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EFFECT OF SALINITY ON THE FATTY ACIDS COMPOSITION OF *NANNOCHLOROPSIS OCULATA* DURING EXPONENTIAL AND STATIONARY GROWTH PHASES

Nagwa G. Mohammady
Botany Department, Faculty of Science, Alexandria University, Egypt

Abstract:

The total fatty acids of *Nannochloropsis oculata* grown under optimum growth conditions and different salinity grades ranged from 25‰ to 45‰ during both exponential and stationary growth phases were analysed using Gas liquid chromatography (GlC) During the exponential growth phase, the domenant fatty acids were monounsaturated chains, while during the stationary growth phase, the acids were saturated, monounsaturated and polyunsaturated long chains. The optimum salinity for the production of the essential polyunsaturated C_{18} and C_{20} components for the fish larvae was 45‰ during the stationary growth phase.

Introduction

The marine estigmatophyte *Nannochloropsis oculata* is a unicellular alga that is widely used in many mariculture systems as a primary food source that supplies essential polyunsaturated fatty acids of the omega- 3 groups, as the chief food of molluscan larvae particularly in the initial stages (Depauw and Persoone, 1988). Oyster larvae can ingest noting larger than 10 microns and appear to rely for food on minute marine algae (Gopinathan, 1984), the reason by which EL- said, 1998 considered *Nannochloropsis oculata* to be the suitable alga for this purpose. Physiological studies demonstrated that fish cannot synthesize omega-3 fatty acids, but accumulate them via the food chain, where *Nannochloropsis oculata* is the primary source of these fatty acids (EL-said, 1998). These fatty acids are essential for the development and growth of marine fish larvae shrimp and molluscs (Koven *et al.*, 1989). The influence of salinity on fatty acids composition of the marine microalgae *Isochrysis* Sp. and *Nitzschia frustulum* was investigated by Renaud and Parry (1994) over the experimental range of salinity (10-35‰). However, EL-said (1998) estimated the optimum salinity concentration for the growth and the synthesis of the essential fatty acids in both *Chlorella salina and Nannochloropsis salina.* Shamsudin (1992) found a relation between the culture age and the production of the polyunsaturated C_{18} andC_{20} fatty acid components of some microalgae used in malysain aquaculture as a live food for early stage of *penaeid larvae.*

The purpose of this work is to study the effect of different salinity concentrations on the production of polyunsaturated fatty acids mainly C_{18} and C_{20} components, by *Nannochloropsis oculata* under optimum growth conditions during both exponential and stationary growth phases.

Materials And Methods

Biological Material: The green unicellular *eustigmatophyte Nannochloropsis oculata* was obtained from Maryut fish farming company.

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Culturing: The algal material was grown axenically in enriched sea water medium as described by Boussiba *et al.*, (1987).

Culture conditions: According to EL- said (1998).
Salinity concentration: The different salinity concentrations used were 25, 30, 35, 40, & 45‰ prepared according to Starr (1964).

Harvesting of culture: During both the exponential (after 7 days culturing) and stationary growth phase (after 13 days culturing), the cells of *Nannochloropsis oculata* at each salinity concentration were spinned down by centrifugation at 700 rpm for 15 minutes. The pellets washed several times with sterile medium and the cell pellets were kept at (-20 C°) in deep freezer until used.

Lipid extraction: The thawed cells were treated with chloroform- methanol (2/1) according to Dembitsky *et al.*, (1991).

Methyl esters of fatty acids: The total lipid extraction containing chloroform was evaporated to dryness and saponified by boiling for 2 hours in 50 ml of 2M NaOH in 50% ethanol (Dembitsky *et al.*, 1991). Fatty acid methyl esters were separated using GLC technique with SE-30 as the stationary phase (Dembitsky *et al.*, 1991)

Identification of fatty acids: Each fatty acid was identified by comparing its retention time with those of authentic standards.

Results and Discussion

The fatty acid profiles produced during both exponential and stationary growth phases of the investigated alga under different salinities were recorded in table (1) During the exponential growth phase most fatty acids were of the short monounsaturated chains. The dominant fatty acid components which represented nearly 50% of the total fatty acids were C_{14} components. C14:1 (tetradecaenoic acid) reached its maximum concentration at higher salinities. However C14:2 (tetradecadienoic acid) reached its maximum concentration at 30 and 35‰ salinities but it was gradually decreased with increasing salinity. C_{14} acids were the only fatty acids existing during both the exponential and stationary growth phases under all the tested salinity concentrations except at 45‰ for the stationary phase. However, during the stationary growth phase, these acids presented in lower values during the exponential phase of growth under all salinities tested, compared with the values of exponential phase. C_{14} fatty acid components were previously estimated by Volkman *et al.*, (1991) from *Pavlova salina* and by Nichols *et al.*, (1986) from the diatom *Nizstchia cylindrus*. Both monounsaturated short chains C8:1 (octaenoic acid) and C12:1(dodecenoic acid) were only detected.

The monounsaturated C16:1 (palmitoleic acid) was detected during both exponential and stationary growth phases only at salinties 25 and 30‰. The disappearance of this fatty acid at salinity concentration above 30‰ may be due to the inactivation of the enzyme respond for building up this fraction, an opinion which is in agree with Volkman *et al.*,(1991). The same authors proposed that the palmitoleic acid occurs in the phosphatidyl glycerol of most fresh water microalgae and it is not found in *Pavlova lutheri* and *Isochrysis* sp. (prymnesiophycean algae) in the marine environment which have been used in mariculture hatcheries. The results of the present investigation indicated the presence of C16:0 (palmitic acid) during both phases but at the exponential phase this fraction disappeared under salinity concentrations 25, 30 and 45‰. Also C18:1(oleic acid) was detected only in exponential phase at salinity 40‰, however both C18:1 and C18:2 were detected in stationary phase under all salinity concentrations.

