

Variation of Fatty Acids in *Isochrysis galbana* (*T-Iso*) and *Tetraselmis suecica*, Cultured under Different Nitrate Availabilities

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Abstract

The use of high protein microalgae obtained by increasing the content of nitrate in the culture medium is recommended to improve the performance of broodstock, larvae and juveniles in bivalve hatchery. However, the effect of these concentrations of nitrate on the composition of fatty acids in microalgae is not known and it is relevant to assess possible changes in its nutritional properties for filtering bivalves. The results of nitrate increase in *Isochrysis aff. galbana* (clone *T-Iso*) showed that in the high nitrate medium, *T-Iso* is high in protein and carbohydrate and low in ash, and also exhibits higher values of polyunsaturated fatty acids (PUFA), especially n-3PUFA. The best culture medium for *Tetraselmis suecica* would also be the one high in nitrate because of it is high in protein and lipid and low in ash, although the best values PUFA were observed in the standard nitrate medium. *T-Iso* was characterized by its tendency to increase the level of n-3 PUFA with the increase of nitrate, while *T.suecica* was characterized by no effect of nitrate on the contents of n-3 PUFA of the cells nor a PUFA pattern related to the increase or decrease of nitrate.

Keywords: Microalgal diets; Essential fatty acids; High nitrogen medium; *Tetraselmis suecica*; *Isochrysis aff. galbana*

Introduction

The high concentrations of protein, carbohydrates, fatty acids and vitamins contained in microalgae make them essential food for zooplankton, larvae and juvenile stages of molluscs, crustaceans and certain herbivorous fish. The variations of culture medium, particularly nitrogen concentration, cause significant changes in the growth and biochemical composition of microalgae species which subsequently affects the growth and survival of the filtering that are consuming them [1-3]. The increase of nitrogen in the culture medium allows the microalgae increase by almost 50% normal protein content of the cells [4], promoting maturity time, female fecundity and balance pectinid energy and also improving the quality of the larvae [5]. The aim of this study was to determine whether nitrate concentration variations in the culture medium, apart from affecting the composition of proteins, lipids and carbohydrates, can affect the fatty acid composition of microalgae, particularly fatty acids that are considered essential for aquatic organisms. The content and ratios of certain polyunsaturated fatty acids (PUFA) synthesized by algae generate an indicator of the quality of microalgae [6].

Materials and Methods

Cultures of the microalgae *Isochrysis aff. galbana* (clone *T-Iso*) and *Tetraselmis suecica* were carried out at high (300 mg NO⁻³L⁻¹), standard (100 mg NO⁻³L⁻¹), and low (20 mgNO⁻³L⁻¹) concentrations of nitrate determined to modify the protein content in *T-Iso* [3]. Microalgae were grown in a closed system, in 500 mL flasks at 28°C and harvested during the exponential growth phase. Three cultures were done for each species under each condition. The cell concentration in the culture was measured daily using an electronic

particle counter (Coulter Z2). The harvested microalgae were centrifuged at 2500 rpm for 20 min at 4°C to remove seawater and then store the cells at -20°C for 24h for subsequent lyophilization for 48h at -40°C. Proteins were determined by the total nitrogen content, using an Elemental Analyzer (LECO 900CHN). The extraction and quantitation of total lipids and fatty acids followed the methodology cited by [7]. Ashes were obtained by calcinations at 500°C for 4 h (Thermolyne muffle) and carbohydrates were determined by calculating the difference between dry weight and the sum of protein, lipid and ash. The effect of nitrate concentration on the biochemical composition of microalgae was statistically analyzed for each microalgal species, after verifying the homogeneity of variance and normality of the data using the Bartlett test. ANOVA was applied when data were parametric, otherwise a Kruskal Wallis test was applied [8].

