Effect of zeolitic products in the nutritive quality of the diatom *Thalassiosira weissflogii*

Efecto de productos zeólicos en la calidad nutritiva de la diatomea *Thalassiosira weissflogii*

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ABSTRACT

The marine diatom *Thalassiosira weissflogii* (Grunow) G. Fryxell *et* Hasle (= *T. fluviatilis* Hustedt) was cultured in a batch system with f/2 medium supplemented with 10 mg l^{-1} of the zeolitic products Zeben 56, Zestec 56 and AZ. After 10 days cell composition was measured. The most important effect was observed with Zestec 56 which induced an increase of octadecatetraenoic acid ($C_{18:4}$) and docosahexaenoic acid ($C_{22:6}$ –DHA). Results suggest that zeolitic products can be used as part of the f/2 medium to improve the nutritional quality of this diatom.

Key words: Polyunsaturated fatty acids, *Thalassiosira weissflogii, T. fluviatilis*, zeolitic products, diatoms.

RESUMEN

La diatomea marina Thalassiosira weissflogii (Grunow) G. Fryxell et Hasle (= T. fluviatilis Hustedt) se cultivó en un sistema discontinuo con el medio f/2 adicionado de 10 mg l-1 de los productos zeólicos Zeben 56, Zestec 56 y AZ. Después de diez días de cultivo se determinó la composición proximal celular. El efecto mas importante se observó con Zestec 56 el cual indujo un incremento del ácido graso octadecatetraenoico (C18:4) y del ácido docosahexaenoico (C22:6 –DHA). Los resultados sugieren que la adición de productos zeólicos pueden ser utilizados como parte del medio f/2 para mejorar la calidad nutricia de esta diatomea.

Palabras claves: Acidos grasos poliinsaturados, *Thalassiosira weissflogii, T. fluviatilis*, productos zeólicos, diatomea.

INTRODUCTION

Marine microalgae constitute the main food source for bivalve mollusks and crustaceans. The specific nutrient requirements of crustaceans and of mollusk larvae and spat are not completely known. Enright *et al.* (1986) noted the role of carbohydrates and lipids, and other authors talk about the importance of polyunsaturated fatty acids (Ackman, 1982; Langdon & Waldock, 1981). Webb and Chu (1982) noted that the impact of the food given to bivalve larvae and juveniles depends more on specific nutrients (fatty acids, amino acids, monosaccharides, minerals, and vitamins) than on the gross biochemical composition.

The w-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA, $C_{20:5}$, *w*-3) and docosahexaenoic acid (DHA, $C_{22:6}$, *w*-3) are the major membrane components of marine animals, and are considered to be essential dietary components (Enright *et al.*, 1986; Watanabe *et al.*, 1983). The *w*-3 PUFA arachidonic acid (AA, $C_{20:4}$, *w*-6) is a significant component of lipids of certain benthic macroalgae and animals which feed directly on them (Dunstan *et al.*, 1988). In most marine animals, EPA and DHA are obtained directly from the diet and small amounts of both PUFA are converted into anti-inflammatory leukotrienes (Tocher & Sargent, 1987) and the prostaglandins necessary for growth and reproduction (Ruggeri & Thoroughgood, 1985).

Diatoms are considered the most important species in marine food chains, and are among the most effective strains used in the nutrition of mollusk larvae because their content of lipids and PUFA, is considered the primary energy storage material. Diatoms generally contain high concentrations of *w*-3 PUFA including EPA and smaller proportions of DHA. Both of these PUFA are essential to the diets of many marine animals (Volkman *et al.*, 1989; Thompson *et al.*, 1992; Brown *et al.*, 1996).

