (Guillard and Lorenzen, 1972). All treatments were carried out in triplicates. The cultures were continuously illuminated by fluorescent lamps at light intensity 1500 Lux on a 12h light: 12h: dark cycle with temperature 25ºC (Hoff and Snell, 1987). Total lipid and fatty acid was extracted according to the method of Bligh and Dyer (1959) and Metcalfe et al. (1966), respectively by harvesting the algal cells at particular growth phases viz. exponential and stationary by flocculation method (Hoff and Snell, 1987).

Statistical Analysis

The data were compared using one way ANOVA with Tukey's post hoc tests. P values <0.05 were used as standard for statistical significance. The experiments were carried out in triplicate and the results were expressed as mean ± standard deviation. Least significant difference (LSD) was used to compare the significant difference between means. SPSS version 22 was used for analysis.

RESULTS AND DISCUSSION

Culture of *T. gracilis* was harvested during the exponential and stationary phase and total lipid was extracted and analyzed for the presence of fatty acids. Analysis using one way ANOVA revealed that the fatty acid composition of *Tetraselmis gracilis* cultured in different culture media showed significant difference ($P<0.05$) between medium and different growth phase viz. exponential and stationary. The major fatty acids found in *T. gracilis* are tridecanoic acid (C13:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), stearic acid (C18:0) (saturated fatty acids), palmitoleic acid (C16:1), oleic acid (C18:1) (mono unsaturated fatty acids), linoleic acid (C18:2), arachidonic acid (C20:4), eicosapentaenoic acid (C20:5) and behenic acid (C22:0) (poly unsaturated fatty acids) (T-1) (figure- 1, 2).

In f/2 medium, in the exponential phase, *T. gracilis* was found to produce 42.93% of saturated fatty acids (SFAs), 32.81% of monounsaturated fatty acids (MUFAs) and 24.44% of polyunsaturated fatty acids (PUFAs). Ohse et al. (2015) also supports this result through their study on *Tetraselmis suecica* and *Tetraselmis chuii*. They also reported dominance of palmitic acid and oleic acid in these microalgae. In f/2 medium, the fatty acid composition is dominated by SFAs and this result is in accordance with the results reported by Sahay et al. (2016) in *Tetraselmis* sp.

In the stationary phase of growth, *T. gracilis* produced 48.50% SFAs, 40.02% MUFAs and 11.43% PUFAs. Similar result was also reported by Ohse et al. (2015) in *T. suecica* cultured in f/2 medium. He observed that SFAs (41.91%) was the major fatty acid family in *T. suecica* followed by MUFAs (30.79%) and PUFAs (26.03%), respectively. In the present study also *T. gracilis* showed higher amounts of myristic acid (6.20± 0.12), palmitic acid (35.59± 0.14) (SFAs), palmitoleic acid (27.23± 0.17), oleic acid (12.79± 0.18) (MUFAs) and EPA (8.43± 0.12) (PUFAs).

In Walne's medium, in the exponential phase, *T. gracilis* was found to produce 48.16% of SFAs, 27.21% of MUFAs and 25.35% of PUFAs. According to the report of Fernandez- Reiriz et al. (1989) *Tetraselmis seucica* cultured in Walne's medium exhibited a higher percentage of SFAs (61.37%) in the exponential phase. The percentage composition of MUFAs in the study of Fernandez- Reiriz et al. (1989) was 25.02% which is in a similar trend with the present report. In contrast, they could not detect any PUFAs in their study. Costard et al. (2012) studied the chemical composition of five marine microalgae viz. *Bellerochea* sp., *Chaetoceros* sp., *Chlorella* sp., *Rhodomonas* sp. and *Thalassiosira* sp. Fatty acid profiles of these microalgae support the results of the present study with comparably high percentages of SFAs in the exponential phase. *T. gracilis* showed higher amounts of myristic acid (7.66% ± 0.25), palmitic acid (21.12%± 0.23), behenic acid (17.53± 0.06) (SFAs), palmitoleic acid (26.39%± 0.08) (MUFAs), and EPA (17.09%± 0.10) (PUFAs) in this phase. *T. gracilis* when grown in Walne's medium and harvested during the exponential phase of the growth, was found to produce more palmitic and palmitoleic acid in comparison to the other fatty acids. This result is in corroboration with the results reported by Lourenco et al. (1997) in *T. gracilis* and Fernandez-Reiriz et al. (1989) in *T. suecica*.

In the stationary phase of growth *T. gracilis* produced 45.54% SFAs, 34.37% MUFAs and 20.05% PUFAs. Fernandez- Reiriz et al. (1989) also reported dominance of SFAs in their study. *T. gracilis* showed higher amounts of myristic acid (6.81%± 0.05), palmitic acid (29.71%± 0.11) (SFAs), palmitoleic acid (26.23%± 0.19) (MUFAs) and EPA (17.11%± 0.09) (PUFAs) in the stationary phase. This is in corroboration with the results reported by Lourenco et al. (1997).

