Microalgae biomass colourings. 2. Toxicological evaluation

Luísa Gouveia[1], M. Luisa Corvo[2], Rui Pinto[3], Cristina Sampayo[3], Beatriz Silva Lima[3], Ana Paula Batista[4], Anabela Raymundo[4], Isabel Sousa[5], Narcisa M. Bandarra[6]

[1] LNEG- Unidade Bioenergia, Estrada do Paço do Lumiar, 1649-038 Lisboa, Portugal
[2] UNFAB-FFUL, and Nano&DDS Group - Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL). Faculdade de Farmácia da Universidade de Lisboa, Campus do Lumiar - Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal
[3] IMED.UL, Faculdade de Farmácia Universidade de Lisboa, Av das Forcas Armadas 1600 Lisboa, Portugal.
[4] Núcleo de Investigação em Engenharia Alimentar e Biotecnologia, Instituto Piaget - ISEIT de Almada, Quinta da Arreinela de Cima, 2800-305 Almada, Portugal
[5] DAIAT – Instituto Superior de Agronomia / Technical University of Lisbon. Tapada da Ajuda, 1349-017 Lisboa, Portugal
[6] INRB/L-IPIMAR, U-VPPA, Av. Brasília 1449-006 Lisboa, Portugal

INTRODUCTION

The use of microalgae as a food ingredient has been tested in several food products due to its health promoting effects, probably related to a general immune-modulating effect (*1*). The main microalgae attributes are their pigment content, being recognized as an excellent source of natural colorants, and their content in nutraceuticals, such as antioxidants, polyunsaturated fatty acids, sterols, tocopherols, among others.

The present work results from a project that aims to use microalgal biomass as source of pigments, antioxidants and omega3-fatty acids in food products.

Chlorella vulgaris (carotenogenic, orange), *Haematococcus pluvialis* (carotenogenic, red) and *Diacronema vlkianum* (green) have been produced and studied by the authors, as colouring agents, source of antioxidants and omega3-fatty acids, respectively, in numerous products, such as egg yolks, rainbow trout, sea bream, ornamental fishes, food emulsions (mayonnaise-type), puddings, biscuits and pastas (*2*).

Chlorella vulgaris (Cv) biomass is widely used as a traditional food in the Orient, as a nutraceutical, and has been used as an alternative medicine in the Far East since ancient times (*3*). Their role as antiviral, antibacterial, anti-tumour, anti-HIV and to have a positive effect on several diseases, infant malnutrition and neurosis (*4*), as well as food additive, have been well established. Besides health aspects, *Chlorella* is also important as source of natural pigments such as carotenoids, which is able to accumulate with high concentrations, under certain culture conditions (light stress, nutrient depletion and high salinity) (*5*).

Haematococcus pluvialis microalga is the best astaxanthin-producing organism, accumulating it in its aplanospore and become blood red in colour, when exposed to harsh environmental conditions. It can be used as a whole biomass for animal feed (*6-7*) and healthy food products as a pigment source and antioxidant agent, with positive effect as antitumor (*8*), blood pressure (*9-10*), as well as has a cardioprotective effect (*11*).

Diacronema vlkianum is a marine green algae with an interesting fatty acid profile, rich in omega 3 long chain polyunsaturated fatty acids (LC-PUFA), mainly eicosapentaenoic acid (EPA, 20:5ω3) and also docosahexaenoic acid (DHA, 22:6ω3), that are accumulated as oil droplets in prominent lipid bodies in the cell. This microalga is potentially promising for the food industry as a valuable source of LC-PUFA's, in alternative to fish oils, supplying also sterols, tocopherols, colouring pigments and other nutraceuticals (*12*).

The aim of the present study was to evaluate if these microalgae biomass present any hazard to be used as human food ingredients.

MATERIALS AD METHODS

Microalgal biomass production

Chlorella vulgaris (INETI 58) (Cv) and *Haematococcus pluvialis* (INETI 33) (Hp) used in this study, were cultivated in appropriated growth medium (*13*) and growing in airlift bioreactors, bubbling air, at 25ºC temperature and at low light conditions (150 μE m^{-2} s−1). After the biomass growths, stress conditions were induced by nitrogen starvation, NaCl addition (30% for Cv (orange) (*5*) and 2% for Hp (red) (*14*), at high luminosity favoured by culture dilution (1000 μE m^{-2} s^{-1}). Microalgal biomass harvesting was produced without flocculation by simply removing agitation, concentration by centrifugation and freeze drying. Total pigment content was 0.4% w/w Cv (orange) and 1.2% w/w for Hp (red) (dry basis), measured by acetone extraction method (*5*).

