Algal Research 29 (2018) 242–248

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Potential of microalga *Isochrysis galbana*: Bioactivity and bioaccessibility

Bonfanti C.[a,b], Cardoso C.[b,c,*], Afonso C.[b,c,*], Matos J.[b,d], Garcia T.[a,b], Tanni S.[a], Bandarra N.M.[b,c]

[a] Botucatu Medical School, UNESP - Universidade Estadual Paulista, Botucatu Campus, Botucatu, São Paulo, Brazil
[b] Division of Aquaculture and Upgrading (DivAV), Portuguese Institute for the Sea and Atmosphere (IPMA, IP), Rua Alfredo Magalhães Ramalho, 6, 1495-006 Lisbon, Portugal
[c] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal
[d] Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 16, 1749-016 Lisbon, Portugal

ARTICLE INFO

Keywords:
Isochrysis galbana
Lipid composition
ω3 PUFA
Anti-inflammatory activity
Bioaccessibility

ABSTRACT

The lipid composition and anti-inflammatory activity of the microalga *Isochrysis galbana* were studied. Moreover, the influence of bioaccessibility on composition and bioactivity was evaluated through the application of an *in vitro* model of the human digestion. The fatty acid (FA) profile was characterized by abundance of polyunsaturated FA (PUFA) and, within PUFA, ω3 PUFA were the most abundant. High contents of myristic, oleic, linoleic, α-linolenic, and stearidonic acids as well as docosahexaenoic acid (DHA) were determined. A low level of hydrolysis of triacylglycerols (TAGs) and polar lipids was observed during digestion. Total lipid bioaccessibility and specific FA bioaccessibility were low (between 7 and 15%). The highest bioaccessibility percentages were determined for palmitic, oleic, and linoleic acids as well as total ω6 PUFA and the lowest bioaccessible percentages were calculated for myristic and stearidonic acids, DHA, and total ω3 PUFA. Chemical affinity phenomena could be an explanation for these results. Regarding anti-inflammatory activity, it was only detected in the lipid extract of *I. galbana* prior to digestion (79 ± 7% of cyclooxygenase-2, COX-2, inhibition). No activity was found in the bioaccessible fraction extract. Apparently, the COX-2 inhibitory compounds were not rendered bioaccessible.

1. Introduction

Microalgae are an important aquatic resource [1]. Moreover, microalgae are a promising and valuable natural source of bioactive compounds, such as ω3 polyunsaturated fatty acids (ω3 PUFA). Due to its potential, microalgal biomass can be incorporated as a functional ingredient in order to enhance the nutritional value of foods and, thus, to positively affect human health.

In particular, the microalga *Isochrysis galbana* is potentially promising to the food industry due to its significantly high lipid content (20–30% w/dw), representing a rich source of ω3 PUFAs, namely eicosapentaenoic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6 ω3) [2]. Accordingly, it can be regarded as an additional source of essential oils to fisheries, thus covering the needs of increasing human population. It also supplies sterols, tocopherols, colouring pigments, and other bioactive substances [2,3].

EPA and DHA are associated with decreased morbidity and mortality from cardiovascular and other diseases as well as with foetal development [4]. Reviewed evidence also pointed to benefits for the development of the neural system in children [5] and prevention of mild cognitive decline in elderly [6]. Furthermore, EPA has been claimed to enhance anti-inflammatory properties of high-density lipoprotein, among other effects [7].

On the other hand, the reports of anti-inflammatory effects on rats due to *I. galbana* [8] may correspond to the action of bioactive substances in *I. galbana*, including EPA and other than EPA. These substances may be peptides, carotenoids, or sulphated polysaccharides [9]. In the case of the microalga *Chlorella*, evidence linking extracts of this organism to anti-inflammatory outcomes has been stronger [1,10]. More specifically, for the microalga *Porphyridium cruentum*, a sulphoglycolipidic fraction has been shown to display an anti-inflammatory effect [11]. A fatty acid (FA) analysis showed that this fraction contained large amounts of palmitic acid, 16:0 (26.1%) and EPA (16.6%) as well as noticeable amounts of the 16:1 ω9 (10.5%) [11]. These results show that microalgae have anti-inflammatory potential, which is stimulating the development of innovative nutraceuticals.

