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ORIGINAL PAPER

Fatty acid composition of 12 microalgae for possible use in aquaculture feed

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Abstract Twelve algal strains representing the classes Cyanophyceae, Prymnesiophyceae, Bacillariophyceae, Rhodophyceae, Cryptophyceae, Chlorophyceae, Xantophyceae and Eustigmatophyceae were selected mainly from the culture collection of the Norwegian Institute for Water Research (NIVA). The algae were grown as continuous cultures in a 1.8 l. reactor, internally illuminated with an 11 W fluorescent tube. The retention time was adjusted in the range 2–4 days to fit the growth rate of the algae. The growth responses and fatty acid composition were analysed. The maximum production rate was obtained with *Pseudokirchneriella subcapitata* (0.63 g 1^{-1} day^{-1}) and the lowest with *Porphyridium cruentum* 0.13 g 1^{-1} day^{-1}. Arachidonic acid (AA) and eicosapentaenoic acid (EPA) were the dominating polyunsaturated fatty acids (PUFAs) in *P. cruentum*, while only EPA accumulated in *Phaeodactylum tricornutum*. Docosahexaenoic acid (DHA) was the major PUFA in *Isochrysis galbana*, while *Pavlova* sp. had both EPA and DHA. This is the first report on the fatty acid profiles of *Nannochloropsis oceanica, Chroococcus* sp., *Synechococcus* sp. and *Tribonema* sp.

Keywords Continuous culture · Growth media · Photobioreactor · Polyunsaturated fatty acids · Productivity

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Introduction

Eicosapentaenoic acid (EPA, C20:5 ω3) and docosahexaenoic acid (DHA, C22:6 ω3) are important ω3 polyunsaturated fatty acids (PUFA), while arachidonic acid (AA, C20:4 ω6), is a vital ω6-PUFA. They have numerous neutraceutical and pharmaceutical applications (Shahidi and Wanasundara 1998; Horrocks and Yeo 1999). EPA and DHA are important in the treatment of atherosclerosis, cancer, rheumatoid arthritis, psoriasis and diseases of old age, such as Alzheimer's and age-related macular degeneration (Drevon et al. 1993; Simopoulos 1999). AA and DHA are of special importance in the brain and blood vessels and are considered to be essential for pre- and post-natal brain and retina development (Crawford 2000). The eicosanoids, such as prostaglandin, prostacyclin and leukotriene, derived from ω3-PUFAs are important for infant development, modulatory vascular resistance and wound healing (Simopoulos 1999; Nettleton 1995). Modern nutritional theory is focusing on the numerous health benefits of maintaining sufficient levels of ω3-PUFAs (Patil and Gislerød 2006).

The principal dietary source of DHA and EPA is marine fish. The global aquaculture production was 547 million tonnes in 2003 (FAO 2006), and it has been growing by roughly 9% annually (Naylor and Burke 2005), with trends towards intensification and greater control over nutritional input that result in increasing demand for fish feed. Feed is the largest production cost for commercial aquaculture plants, so improving feed efficiency in industrial systems has high priority. Moreover, fishmeal prices have risen in real terms in the past three decades and are likely to increase further with continuously growing demand. Fish is a declining resource, and there are serious environmental consequences related to the continued exploitation of fish stocks in order to meet the demands of an expanding market. Moreover, fish accumulate pollutants. The extracted oil often has an unpleasant odour, and the proportion of specific fatty acids in its triacylglycerols is difficult to control. Considerable evidence has indicated that ω3-PUFAs in fish oils are actually derived via the marine food chain from zooplankton consuming ω3 PUFA-synthesising microalgae (Yongmanitchai and Ward 1989). The importance of microalgae as a source of PUFA has been recently reviewed (Patil et al. 2005). The microalgae may have superior lipid stability compared with traditional PUFAs, because they are naturally rich in antioxidant carotenoids and vitamins and because lipids are bioencapsulated by the algal cell wall. Therefore, microalgae are some of the most important feed sources in aquaculture (live feed for larvae of bivalves, crustaceans and marine fish; food for rotifers and shrimps), due to their nutritional value and their ability to synthesise and accumulate great amounts of ω3-PUFA.