Results

The level of nitrate in the culture medium showed significant effects on the protein content in *T-Iso* (Table 1, F =48.6, df= 2, 3, p=0.005), where the highest protein values were registered at 300 mg NO⁻³L⁻¹, followed by intermediate values at 100 mg NO⁻³L⁻¹ and the lowest protein levels were obtained at 20 mg NO⁻³L⁻¹. In the case of *T.suecica*, the highest protein value was obtained at 300 mg NO⁻³L⁻¹ (Table 1, F=6723.4, df=2, 3, p=0.000003), observing the lowest value in the other two nitrate concentrations without differences among them. Total lipid content of *T-Iso* was constant with an average value of 12.0% (±2.1) dry weight between nitrate levels, while *T.suecica* showed significant differences (Table 1, F=13.87, df=2, 3, p=0.03) with the higher lipid content at 300 mg NO⁻³L⁻¹, observing the lowest value under the other two concentrations of nitrate, with no differences between them. The carbohydrate content in *T-Iso* was higher in the medium that was higher in nitrate (Table 1, F=10.92, df=2, 3, p=0.04),

and the values were similar and lower at standard and low concentrations of nitrate. *T.suecica* showed no differences in carbohydrates between all levels of nitrate, yielding an average of 31 % (± 3.1) dry weight. Ash contents in *T-Iso* were lower at high levels of nitrate, intermediate in the standard medium and higher at low levels

of nitrate ($H(2, N=12) = 6.0, p=0.05$), whereas in *T.suecica* the lowest ash content was observed at 300 mg $\text{NO}^{-3}\text{L}^{-1}$ (Table 1, $F=4.26, df=2, 3, p=0.05$), and the highest content was observed in the standard and low media with no difference between them.

Species	Nitrate level	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)
<i>I.aff.galbana</i>	High	19.8 \pm 0.87 ^a	14.8 \pm 0.36	51.7 \pm 0.51 ^{ab}	13.5 \pm 0.14 ^a
	Standard	12.4 \pm 0.24 ^b	15.3 \pm 0.44	51.2 \pm 0.68 ^{ab}	20.9 \pm 1.48 ^{bc}
	Low	6.3 \pm 1.11 ^c	5.7 \pm 3.34	36.2 \pm 4.46 ^c	51.7 \pm 2.29 ^c
<i>T. suecica</i>	High	23.6 \pm 0.18 ^{ab}	16.6 \pm 0.08 ^{ab}	40.4 \pm 0.10	19.2 \pm 4.01 ^a
	Standard	24.0 \pm 0.05 ^{ab}	15.5 \pm 1.37 ^{ab}	23.8 \pm 1.32	36.6 \pm 5.51 ^{ab}
	Low	7.0 \pm 0.09 ^c	11.2 \pm 0.09 ^c	28.6 \pm 0.01	53.0 \pm 12.79 ^c

Table 1: Protein, lipid, carbohydrate and ash content of *I.aff. galbana* and *T.suecica* acclimated to high, standard, and low concentration of nitrate in the culture medium. Superscripts within each specie sand column indicate significant differences with $p<0.05$.

Species	Nitrate level	SFA	MUFA	PUFA	EPA	DHA	n-3
<i>I.aff. galbana</i>	High	42.6 \pm 12.5	26.4 \pm 10.4	31.0 \pm 2.1 ^b	nd	3.7 \pm 0.5	8.8 \pm 1.3 ^b
	Standard	31.7 \pm 9.3	39.2 \pm 10.8	29.1 \pm 1.5 ^b	nd	3.3 \pm 0.4	7.0 \pm 0.6 ^{ab}
	Low	46.9 \pm 3.6	38.6 \pm 4.3	14.5 \pm 0.7 ^a	nd	2.1 \pm 0.2	4.1 \pm 0.3 ^a
<i>T. suecica</i>	High	38.9 \pm 0.2	27.3 \pm 0.1 ^b	33.8 \pm 0.1 ^{ab}	2.3 \pm 0.6 ^{ab}	nd	15.9 \pm 3.6
	Standard	43.7 \pm 5.7	14.9 \pm 1.5 ^a	41.4 \pm 4.2 ^b	1.5 \pm 0.5 ^a	nd	19.7 \pm 1.2
	Low	43.2 \pm 3.5	32.9 \pm 2.0 ^b	23.9 \pm 1.5 ^a	4.3 \pm 0.1 ^b	nd	13.3 \pm 1.1