Thalassiosira weissflogii (Grunow) G. Fryxell *et* Hasle (= *T. fluviatilis*, Hustedt) (Fryxell & Hasle, 1997) has been used successfully for feeding shrimp larvae (Alfonso *et al.*, 1988). Emmerson (1980) found that larvae of *Penaeus* (*Fenneropenaeus*) indicus (Milne Edwards, 1837) fed with T. *weissflogii* (as *T. fluviatilis*) showed faster growth compared to feeding with other species of microalgae, and they concluded that this was because of lipid and protein content. Olivera (1991) and Olivera *et al.* (1993) reported similar results and also concluded that *T. weissflogii* is better than other microalgae used as food for shrimp larvae. Leal *et al.* (1990) compared cell composition of *Chaetoceros ceratosporum Ostenfeld, Chaetoceros gracilis* Schütt, *T. weissflogii* (as *T. fluviatilis*) and *Tetraselmis tetrathele* (West) Butcher, and observed that high levels of protein, carbohydrates, and lipids are contained in *T. weissflogii* (as *T. fluviatilis*).

Zeolites are a broad classification of minerals formed from the alteration of silicic volcanic ash. Zeolitic products are sodium and calcium aluminosilicates, with one or more alkaline or ferrous-alkaline compounds, which are easily exchanged for other cations. They are crystals with high uniformity of pore length and channels that define their honeycomb structure (Breck, 1974). This structure is formed by a system of interconnecting channels. The diameter of these channels is what, in part, differentiates each species of natural zeolite and gives relevance to their unique properties. Zeolites can be natural or synthetic. Some examples of natural sources are: modernites, faujacites, chabacites, montmorillonites and clinoptilolites. Due their anthropogenic source these zeolites are defined as natural zeolites products –NZ– (García-Sánchez, 2000). Synthetic forms are named with letter as part of their key identification.

There are few studies that involve the use of zeolites in the aquaculture field, and the results are sometimes contradictory. In general, the reports related to the use of zeolitic products are focused on the capture of heavy metals in waste water (Andrews *et al.*, 1991; Malliou *et al.*, 1994; Misaelides & Godelitsas, 1995; Jain *et al.*, 1996; Gómez-Villa, 1999; Quevedo-Aguillén, 2000; Pollorena-Audeves, 2001), the transformation of nitrates into nitrites (López-Ruiz & Gómez-Garrudo, 1994), and the exchange of their metals with the ammonium ion (López-Ruiz & Fernández del Barrio, 1987; Sand & Mumpton, 1978; Mumpton & Fishman, 1977; Mumpton, 1984).

Recent works suggest that yields of microalgae cultures might be improved by the addition of natural zeolites to the culture, due to their attribute of increasing the reproduction rate, and that significant applications can be made due to this positive influence on microalgae growth (Cháves-Sanz & López-Ruiz, 1990; López-Ruiz *et al.*, 1995a, 1995b; Voltolina *et al.*, 1997; Escalante-Gastelum, 1999; Villegas-Beltrán, 1999; García-Sánchez, 2000, Nieves *et al.*, 2000). Until now, there have been no reports related to the influence of these products on the chemical composition or in the concentration of fatty acids in microalgae. In the present work, we report the effect of Zestec 56, Zeben 56 and AZ, on the cell composition of the diatom *T.* weissflogii with emphasis on the fatty acids profile.

MATERIALS AND METHODS

The non-axenic marine microalga *T. weissflogii* was obtained from the "Colección del Centro de Investigaciones Marinas de la Universidad de La Habana", Cuba, and incorporated as THF-1, in the "Colección de Microalgas del Centro de Investigaciones Biológicas del Noroeste".

The zeolites Zeben 56 and Zestec 56 were synthesized from bentonite and coal wastes mines with an alkaline treat-

ment, at 160 °C for 72 h. These sodium products contain 61 and 44 % SiO_2, and 20 and 22 % Al_2O_3, respectively (Ferreiro-Almeda *et al.*, 1998).

Zestec 56 contains 11 % organic carbon substances. AZ is a natural zeolite prepared in the Laboratory of Zeolites (University of La Havana) from Cuban natural zeolite –NZ– by a sodium treatment. It is a clinoptilolite-heulandite (Mines of Tasajeras, Cuba) containing 64 % SiO_2 and 12 % Al_2O_3.

Thalassiosira weissflogii (THF-1) was previously synchronized in glass tubes with 50 ml of complete f/2+Si medium (Guillard & Ryther, 1962), at 23 °C and pH 7.5 – 7.8. After four seven-day synchronization periods at the above conditions, experiments were carried out in batch cultures, following addition of 15 % v/v of an inoculum.