Diacronema vlkianum (IPIMAR) was grown in Wallerstein & Míquel medium (3:1) in airlift bioreactors at 18ºC, according to the method described by Donato *et al.* (*12*).

Toxicological studies

Animals

BDF1 male mice weighting 25-30 g were obtained from Charles Rives, Barcelona, Spain. Animals were fed with standard laboratory food and water *ad libitum*. All animal experiments were carried with the permission of the local animal ethical committee, and in accordance with the Declaration of Helsinki, the EEC Directive (86/609/EEC) and the Portuguese laws D.R. nº 31/92, D.R. 153 I-A 67/92, and all following legislations.

Treatment

C. vulgaris and *H. pluvialis* carotenogenic biomass were administered orally at two different schedules to BDF_1 male mice at doses of 10, 50 and 125 mg kg^{-1} of body weight, with 5 animals per group. The first schedule consisted in a single administration and the second schedule consisted in a 5-days daily administration.

The evaluation parameters used were the animal behaviour changes (2 hours following administration and twice a day during the following 7 days after last administration), the monitoring of food and water uptake during the study period and statistical evaluation of significance (95%) of variations on animal weight and relative weight of liver, spleen, heart and kidneys seven days after oral administration. Histological observation of liver and spleen were performed randomly in 2 animals per group. Animals were also monitored seven days before treatment to establish a baseline behavior.

D. vlkianum biomass suspended in water (75mg/ml; 3mg/ml of EPA+DHA) was orally administered, by gavage, with single doses of 0, 10, 50 and 100 mg/Kg body weight, to 5 male mice. After 5 days post dose the animals were sacrificed and the main organs were visually observed. Behaviour and clinical signs were monitored twice/ day.

RESULTS

During seven days after algae administration signs of toxicity as well as animal behaviour modifications were evaluated. For all mice at the three doses studied, no statistical variations on animal weight, food and water uptake were observed when compared to the control group (water administration). Furthermore, analyzing the statistical evaluation of significance (95%) of variations on relative organ weight (liver, spleen, heart and kidneys) no variations were also observed for the algae treatment when compared to the control group. Histological studies on liver and spleen did not show differences between treated and control animals, and no signs of toxicity were observed.

With *D. vlkianum* no mortalities, relevant clinical signs or behavioural changes were observed in mice fed with microalgal biomass.

Acknowledgements: This work was supported by "Fundação para a Ciência e a Tecnologia" (FCT) through the research project PTDC/AGR-ALI/65926/2006.

REFERENCES

1. Belay (1993) *Journal of Applied Phycology*, *5*, 235-240.
2. Gouveia, L.; Batista, A.P.; Sousa, I.; Raymundo, A.; Bandarra, N.M. (2008). Microalgae in Novel Food Products. In Food Chemistry Research Developments. Frank Columbus (Ed). Nova Publishers.
3. Otles and Pire
4.Yamaguchi.
5. Gouveia L.; Veloso V.; Reis A.; Fernandes H.L.; Empis J.; Novais J.M. (1996). *Biores Technol, 57,* 157–163.
6. Gomes E.; Dias J.; Silva P.; Valente L.; Empis J.; Gouveia L.; Bowen J.; Young A. (2002). *Eur Food Res Technol. 214*, 287–293.
7. Gouveia L.; Rema P.; Pereira O.; Empis J. (2003). *Aquac Nutri, 9,* 123–129
8. Tanaka T.; Makita H.; Ohnishi M.; Mori H.; Satoh K.; Hara A. (1995). *Cancer Res, 55*, 409-064.
9. Hussein G.; Goto H.; Oda S.; Iguchi T; Sankawa U.; Matsumoto K.; Watanabe H. (2005). *Biol Pharm Bull, 28*, 967-971.
10. Hussein G.; Nakamura M.; Zhao Q.; Iguchi T; Goto H.; Sankawa U.; Watanabe H. (2005). *Biol Pharm Bull, 28*, 47-52.
11. Gross G.J.; Lookwood S.F. (2004). *Life Science, 75,* 215-224.
12 Donato, M.; Vilela, M. H.; Bandarra, N. M., 2003**.** Fatty acids, sterols, α-tocopherol and total carotenoids of *Diacronema Vlkianum*. *Journal of Food Lipids* 10(4): 267-276.
13. Vonshak A. (1986) *In A. Richmond, CRC Handbook of Microalgal Mass Culture*, (pp 117-143). CRC Press, USA
14. Gouveia L.; Empis J. (2003). *Innov Food Sci Emerg Technol., 4*, 227–233.