The objectives of the present study were twofold: (1) mainly to screen microalgae from the Norwegian Institute for Water Research (NIVA) culture collection for PUFA (DHA, EPA & AA) and (2) to investigate the growth performance and production potential in continuous culture.

Material and methods

Algal cultures

Twelve algal strains representing Cyanophyceae, Prymnesiophyceae, Bacillariophyceae, Rhodophyceae, Cryptophyceae, Chlorophyceae and Xantophyceae were

selected from the culture collection of NIVA. *Nannochloropsis oceanica* (Eustigmatophyceae) was obtained from Grieg Marine Farms AS, Nedstrand, Norway. All of these are unicellular microscopic forms with *Tribonema* sp. being the exception; it is filamentous. Table 1 lists the strains used in the present study, with mean cell size and cell dry weight.

Growth conditions

The algae were produced in a photobioreactor with continuous internal illumination by an 11 W fluorescent tube. The culture volume was 1.8 l. The culture was continuously aerated, and CO_2 was added to the air stream. Addition of CO_2 was triggered by a pH meter set at pH 7.5 (freshwater algae) or pH 8.0 (seawater algae). Temperature was controlled at 20 ± 2°C. Half-strength ''Z8'' (Staub 1961; Kotai 1972) was the growth medium used. The marine algae were cultured in seawater medium prepared from natural seawater (collected from Oslofjord from 60 m depth), the salinity adjusted to 25 g l^{-1}. Media for culturing fresh water algae were prepared with distilled water.

The production reactor was inoculated from stock cultures of various marine and freshwater microalgae and operated as a batch culture until the algae had passed the exponential growth phase. At this stage, continuous supply of fresh medium was initiated. The dilution rate was adjusted according to the growth rate of the algae, to obtain steady state.

Biomass measurements

Algal cell density was recorded daily for 10–20 days with a Coulter Multisizer equipped with a 100 μm-orifice tube. *Tribonema* sp. could not be measured with the electronic particle counter due to formation of filaments, and, for this alga, the cell density was measured as optical density at 710 nm, with a Pharmacia Novospec II

Table 1 Overview of microalgae with their cell size and cell dry weight used in the study

Class	Genus/ species	Fresh/ marine	Strain	Mean cell size (μm^3 $cell^{-1}$)	Cell dry weight (10^{-9} mg $cell^{-1}$)
Cyanophyceae	*Chroococcus* sp.	Marine	NIVA-CYA 330	12	8.6
	Synechococcus sp.	Marine	NIVA-CYA 379	1.8	1.2
Prymnesiophyceae	*Isochrysis galbana*	Marine	NIVA-4/91	45	16
	Pavlova sp.	Marine	NIVA-4/92	49	13
Bacillariophyceae	*Phaeodactylum tricornutum*	Marine	NIVA-BAC 2	44	19
Rhodophyceae	*Porphyridium cruentum*	Marine	NIVA-1/92	227	150
Cryptophyceae	*Rhodomonas baltica*	Marine	NIVA-5/91	262	120
	Oocystis sp.	Fresh	NIVA-CHL 154	21	19
Chlorophyceae	*Pseudokirchneriella subcapitata*	Fresh	NIVA-CHL 1	62	30
	Tetraselmis suecica	Marine	NIVA-3/92	450	220
Xanthophyceae	*Tribonema* sp.	Fresh	NIVA-1/84	200	140
Eustigmatophyceae	*Nannochloropsis oceanica*	Marine	NIVA-2/03	15	6.4

spectrophotometer and a cell with a 1 cm path length. The average daily production was calculated over a period of at least 6 days of approximate steady-state conditions. Dry weight was determined by the filtering of different volumes of algal culture through Whatman GF/C glass fibre. The filters were washed with 0.5 M ammonium formate, dried at 102°C overnight and weighed.

