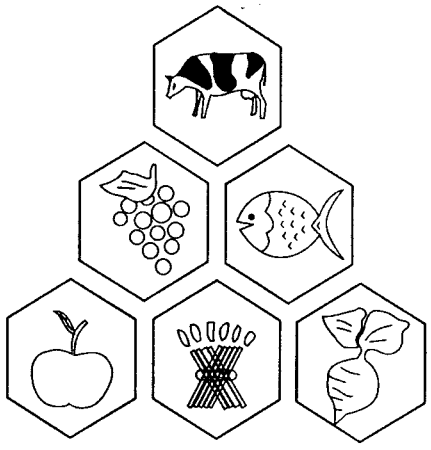
3º ENCONTRO DE QUÍMICA DE ALIMENTOS

ALIMENTAÇÃO MEDITERRÂNICA

Federação Europeia de Sociedades de Química
Sociedade Portuguesa de Química
Escola Superior de Tecnologia da Universidade do Algarve

LIVRO DE ACTAS



Auditório de Gambelas da Universidade do Algarve

FARO

23 a 26 de Março de 1997

MICROALGAE AS ALTERNATIVE SOURCES OF POLYUNSATURATED FATTY ACIDS FROM MARINE ORIGIN

Carvalho, A. P.; Meireles, L.; Malcata, F. X.*

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200 Porto

* To whom correspondence should be addressed

INTRODUCTION

Functional foods are food products which have the same appearance, texture and flavour of conventional food but which, due to addition of extra ingredients during formulation, are especially tailored to certain groups of people for whom beneficial effects in disease prevention have been statistically documented following consistent inclusion in the daily diet.

Two polyunsaturated fatty acids (PUFA) of the ω-3 type, eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) acids, have been shown to cause significant biochemical and physiological changes in the body: DHA is crucial for the normal development of the retina and the brain nervous tissues in the foetus, while EPA has important inhibitory effects on platelet coagulation. Animals require (but do not have the capacity to synthesize) these fatty acids and must therefore rely solely on dietary sources for their required uptake. Although fish oil is an abundant source of both EPA and DHA, its strong odour and taste, oxidative instability, limited and unpredictable supply, and potential for chemical contamination when fish is harvested in near-shore environments have all prevented its extensive use as a functional food ingredient.

Microalgae have been claimed to possess the potential to circumvent most of these disadvantages: some microalgae are indeed rich in PUFA and their content can, to a certain extent, be modulated by varying the culture conditions, thus allowing a more constant production rate to be achieved in fermentors, which is independent of weather conditions and geographical location. In this work, the contents of some microalgae and fresh sardines were compared in terms of EPA and DHA, as well as other long chain fatty acids, using resolution by gas chromatography after appropriate derivatization.

MATERIALS AND METHODS

ORGANISMS: The microalgae utilized were *Phaeodactylum tricornutum* (s/PHAEO-1) from Solar Energy Research Institute (SERI), Golden, CO, USA, *Phaeodactylum tricornutum* (UTEX640) from Texas University, USA, and *Pavlova lutheri* from Instituto Português de Investigação Marítima (IPIMAR), Lisboa, Portugal. Fresh sardines were purchased in local markets.

CULTURE CONDITIONS

Phaeodactylum tricornutum and *Pavlova lutheri* cultures were grown in 500 ml Erlenmeyer flasks and 1 l balloons, respectively. The media used to cultivate the algae were: (i) GPM, following F. Haxo Scripps Institution of Oceanography (SERI, 1986) without soil extract and with seawater substituted by deonized water with 3-3.5% salt for *Pavlova lutheri*, and (ii) ASW (Darley and Volcani, 1969) (obtained in SERI, 1986) for both strains of *Phaeodactylum tricornutum*.

Cultures were incubated at 20±2°C, at light powers of ca. 50 µmol/m^2.s for both *Phaeodactylum tricornutum* and ca. 150 µmol/m^2.s for *Pavlova lutheri* , in continuous light cycles. Filtered air was bubbled in the flasks in order to prevent fouling. Cells were harvested after ca. 16 days (late exponential phase) and were analysed for fatty acid composition.