Table 2: Characteristics of fatty acid per species and nitrate level. Content expressed as a percentage of total fatty acids area. SFA, MUFA and PUFA are saturated, monounsaturated and polyunsaturated fatty acids, respectively. EPA and DHA are eicosapentaenoic and docosahexaenoic acids, respectively. The total polyunsaturated fatty acids of family n-3 is shown as n-3. All values represent the average and standard error of two replicates. Non-detected compounds are indicated as nd. Superscripts within each species columns indicate significant differences with $p<0.05$

The fatty acid profile of both species showed no effect of the nitrate levels on the percentage of saturated fatty acids (SFA). Monounsaturated fatty acids (MUFA) were affected by nitrate level only in, *T.suecica* which had a low MUFA content at the standard level of nitrate (Table 2, $F=15.96, df=2, 3, p=0.03$). Polyunsaturated fatty acids showed differences between nitrate levels for both species, in *T-Iso* the highest values were found in microalgae grown with 100 and 300 mg $\text{NO}^{-3}\text{L}^{-1}$ (Table 2, $F=28.04, df=2, 3, p=0.01$), with no differences between these two treatments. The highest PUFA value in *T.suecica* was found under the standard nitrate condition, while the lowest content was found at low level (Table 2, $F=6.64, df=2, 3, p=0.05$). Only in *T-Iso* the contents of n-3 PUFA showed a tendency to decrease when lowering the nitrate content of the culture medium (Table 2, $F=7.42, df=2, 3, p=0.07$). The n-6/n-3 ratio had an average value of 2.7% in *T-Iso* and 0.38% in *T.suecica*, with no differences between the levels of nitrate.

Discussion

The protein content during the exponential growth phase of *T-Iso* was the only showing an increase that was proportional to the increase of nitrate in the medium, as reported for *I.galbana* [1]. The

biochemical composition of planktonic microalgae can be manipulated by varying the content of nitrogen in the culture medium, thus increasing nitrate medium will increase the concentration of protein [1,9], which was observed clearly in *T-Iso* in our study. Same protein values were obtained with normal and high concentrations of nitrate in *T. suecica*, but there was a significant decrease of protein with low nitrate condition. Carbohydrates, but not lipid concentrations were affected in *T-Iso* under the variations of nitrogen content in culture media, while effect on lipids, but not in carbohydrates was observed in *T. suecica*. The content of ashes showed a proportional decrease with the increase of nitrate content in the culture media in both microalgal species.

The SFA in *T-Iso* and *T.suecica* did not vary significantly with the availability of nitrate, showing mean values of 40.4% (± 8.5) and 41.9% (± 3.1), respectively. These values are considered low for phytoplankton, and are associated with good nutritional quality for bivalve larvae [10]. MUFA in *T.suecica* decreased with the standard nitrate condition, while PUFA increased under high and standard conditions of nitrate for *T-Iso* and *T.suecica*. These results are relevant regarding the digestibility of microalgae, since a better digestibility of fatty acids for cold water marine species is related to a low presence of SFA and a high presence of MUFA and PUFA [11]. Carrasco reported

that mussels fed low protein (L) microalgal diets (diet 1: 100% *T-Iso* L and diet 2: MixL=50% *T-Iso* L: 50% *T. suecica* L) showed no growth, and showed very low digestibility at these diets [12]. This could be due to the lower protein, lower energy, reduced availability of essential fatty acids and more ash observed in *T-Iso* and *T. suecica* acclimated to low concentration of nitrate in the culture medium.

Since n-3 PUFAs contain the two main essential fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), it is interesting that both species behave differently with regard to these features. *T-Iso* was characterized by its tendency to increase the level of n-3 PUFA with the increase of nitrate but without causing a significant effect on the concentrations of DHA. On the other hand, in *T.suecica* there was no effect of nitrate on the contents of n-3 PUFA of the cells and the EPA, which is the essential fatty acid contained in this microalga, shows differences between the different culture media but without following a pattern related to the increase or decrease of nitrate.

In summary, the strategy of cultivating microalgae in high concentration of nitrate in the culture medium should be adopted by the hatchery of filtering feeders because improves the composition of energy nutrients in the microalgal cells and because depending on the microalgal species, can also increase the concentration of PUFA.

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