Analytical methods

The harvested algae were stored at 4°C until the production was terminated and then concentrated by continuous centrifugation. The concentrated algae were freeze-dried and stored at –80°C until analysis of fatty acids was performed. Freeze-dried algae mass was accurately weighed, an internal standard (trinonadecanoin C19:0, Larodan Fine Chemicals, Malmö, Sweden) was added and lipids were extracted by the Bligh and Dyer (1959) method and converted to methyl esters with methanolic HCl and 2,2-dimethoxy propane (Mason and Waller 1964). The methyl esters were analysed in a GC (HP G1530A) equipped with a DB-WAX column (0.1 mm i.d., 10 m, 0.1μm film, produced by J&W Scientific). The temperature programme started at 70°C for 0.2 min, increased by 60°C min^{-1} to 200°C, then 10°C min^{-1} to 240°C (2 min) and 60°C min^{-1} to 250°C, with a final hold time of 5 min. Peaks were integrated with HP GC ChemStation software (rev. A.05.02) and identified by comparison of the retention times with those of pure standards. System performance was checked with blanks and standard samples prior to analysis and regularly within the sample series. The concentration of individual fatty acids was expressed in milligrammes per gramme dry-weight algae.

Results

Growth kinetics

Results of the production trials for the different algae where sufficient steady-state conditions were obtained are presented in Table 2. The highest daily yield was observed in *P. subcapitata* (1.13 g day^{-1}), followed by *Tribonema* sp. (0.92 g day^{-1}), and *Nannochloropsis oceanica* (0.89 g day^{-1}). In relation to the energy input for illumination (0.264 kWh day^{-1}), *P. subcapitata* had a productivity of 4.3 g kWh^{-1} and was the most efficient species, followed by *Tribonema* sp. (3.5 g kWh^{-1}) and *N. oceanica* (3.3 g kWh^{-1}). *Porphyridium cruentum* was the least productive of the ten algae. Steady-state conditions were not achieved in both the algae from Cyanophyceae viz., *Chroococcus* sp. and *Synechococcus* sp., indicating that the culturing conditions were not optimal for these strains.

Fatty acid content

The fatty acid profiles of the algae are presented in Table 3. The highest lipid content was observed in *P. tricornitum* followed by *I. galbana* and *P. subcapitata*, while *Tribonema* sp. had the lowest lipid content. The fatty acid profiles varied between the classes. In Cyanophyceae, *Chroococcus* sp. had C16:0 and C18:2 $\omega 6$ as the dominating fatty acids, with lower amounts of C16:1 and C18:3 $\omega 3$ (Table 3),

Table 2 Productivity of algae in continuous culture

Alga	Retention time (days)	Density (g l^{-1})	Total yield (g day^{-1})	Volumetric yield (g l^{-1} day^{-1})	Energy yield (g kWh^{-1})
Chroococus sp.	2.6	–	–	–	–
Synechococcus sp.	3.3	–	–	–	–
Isochrysis galbana	3.1	0.47	0.28	0.16	1.06
Pavlova sp.	4.1	1.1	0.5	0.28	1.89
Phaeodactylum tricornutum	2.1	0.86	0.77	0.43	2.92
Porphyridium cruentum	3.2	0.24	0.14	0.08	0.53
Rhodomonas baltica	4.1	0.36	0.16	0.09	0.61
Oocystis sp.	2.9	0.68	0.43	0.24	1.63
Pseudokirchneriella subcapitata	2.2	1.03	1.13	0.63	4.28
Tetraselmis suecica	3.3	0.84	0.48	0.27	1.82
Tribonema sp.	2.9	1.39	0.92	0.51	3.48
Nannochloropsis oceanica	2.2	1.09	0.89	0.49	3.34

Dashes indicates that steady state conditions were not achieved

while C14:0, C16:0 and C16:1 were the most abundant fatty acids in *Synechococcus* sp. The diatom *P. tricornutum* was dominated by C14:0, C16:0, C16:1 and C20:5 ω3 fatty acids. In Prymnesiophyceae, *Isochrysis galbana*, C14:0, C16:0, C18:1, C18:4 ω3 and C22:6 ω3 were the major fatty acids, with lower amounts of C16:1, C18:2 ω6 and C18:3 ω3, while C14:0, C16:0, C16:1, C20:5 ω3 and C22:6 ω3 were the most abundant fatty acids, with lower amounts of C18:1, C18:2 ω6, C18:3 ω3 and C18:4 ω6 in *Pavlova* sp. The red algae *Pophyridium cruentum* had mainly C16:0, C20:4 ω6 and 20:5ω3 fatty acids. *Rhodomonas baltica* (Cryptophyceae) had C14:0, C16:0, C24:0, C18:2 ω6, C18:3 ω3, C18:4 ω6 and C20:5 ω3 as the major fatty acids. There was no variation in fatty acid distribution in the two fresh water Chlorophyceae genera. Both *Oocystis* sp. and *Pseudokirchnella subcapitata* had mainly C16:0, C18:1, C18:2 ω6 and C18:3 ω3 as the dominant fatty acids. The major fatty acids in *Tetraselmis suecica* were C16:0, C18:1, C18:3 ω3, C18:4 ω6 and C20:5 ω3. *Tribonema* sp. (Xanthophyceae) had mainly C16:1, with small amounts of C16:0 and C20:5 ω3 fatty acids. The fatty acids C14:0, C16:0, C16:1, C18:2 ω6 and C20:5 ω3 dominated in *Nannochloropsis oceanica*

