International Conference on Food Innovation
foodInnova 2010
25-29 OCTOBER 2010
UNIVERSIDAD POLITECNICA DE VALENCIA

HEALTHIER FOODS WITH NATURALLY ENCAPSULATED FUNCTIONAL INGREDIENTS - MICROALGAE

Ana Paula Batista[1], Anabela Raymundo[1], Narcisa M. Bandarra[2], Isabel Sousa[3], José Empis[4], Luísa Gouveia[5]

[1] Núcleo de Investigação em Engenharia Alimentar e Biotecnologia, Instituto Piaget - ISEIT de Almada, Quinta da Arreinela de Cima, 2800-305 Almada, Portugal (pbatista@almada.ipiaget.org; araymundo@almada.ipiaget.org)
[2] IPIMAR – INRB. Departamento de Inovação Tecnológica e Valorização dos Produtos da Pesca. Av. Brasília 1449-005 Lisboa, Portugal (narcisa@ipimar.pt) .
[3] CEER – Biosystems Engineering, Instituto Superior de Agronomia/Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal (isabelsousa@isa.utl.pt)
[4] IBB - Centro de Engenharia Biológica e Química, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal (jempis@ist.utl.pt)
[5] Instituto Nacional de Engenharia e Tecnologia Industrial - INETI-DER - Unidade Biomassa, Estrada do Paço do Lumiar, 1649-038 Lisboa, Portugal (luisa.gouveia@ineti.pt)

Introduction

The use of microalgae as food ingredients has been tested in several food products due to their potential health promoting effects, probably related to a general immune-modulating effect (Belay, 1993). One of the main attributes of microalgae is their pigment content, and they are recognized as an excellent source of natural colourings and nutraceuticals. Beside chlorophylls, their primary photosynthetic pigment, microalgae also form various secondary pigments, such as phycobiliproteins and a wide range of carotenoids. The present work stems from a project that aims to use microalgal biomass as source of pigments, antioxidants and omega3-fatty acids in food products.

Production and characterization of microalgae biomass

Chlorella vulgaris, *Haematococcus pluvialis*, *Spirulina maxima*, *Isochrysis galbana* and *Diacronema vlkianum* were cultivated and grown under optimum conditions for each microalgae (Gouveia *et al.*, 1996 ; Gouveia & Empis, 2003 ; Reis, 2001; Bandarra *et al.*, 2003; Donato *et al.*, 2003).

A carotenogenesis process was induced in *Chlorella* and *Haematococcus*, by salt, light and nutritional stress, resulting in a massive accumulation of secondary carotenoids, *e.g.* canthaxanthin and astaxanthin (Gouveia *et al.*, 1996 ; Gouveia & Empis, 2003).

Microalgal biomass nutrient profile was determined by AOAC standard methods (AOAC, 2006) in terms of moisture, total ash, crude protein, total fat and fatty acid profile (Bandarra *et al.*, 2003). The microalgae presented distinct biomass nutrient profiles (**Table 1**), which is particularly evident for the carotenogenic microalgae (*Chlorella* orange and *Haematococcus*), with significantly ($p<0.05$) lower protein and higher fat content. *Isochrysis* and *Diacronema* presented a fatty acid profile rich in long chain polyunsaturated ω3-fatty acids, mainly eicosapentaenoic (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3), with EPA+DHA contents from 4000 to 9700 mg/100g, and are therefore potential commercial sources for these compounds as an alternative to fish oils.

Total carotenoids were determined by extraction with acetone, spectrophotometric quantification, TLC and HPLC separation (Gouveia *et al.*, 1996). *Haematococcus* presented the highest pigment yield (3.0%), astaxanthin being the dominant pigment. The major carotenoids found in all microalgae were lutein, β-carotene, canthaxanthin and astaxanthin. Chlorophyll was also extracted by acetone along with carotenoids, which contributed to a high total pigment values for some green microalgae, particularly *Isochrysis* and *Diacronema* (2-3%). Phycocyanin content of *Spirulina* was also determined by extraction with phosphate buffer at low temperatures and spectrophotometric quantification (Reis, 2001). This pigment accounts for 7.1% of the microalgal dry matter, corresponding to about 15% of the total protein.