FATTY ACID COMPOSITION: Fatty acid methyl esters (FAME) were obtained by direct transesterification of freeze-dried samples, according to the acidic method described by Reis (1996), using heptadecanoic acid (SIGMA) as internal standard. The analysis of FAME was carried out by gas chromatography in a 50 m capillary column of fused silica (CP-Sil 88, Chrompack, The Netherlands) using helium as carrier gas at a flow rate of 100 ml/min and a split ratio of 1:100, injector and detector temperatures of 250° C, and detection by flame ionization (FID). The column temperature was programmed for an initial temperature of 170 °C, which was increased up to 220°C at 1.0 °C.min^{-1}. Fatty acid identification was based on comparison between sample peak retention times and those of known pure standards (SIGMA).

RESULTS AND DISCUSSION

All cultures were grown in duplicate. Freeze-dried biomass from each flask was transesterified in duplicate and injected in the chromatograph. The average amounts of fatty acid composition for the more important standards screened are presented in Table 1.

TABLE 1: Average fatty acid composition (in mg/g dry algae/sardine)

	16:0	16:1ω7	18:0	18:1ω9	20:5ω3	22:6ω3
P. lutheri	8.89	9.74	1.20	1.00	9.05	5.50
P. tricornutum (UTEX640)	5.21	10.3	2.33	3.67	6.56	0
P. tricornutum (SERI)	1.14	2.53	0	0.85	1.70	0
Sardina pilchardus	9.76	2.52	1.91	1.85	4.52	6.07

The major fatty acids in *P.lutheri* were palmitic (16:0), palmitoleic (16:1ω7), stearic (18:0), oleic (18:1ω9), eicosapentaenoic (20:5w3), and docosahexaenoic acid (22:6ω3), which

agrees with the literature (Volkman, 1989). *P. tricornutum* (UTEX640) exhibits somewhat lower values of EPA than the former algae and no DHA at all, which is in disagreement with other results for the same species (but grown under different conditions). The strain from SERI exhibited considerably lower FA values, which can be explained by the long storage time of the freeze-dried samples. Sardine (*Sardina pilchardus*) exhibits a vast profile of fatty acids with 16:0, 16:1, 18:0, 18:1, EPA and DHA as the most abundant. These results are consistent with literature, except for the case of stearic acid (18:0) which is important in our case but not in other studies (Bandarra, 1992; Barlow, 1982). The values for sardine are lower than expected, probably because of the time in the fishing season; in fact, the FA composition and the total fat changes during the year, and the latter is lower in winter.

An unpaired t-test at the 5% significance level was performed with these results in order to determine whether the differences observed in the EPA and DHA levels of the different species were statistically significant. It could be concluded that all results are statistically different at the aforementioned level. As a general conclusion, one found that microscopic algae are suitable alternative sources of PUFA in technical terms.

ACKNOWLEDGEMENTS

Financial support (Ph.D. grant ref. BD/2838/93-IF by PRAXIS XXI (Portugal) is hereby gratefully acknowledged.

REFERENCES

- Bandarra, N. M.; Nunes, M. L.; Campos, R. M.; Batista, I. (1992) Variação sazonal dos lípidos de sardinha *Sardina pilchardus*, Rel. Técnico e Científico No. 54, Instituto Nacional de Investigação das Pescas, Lisboa

- Barlow, S. M. and Starsby, M. E. (1982) Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil, Academic Press

- Carvalho, A. P. and Malcata, F. X. (1996) World of Ingredients, March-April, 22

- Cohen, Z., Vonshak, A. and Richmond, A. (1988) J. Phycol. 24, 328

- Lepage, G. and Roy, C. (1984) J. Lipid Research 25, 1391

- Reis, A.; Gouveia, L.; Veloso, V.; Fernandes, H. L.; Empis, J. A.; Novais, J. M. (1996) Eicosapentaenoic acid-rich biomass production by the microalga *Phaeodactylum tricornutum* in a continuous-flow reactor, Bioresource Technology 55, 83

- SERI 1986 Microalgae Culture Collection 1985-1986, Solar Energy Research Institute, Golden, CO, USA

- Volkman, J. K., Jeffrey, S. W.; Nichols, P. D.; Rogers, G. I.; Garland, C. D. (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture, J. Exp. Mar. Ecol. 128, 219

-Yongmanitchai, W.; Ward, O. P. (1991) Screening of algae for potential alternative sources of eicosapentaenoic acid, Phytochemistry 30,2963