Discussion

Most of the algae were isolated at NIVA and originated from Norwegian lakes, rivers and coastal waters. The strains were selected to include some species known to produce high levels of PUFAs, viz. *Isochrysis galbana, Pavlova* sp., *Phaeodactylum tricornutum* and *Porphyridium creuntum* as well as *Chroococcus* sp., *Synechococcus* sp. and *Tribonema* sp. for which the fatty acid composition has not been previously described. These strains were selected on the basis of their appearance in stock culture which indicated high lipid content. Modified 'Z8' (Staub 1961; Kotai 1972) was the culture medium of choice, as it has been used routinely in NIVA for over three decades. *Nannochloropsis* species are widely used as food in aquaculture (Maruyama et al. 1986; Apt and Behrens 1999) and have been proposed for the commercial production of eicosapentaenoic acid (Sukenik 1991). However, the strains used for such applications are sometimes not adequately identified to species.

Table 3 Fatty acid (FA) profile of the microalgae tested. Data are given as mg g^{-1} of dry weight

	Chroococcus sp.	*Synechococcus* sp.	*Isochrysis galbana*	*Pavlova* sp.	*Phaeodactylum tricornutum*	*Porphyridium cruentum*	*Rhodomonas baltica*	*Oocystis* sp.	*Pseudokirchneriella subcapitata*	*Tetraselmis* sp.	*Tribonema* sp.	*Nannochloropsis oceanica*
Saturated												
C12:0	2.1	0.7	–	–	–	–	2.0	–	–	–	–	1.2
C14:0	0.1	5.6	8.9	7.5	8.8	–	4.1	0.2	0.1	0.5	1.1	16.9
C16:0	21.3	3.4	11.5	13.4	16.6	5.9	6.0	3.8	16.2	6.3	2.5	17.2
C 18:0	0.3	–	–	0.4	0.6	0.2	0.8	–	1.3	1.2	0.1	1.8
C 20:0	0.2	–	–	–	–	–	0.1	–	0.2	–	–	–
C 24:0	–	–	–	–	1.6	–	4.0	0.1	0.7	–	–	–
Sum	24.0	9.7	20.4	21.3	27.6	6.1	17	4.1	18.5	8.0.	3.7	37.1
Monosaturated												
C 16:1	1.1	10.8	3.3	12.8	26.0	–	0.4	1.5	1.0	1.3	5.1	18.2
C18:1[c]	0.4	–	13.1	2.9	1.8	0.1	3.4	3.9	31.1	10.7	0.2	4.1
C 20:1[c]	–	–	–		–	–	0.1	–	0.9	0.9	–	0.5
C 22:1[c]	0.1	–	0.6	0.8	0.3	–	0.1	–	0.8	–	–	
Sum	1.6	10.8	17.0	16.5	28.1	0.1	4.0	5.4	33.8	12.9	5.3	22.8
Polyunsaturated												
C 18:2[b]	10.7	–	7.0	2.1	1.5	2.1	11.7	6.4	5.1	2.5	0.2	9.7
C 18:3[a]	1.0	–	3.8	1.8	0.3	–	12.0	8.1	11.4	6.4	–	0.5
C 18:4[a]	–	–	12.5	4.3	3.3	–	5.1	0.7	3.0	4.1	0.1	
C 20:2[b]	0.1	–	–		–	0.3	0.1	–	–	–	–	0.5
C 20:4[b]	0.1	–	–	0.4	2.2	6.0	0.2	0.5	–	0.6	–	3.7
C 20:5[a]	–	–	0.8	18.0	28.4	6.1	4.4	1.1	–	4.8	3.2	23.4
C22:5[b]	–	–	–		1.3	–	0.2	–	2.1	–	–	–
C22:6[a]	–	–	15.8	13.2	0.2	–	–	–	0.1	0.2	–	–
Sum	11.9	–	39.9	39.8	37.2	14.5	33.7	16.8	21.7	18.6	3.5	37.8