In Chu medium, in the exponential phase, *T. gracilis* was found to produce 57.24% of SFAs, 30.92% of MUFAs and 11.80% of PUFAs. In the stationary phase of growth *T. gracilis* produced 51.91% SFAs, 35.81% MUFAs and 12.23% PUFAs. In WC medium, in the exponential phase *T. gracilis* was found to produce 66.27% of SFAs, 32.55% of MUFAs and 1.16% of PUFAs. In the stationary phase of growth *T. gracilis* produced 43.81% SFAs, 50.78% MUFAs and 5.37% PUFAs. *T. gracilis* showed higher amounts of palmitic acid (36.45%± 0.43) (SFAs) and palmitoleic acid (29.40%± 0.04) (MUFAs) in this phase.

From the results it has been found that the growth phase of the organism affected its fatty acid composition and production. When *T. gracilis* was harvested during the exponential and stationary phases of growth it was found out that saturated fatty acids tend to decrease when enter into the stationary phase of growth in Walne's, Chu and WC media. Whereas in f/2 medium, saturated fatty acids showed an increase in the stationary phase of growth. In case of monounsaturated fatty acids an increase in production from exponential to stationary phase has been observed in all the culture media. Polyunsaturated fatty acids showed a tendency to increase in amount from exponential to stationary phase in Chu medium and WC medium whereas the amount of PUFA got decreased in f/2 and Walne's medium as the culture aged.

The above results and discussion revealed a visible difference in quantity and quality of fatty acids produced by the microalgae with respect to the culture medium. Likewise, the growth phase also affects the profile of fatty acids in microalgae. In the present study SFAs were found to decrease in the stationary phase in Walne's, Chu and WC media as reported by Fernandez- Reiriz et al. (1989) in *Tetraselmis suecica* culturerd in Walne's medium. In f/2 medium, SFAs increased in the stationary phase of growth, and this is in accordance with the results reported by Pratoomyot et al. (2005) who cultured *Tetraselmis* sp. in f/2 medium and found that SFAs increased in the stationary phase. Supporting results were published by Huerlimann et al. (2010) who cultured *Tetraselmis* sp. in f/2 medium.

In case of MUFAs, an increase in quantity was found when the culture entered the stationary phase of growth in all the culture media. Similar trend was observed in *Tetraselmis* sp. when cultured in f/2 and Walne's medium (Fernandez- Reiriz et al., 1989: Huerlimann et al., 2010 and Pratoomyot et al., 2005).

In the present study PUFAs was found to decrease in f/2 and Walne's medium as the culture aged which is in agreement with the study of Huerlimann et al., (2010) and Pratoomyot et al. (2005).

The PUFAs, 20:5(n - 3) and 22:6(n - 3) have been shown to be essential for a variety of mollusk, prawn and fish larvae, and may also be essential for other marine animals (Castell et al., 1986). The present work revealed that *T. gracilis* can produce up to 18% of EPA.

The fatty acid profiling of *T. gracilis* was found to produce more PUFAs when cultured in Walne's medium. So Walne's medium can be considered as the best medium for providing better growth and nutritional quality to *T. gracilis* which in turn results a good nutrient composition especially EPA content which can provide better growth and metamorphosis in bivalve molluscs and shrimp larvae.