Development of coloured food products with microalgal biomass

In the last years, our research group in Portugal has aimed to develop a range of novel coloured foods, prepared with microalgal biomass, rich in carotenoids and ω3 polyunsaturated fatty acids (PUFA-ω3) with antioxidant effect and other beneficial properties. At the same time toxicological studies involving all the microalgae to be incorporated are being conducted. Traditional foods, like mayonnaises/salad dressings, puddings/gelled desserts, biscuits/cookies and pasta, largely consumed on daily basis on different European diets, were studied. The effect of microalgal concentration on the products colour parameters was investigated, as well as its stability throughout the processing conditions and during storage time. Obtaining of appealing and stable colorings is an important innovation for these types of products.

According to previous studies (Raymundo *et al.*, 2002) vegetable proteins (pea protein isolate) were used as substitutes of egg yolk in oil-in-water emulsions. These *"vegetable mayonnaises"* were further coloured with natural pigments (lutein and phycocyanin) (Batista *et al.*, (2006a. & b.), *Chlorella vulgaris* (green/carotenogenic) and *Haematococcus pluvialis* biomass (carotenogenic) (Raymundo *et al.*, 2005 and Gouveia *et al.*, 2006).

Pea protein isolate (Pisane F9®, Cosucra, Belgium) was also used, in combination with kappa-carrageenan and starch polysaccharides, to develop *"animal-free"* gelled desserts, as an alternative to dairy-desserts (Nunes *et al.*, 2006). The gels were coloured with different microalgae - *Chlorella vulgaris* (green/carotenogenic), *Haematococcus pluvialis* (carotenogenic), *Spirulina maxima* and *Diacronema vlkianum* (Batista *et al.*, 2008a. and Gouveia *et al.*,2010) and compared with gels prepared with commercial pigments (phycocyanin, astaxanthin, β-carotene, canthaxanthin and lutein) (Batista *et al.*, 2008b.).

Chlorella vulgaris (green) (Gouveia e*t al.*, 2007) and *Isochrysis galbana* (Gouveia et al., 2008) have been studied as colouring and PUFA-ω3 sources in short dough butter cookies, previously optimized (Piteira *et al.*, 2004).

More recently, the addition of microalgal biomass – *Chlorella vulgaris* (green/carotenogenic), *Spirulina maxima*, *Isochrysis galbana* and *Diacronema vlkianum* - on *durum* wheat semolina pasta products is being studied (Fradique *et al.*, 2008).

In general, the developed products presented appealing and stable colours with added value in terms of health benefits, considering the antioxidant properties and PUFA-ω3 content of the microalgae. The results obtained are promising since it was possible to obtain common food products enriched with microalgae, resulting stable, attractive and healthier foods with enormous potential in the functional food market.

Acknowledgements

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

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Table 1. Chemical composition of microalgae biomass (% w/w, DM) (average ± standard deviation).

	Chlorella vulgaris (green)	*Chlorella vulgaris* (orange)	*Haematococcus pluvialis*	*Spirulina maxima*	*Diacronema vlkianum*	*Isochrysis galbana*
Crude Protein	38.0 ± 1.5 [a]	12.3 ± 0.1 [b]	10.2 ± 0.2 [b]	44.9 ± 1.8 [c]	38.4 ± 0.2 [a]	39.6 ± 0.3 [a]
Crude Fat	5.1 ± 0.01 [a]	27.6 ± 1.4 [b]	40.7 ± 1.2 [c]	3.6 ± 0.1 [a]	17.9 ± 0.5 [d]	23.9 ± 0.02 [e]
Total Ash	24.2 ± 0.6 [a]	34.8 ± 1.8 [b]	8.9 ± 0.2 [c]	30.9 ± 1.5 [d]	18.4 ± 0.8 [e]	14.5 ± 0.1 [e]
Carbohydrates*	16.2	29.7	18.8	33.9	17.4	21.7

*Calculated by difference from 100% and the total sum of the determined components. **Different letters in the same row correspond to significant differences ($p<0.05$) - Anova Post-Hoc Comparison, Scheffé test.