Separate batch cultures of *T. weissflogii* were supplemented with 10 mg l^{-1} of Zeben 56, Zestec 56, and AZ. Continuous illumination with daylight Phillips tubes was done at 150 µmol photon m^{-2} s^{-1}, and cultures were stirred at 125 rpm at 23 °C. All experiments were done in triplicate with three repetitions. Control cultures were carried out at the same conditions with no zeolitic products.

After 10 days of culture at the above conditions, biomass was harvested by centrifugation at 3,000 rpm/15 °C for 15 min, washed with 3 % ammonium formiate in order to eliminate salts of the medium, and finally freeze-dried. Analyses of proteins (Lowry *et al.*, 1951), carbohydrates (Dubois *et al.*, 1956), lipids (Bligh & Dyer, 1959; Marsh & Weinstein, 1966) and fatty acids (Sato & Murata, 1988) were performed on lyophilized biomass.

For fatty acid analysis, lipid extracts (Bligh & Dyer, 1959) were methylated with 5 % HCl in methanol for 2.5 h at 85 °C (Sato & Murata, 1988). After extraction with HPLC grade hexane, samples were analyzed with an HP GCD System (G1800B) using an Omegawax TM250 (fused silica column, SUPELCO). Triheptadecanoylglycerol ($C_{17:0}$) was added to samples as an internal standard before methylation. Fatty acids were identified by comparison of the mass spectra obtained with mass spectra in the NBS75K, NIST98 and CIBNOR libraries, and by comparison of the retention time with a commercial mixture of fatty acid methyl esters (Sigma).

Statistic 5.0 for Windows was used for analysis of the results, which were compared by an overall multivariate one-way analysis of variance (ANOVA) to determine the differences between treatments. The Tukey test ($\infty = 0.05$) was used to compare treatments with different numbers of repetitions (Zar, 1996).

Table 1. Gross proximate composition (in percentage of organic dry weight) and standard deviation at the beginning of stationary phase growth of *Thalassiosira weissflogii.*

Gross composition	Control	Zeben 56	Zestec 56	AZ
Proteins	28±3.3	26±2.7	20±1.9	20±9.4
Carbohydrates	23±4.5	40*±1.7	30±4.5	32±3.6
Lipids	22±2.9	20±0.7	25±3.1	25±2.5
Ash	27±0.7	14±1.3	25±1.4	23±2.2

*æ ≤ 0.05

RESULTS

After ten days of batch culture *T. weissflogii* showed growth in presence of all zeolitic products tested. At the beginning of the stationary growth phase, proteins, carbohydrates and lipids were extracted. Proximate composition (Table 1), and fatty acids concentration (Table 2) were calculated in organic dry weight, and all comparisons were made with respect to the control culture.

Thalassiosira weissflogii cultured with zeolitic product Zeben 56 showed a significant increase with the carbohydrates and significant decrease with ash (Table 1).

Long chain polyunsaturated fatty acids showed strong significant differences (Table 2). With Zeben 56 the pentadecanoic acid ($C_{15:0}$) and octadecatetraenoic acid ($C_{18:4}$) showed increases of 1.8 and 3.6 times, respectively. The most important effect was obtained with Zestec 56 that gave an increase in the concentration of octadecatetraenoic acid ($C_{18:4}$) and docosahexaenoic acid ($C_{22:6}$, DHA, *w3*) of 3.6 and 32 times, respectively (Table 2). With AZ there were increases of palmitic acid ($C_{16:0}$), hexadecaenoic acid ($C_{16:1}$) and octadecatetraenoic acid ($C_{18:4}$ *w*-3) of 2.1, 1.5 and 10 times (Table 2).

DISCUSSION

Microalgae can change their biochemical cell composition in response to different culture conditions, including variations in light, temperature, and nutrient availability (Cohen *et al.*, 1988; Mortensen *et a*l., 1988; Volkman *et al.*, 1989; Harrison *et al.*, 1990; Sukenick & Wahnon, 1991; Thompson *et al.*, 1992; Yang & Zhu, 1993; Dunstan *et al.*, 1994).