Table 1: Percentage of fatty acids in *T. gracilis* cultured in four media

Fatty acid	Medium							
	f/2		Walne's		Chu		WC	
	EP	SP	EP	SP	EP	SP	EP	SP
C 13:0	1.42±0.32	0.24±0.06	0.81±0.08	1.12±0.11	0.34±0.03	0.72±0.02	3.30±0.20	0.65±0.03
C 14:0	6.35± 0.12	6.20± 0.12	7.66±0.25	6.81±0.05	6.43±0.86	5.39±0.79	3.46±0.10	1.94±0.07
C 15:0	0.36±0.01	0.58±0.04	0.02±0.01	3.77±0.14	0.50±0.03	0.54±0.08	8.20±0.09	0.36±0.02
C 16:0	27.86±0.37	35.59±0.14	21.12±0.23	29.71±0.11	41.47±0.60	36.27±0.53	32.68±0.08	36.45±0.43
C 18:0	1.96±0.06	2.11±0.08	1.02±0.08	0.05±0.31	2.58±0.15	0.81±0.05	1.96±0.20	2.98±0.10
C 22:0	4.98±0.13	3.84±0.09	17.53±0.06	4.08±0.04	5.90±0.57	8.18±0.40	16.67±0.93	1.41±0.02
SFA	**42.93**	**48.50**	**48.16**	**45.54**	**57.24**	**51.91**	**66.27**	**43.81**
C 16:1	24.66±0.28	27.23±0.17	26.39±0.08	26.23±0.19	16.57±0.69	26.98±0.54	22.17±0.21	29.40±0.04
C 18:1	8.15±0.08	12.79±0.18	0.82±0.05	8.32±0.24	14.35±1.13	8.83±0.03	10.38±0.20	21.38±1.16
MUFA	**32.81**	**40.02**	**27.21**	**34.37**	**30.92**	**35.81**	**32.55**	**50.78**
C 18:2	1.73±0.10	0.90±0.10	3.08±0.09	2.54±0.16	1.33±0.04	1.36±0.02	0.57±0.04	1.48±0.04
C 20:4	3.74±0.17	2.10±0.50	5.18±0.04	0.40±0.10	1.21±0.03	2.36±0.25	0.11±0.02	1.17±0.05
C 20:5	18.75±0.36	8.43±0.12	17.09±0.10	17.11±0.09	9.26±0.40	8.51±0.62	0.48±0.04	2.72±0.06
PUFA	**24.44**	**11.43**	**25.35**	**20.05**	**11.80**	**12.23**	**1.16**	**5.37**

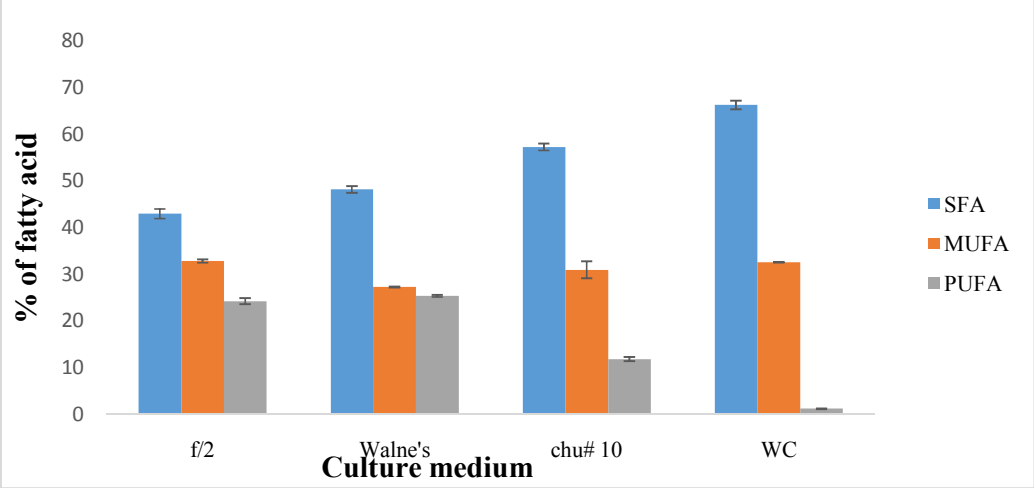

Figure 1: Fatty acid composition of *T. gracilis* during the exponential phase of growth

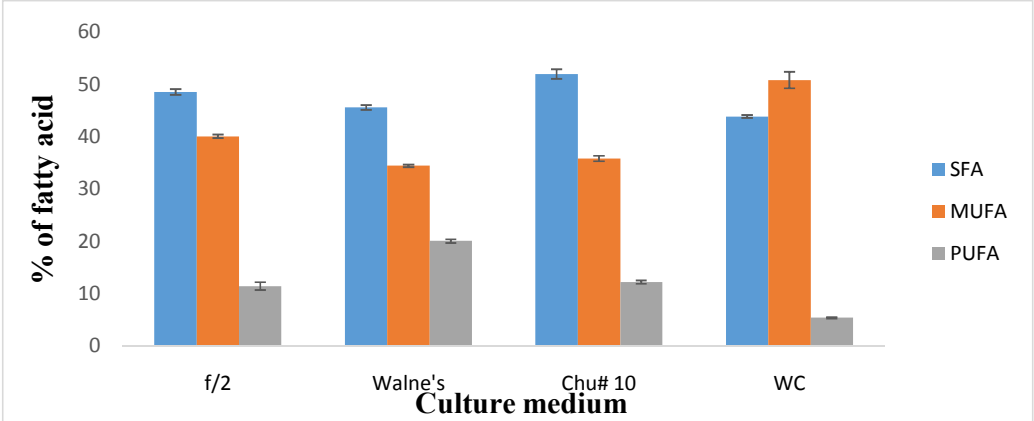

Figure 2: Fatty acid composition of *T. gracilis* during the stationary phase of growth

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