On the other hand during the stationary growth phase, most fatty acids produced were relatively of the long chains saturated, monounsaturated and polyunsaturated fractions. However, the saturated fatty acid C18:0 (Stearic acid) was detected at only this phase. C18:2 (linoleic acid) increased with elevation salinity levels. Nichols *et al.*, (1986) suggested that the presence and distribution of the long chain monounsaturated fatty acids during stationary growth phase might occurred as a results of the chain elongation of the short monounsaturated fatty acids during the exponential growth phase in microscopic algae. Since the presence of C16: 0 in relatively small value (compared with the sum of the amount of C18: 1 and C18: 2 confirmed the explanation of Nichols *et al.*, (1986) that the desaturase enzyme acts almost exclusively on palmitic acid and the chain elongated to unsaturated C_{18} fatty acid components.

Table 1. Fatty acids composition of *Nannochloropsis oculata* during both exponential and stationary growth phases under different salinity concentrations (data were expressed as percentage).

Salinity conc.	25‰		30‰		35‰		40‰		45‰	
Carbon No. of fatty acid Methyl esters	exp	st	exp	st	exp	st	exp	st	Exp	st
C 8: 1	10.4	-	11.5	-	12.2	-	20.2	-	30	-
C12: 1	15.6	-	18.5	-	19.6	-	19.8	-	20	-
C14: 1	34.1	20	33.4	20.7	35.2	15.4	35	10.3	35	10.3
C14: 2	22.6	14.3	25	12	25	8.8	15.1	8.8	15	-
C16: 0	-	10.7	-	17.3	5.2	16.7	4.8	15.2	-	15.2
C16: 1	15.3	14.2	11.6	6.75	-	-	-	-	-	-
C18: 0	-	10.9	-	11.5	-	12.95	-	-	-	-
C18: 1	-	10.8	-	11.6	-	12.1	5.1	10.4	-	10
C18: 2	-	19.1	-	20.15	-	21.05	-	24.7	-	25
C20: 2	-	-	-	-	-	6.2	-	16.1	-	24.5
C20: 5	-	-	-	-	-	6.8	-	14.5	-	15

exp: exponential growth phase, st:stationary growth phases.

In the present work, the production of polyunsaturate 20:2 (eicosadienoic acid) and C20: 5 (eicosapentaenoic acid) started only during the stationary growth phase and under high relatively concentration. The values of these fractions increased gradually until reached their maximum amount at salinity 45‰. These important components (beside C18:2) are useful for aquaculturing since the fish larvae cannot synthesize them (EL-said, 1998). It is well known that during the stationary growth phase, the total nutrients of the culture media and in particular nitrogen are decreasing and affect markedly in lipid metabolism during growth phases. A redistribution from polar to neutral lipid and from saturated to unsaturated fatty acids occurred. An accumulation of fats was described for ageing culture of diatoms lead to the formation of more fatty acids as a result of insufficient nutrient media and salt stress (Piorreck and Pohl, 1984).

In general, the present results indicated that most fatty acids composition of *Nannochloropsis oculata*_increased with the increase in salinity during the different phases of growth. During the exponential growth phase, most fatty acids were monounsaturated with short chains. Since all vital activities occur in this phase, and the fatty acids considered to be the main source of the energy required for this purpose, a breaking down of the fatty acid chains must be occured (Kates, 1975). While during the stationary growth phase, the fatty acids were of long chains and the production of polyunsaturated C18 and C20 components was obtioned (specially at highly saline medium), the results agreed with Shamsudin (1992) who concluded that there was an increase in the proportion of the total C18 and C20 fatty acid components with the culture age. These polyunsaturated fatty acids are necessary for the growth and development of the early stages of fish larvae, a conclusion agree also with Volkman, et al. (1991).

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تأثير درجات الملوحة المختلفة على محتوى الأحماض الدهنية لطحلب نانو كلوربسيس أوكيولاتا أثناء مرحلتى النمو التزايدي والثابت

نجوى جمال الدين محمدي

قسم النبات – كلية العلوم – جامعة الإسكندرية

تم تحليل الأحماض الدهنية لطحلب نانو كلوربسيس أوكيولاتا تحت درجات من الملوحة تتراوح ما بين ٢٥ ٪ و ٤٥ ٪ أثناء كل من طوري النمو التزايدي والثابت للطحلب ولقد أثبتت النتائج أنه أثناء طور النمو التزايدي للطحلب تكون الأحماض الدهنية السائدة هي من الأنواع أحادية التشبع بينما أثناء الطور الثابت فقد وجدت الأحماض الدهنية المشبعة وأحادية التشبع بجانب الأحماض الغير مشبعة طويلة السلسلة – كذلك وجد من النتائج أن درجة الملوحة ٤٥ ٪ هي التي تسمح بإنتاج الأحماض الدهنية غير المشبعة C18, C20 وذلك أثناء طور النمو الثابت والتي تعتبر الأحماض الأساسية لتغذية يرقات الأسماك.