Dashes indicate FA not detected

[a] ω-3 fatty acids

[b] ω-6 fatty acids

[c] ω-9 fatty acids

We obtained a *Nannochloropsis* strain from a hatchery in the west coast of Norway; this was identified as *Nannochloropsis oceanica* (Suda et al. 2002) from the 18S rDNA gene sequence by Hart et al. at the Dunstaffnage Marine Laboratory, Oban, Argyll, UK.

To our knowledge, this is the first report on the fatty acid profiles of *Nannochloropsis oceanica, Chroococcus* sp., *Synechococcus* sp. and *Tribonema* sp. However, our fatty acid profiles of the diatom *P. tricornutum* were in agreement with previous results (Kates and Volcani 1966; Ackman et al. 1968; Volkman et al. 1989; Yongmanitchai and Ward 1991; Reitan et al. 1994). The fatty acid distribution in Prymnesiophyceae *(Isochrysis galbana* sp. and *Pavlova* sp.) was in accordance with that of previous studies (Ackman et al. 1968; Volkman et al. 1989; Reitan et al. 1994). In *Porphyridium cruentum* the dominant PUFAs were C20:4 w6 and C20:5 ω3; similar results were reported by Cohen et al. (1988) and Robelloso Fuentes et al. (2000). The EPA content observed in *Rhodomonas baltica* (Cryptophyceae) was on a par with that reported by Kitano et al. (1997). The fresh water strains in Chlorophyceae, *Oocystis* sp. and *Pseudokirchneriella subcapitata* had mainly C16:0, C18:1, C18:2ω6 and C18:3ω3 as the dominant fatty acids. Of these, C16:0 and C18:1 accounted for 50% of the total fatty acids, which is typical of freshwater green algae (Volkman et al. 1989; Cranwell et al. 1990; Renaud et al. 1994) and is in broad agreement with the results of McLamon-Riches et al. (1998). The fatty acid profiles for *Tetraselmis suecica* were in accordance with those in earlier reports (Ackman et al. 1968; Volkman et al. 1989; Reitan et al. 1994).

These findings reveal that C16:0 was the major saturated fatty acid in all the algae; however, *P. tricornutum* had C14:0 in addition. The major monosaturated fatty acids were C16:1 in *P. tricornutum, Synechococcus* sp. and *Tribonema* sp., and C18:1 in the others. The abundance of PUFAs showed pronounced variation between algal species and classes. The highest amount of EPA was found in *P. tricornutum* (28.4 mg g^{-1}), followed by *Nannochloropsis oceanica* (23.4 mg g^{-1}), *Pavlova* sp. (18.0 mg g^{-1}), *P. cruentum* (6.1 mg g^{-1}), *T. suecica* (4.8 mg g^{-1}) and *R. baltica* (4.4 mg g^{-1}), while DHA was abundant only in *I. galbana* (15.8 mg g^{-1}) and *Pavlova* sp. (13.2 mg g^{-1}) (Table 3). In general, our data were in good accordance with results from the literature. The results shown here were also representative of the growth and composition of the same algae cultured in earlier series (data not shown).

Microalgae are utilised in aquaculture as live feed for bivalve larvae and spat (Knauer and Southgate 1999), early larvae of both crustaceans (Kanazawa et al. 1985) and marine fish (Reitan et al. 1997), and as food for rotifers (Watanabe 1983) and brine shrimp (Lavens et al. 1995). Our results demonstrate that there is variation in the growth rate as well as in the EPA and DHA content between the algal species. Marine algae are major producers of ω3-PUFAs (EPA and DHA), the fresh water algae predominantly having saturated or monosaturated fatty acids. When algae are used as food in the marine chain, it is important to know the lipid and fatty acid composition of the actual algal species used. Analysis of the protein and carbohydrate content of these algae is in progress, and a further two algae (*I. galbana* and *N. oceanica*) will be tested as live feed and in formulations.

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