Diatoms are unique among the algae requiring silicon for growth, and are characterized by unusual distribution of fatty acids compared to green algae. The C_{14}, C_{16} and C_{20} acids comprise the bulk of their fatty acids. Unsaturated fatty

Table 2. Fatty acid concentration (in percentage of organic dry weight) and standard deviation, at the beginning of stationary phase growth of *Thalassiosira weissflogii.*

Fatty acid	Control	Zeben 56	Zestec 56	AZ
Saturated				
14:0	14±2.9	14±1.2	14±2.2	14±0.9
15:0	9±2.2	17*±0.3	3*±0.6	3±0.9
16:0	16±3.7	17±6.4	20±6.1	35**±4.2
18:0	0.6±0.1	0.8±0.2	0.6±0.0	0.6±0.0
Monounsaturated				
16:1 (w-9)	9±1.0	6±0.2	2*±0.4	14**±1.1
18:1 (w-9)	0.6±0.1	0. ±0.0	0.8±0.0	0.6±0.0
Polyunsaturated				
16:2 (w-3)	0.8±0.2	0.7±0.2	1.6*±0.1	1.1±0.1
18:2 (w-6)	0.1±0.01	0.07±0.01	0.12±0.02	0.2*±0.01
16:3 (w-3)	1.5±0.2	2.1±0.5	1.8±0.1	1.5±0.2
18:3 (w-3)	0.4±0.01	0.4±0.014	0.3±0.003	0.2±0.02
18:4 (w-3)	0.03±0.005	0.11*±0.014	0.11*±0.021	0.3**±0.024
20:5 (w-3)	1.1± 0.13	1.6 ± 0.07	1.5 ± 0.2	1.6 ± 0.08
22:6 (w-3)	0.002±0.0002	0.003±0.0002	0.064**±0.013	0.003± .000

*æ ≤ 0.05

*æ ≤ 0.001

acids such as $C_{18:3}$ are either absent or present at very low levels (Ackman *et al.*, 1968).

The culture medium f/2 (Guillard & Ryther, 1962) contains 30 mg l^{-1} of silicon. In the experiments that we carried out, silicon concentration in the medium was increased by the contribution of zeolitic products added in the range of 44 to 64 %. Several doses of zeolites, that is, between 5 and 100 mg l^{-1} have been previously tested with different microalgae, showing that 10 mg zeolite l^{-1} is a better dose with which was observed an increase in the cellular division (López-Ruiz *et al.*, 1995a; Voltolina *et al.*, 1997).

Works involving the use of zeolitic products suggest that the presence of silicon in the medium could enhance its usefulness (López-Ruiz, *et al.*, 1995b). The organic compounds released by microalgae can be recycled by bacterial activities associated with zeolitic products (López-Ruiz & Gómez-Garrudo, 1994), increasing the availability of inorganic carbon and nitrogen sources, for microalgae use and cell yield (Voltolina *et al.*, 1997).

It is known that heterotrophic nutrition can influence cell composition. Also known is the ability of photosynthetic diatoms to grow on organic substances (Hellebust & Lewin, 1977). The Zestec 56 contents of 11 % organic carbon could be a factor in the increase observed in polyunsaturated fatty acid DHA that was obtained (Table 2).

The use of zeolitic products as part of the culture medium in microalgae growth has been tested recently (Voltolina *et al.*, 1997; Escalante-Gastelum, 1999; Villegas-Beltrán, 1999; García-Sánchez, 2000). Voltolina *et al.* (1997) showed that some zeolitic products enhance cell yield of the diatom *C. gracilis* Schütt, and concluded that a slight increase in the cost of production caused by the addition of small amount of a fairly inexpensive compound could increase the daily output of a microalgal production unit.

We conclude that the different zeolitic products used in this research influenced the concentration of polyunsaturated fatty acids in *T. weissflogii*. We suggest that zeolitic products can be used as part of the f/2 medium to improve the enrichment of PUFA content of *T. weissflogii* destined for feeding shrimp larvae.

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