However, in future applications as nutraceuticals, it must be taken into account that the absorbable quantity of a compound in the gastrointestinal (GI) tract is not accurately predicted by its total content in

* Corresponding authors at: Division of Aquaculture and Upgrading (DivAV), Portuguese Institute for the Sea and Atmosphere (IPMA, IP), Rua Alfredo Magalhães Ramalho, 6, 1495-006 Lisbon, Portugal.
E-mail addresses: carlos.cardoso@ipma.pt (C. Cardoso), cafonso@ipma.pt (C. Afonso).

https://doi.org/10.1016/j.algal.2017.11.035
Received 16 September 2017; Received in revised form 27 November 2017; Accepted 27 November 2017
Available online 11 December 2017

the microalgae. Bioaccessibility corresponds to the share of the initial content that is rendered free from the microalgal structure into the GI tract [12]. Thus, determining bioaccessibility may contribute to the assessment of the effective nutraceutical potential of any given microalgal biomass. A bioaccessibility study requires the utilization of an adequate *in vitro* digestion model that reliably simulates human digestion. Different techniques have been developed [13] and optimized [12,13], being the static model with digestive compartment distinction and complete digestive juices, including enzymes in all steps, one of the best models.

The objective of this study was to determine the lipid composition, anti-inflammatory activity, and bioaccessibility effects on these aspects for the microalgae *I. galbana*, paving the way for a deeper knowledge of the biochemical processes involved in any anti-inflammatory effects and a more realistic assessment of the nutraceutical potential.

2. Material and methods

2.1. Sample collection and preparation

Samples of *Isochrysis galbana* were supplied by Necton (Necton, Companhia Portuguesa de Culturas Marinhas, SA, Olhão, Portugal). This material was already freeze-dried and was analysed without further processing.

2.2. Proximate composition

The moisture and ash contents were determined according to Association of Official Analytical Chemists (AOAC) methods [14]. The protein level was quantified according to the Dumas method [15]. Crude lipid content was determined following the Bligh and Dyer extraction method [16]. Carbohydrate content was determined by difference.

2.3. Fatty acid profile

Fatty acid methyl esters (FAME's) were prepared by acid-catalysed transesterification using the methodology described by Bandarra et al. [17]. Samples were injected into a Varian Star 3800 CP gas chromatograph (Walnut Creek, CA, USA), equipped with an auto sampler with a flame ionisation detector at 250 °C. FAME's were identified by comparing their retention time with those of Sigma–Aldrich standards (PUFA-3, Menhaden oil, and PUFA-1, Marine source from Supelco Analytical). The limit of detection (LOD) is 1 mg/100 g. Results were calculated in mg/100 g of edible part using the peak area ratio (% of total fatty acids) and the lipid conversion factors, which were, in turn, calculated according to Weihrauch et al. [18].

2.4. Lipid class determination

The relative weight of each lipid class was determined by analytical thin-layer chromatography (TLC) using a previously described method [19]. An eluent mixture of hexane:diethyl ether:acetic acid (50:50:2 by volume) and a plate coated with 0.25 mm silica gel G were used. Lipid class identification was done by comparison with standards (Sigma Chemical Co., St. Louis, MO, USA). Specifically, glyceryltrioleate (triacylglycerol, TAG), glyceryl 1,3-dipalmitate (diacylglycerol, DAG), DL-α-monoolein (monoacylglycerol, MAG), oleic acid (free fatty acid, FFA), and L-α-phosphatidylcholine (phospholipid, PL, polar lipid) were used. The relative percentage of each lipid class was determined using a GS-800 densitometer and version 4.5.2 of Quantity One 1-D Analysis software from Bio-Rad (Hercules, CA, USA).

2.5. Anti-inflammatory activity

To extract lipids from freeze-dried *I. galbana* microalgae and respective bioaccessible fraction, the Bligh and Dyer method [16] and the methodology described in Section 2.7. were used, respectively. Extracted lipids were directly dissolved in 100% dimethyl sulfoxide (DMSO) (see below Section 2.5.2).

2.5.1. Aqueous extract preparation for in vitro anti-inflammatory activity

An aqueous extract of the freeze-dried *I. galbana* microalgae was prepared with the purpose of attaining a fraction with anti-inflammatory properties to be tested *in vitro*.

Accordingly, approximately 200 mg of freeze-dried *I. galbana* microalgae was weighed and homogenized with 2 ml of Milli-Q water using a model Polytron PT 6100 homogenizer (Kinematica, Luzern, Switzerland) at a velocity of 30,000 rpm during 1 min. Afterwards, the mixture was subjected to a thermal treatment (at 80 °C for 1 h). Both the microalgae and bioaccessible extraction mixtures were centrifuged (3000 × *g* at 4 °C during 10 min) and the respective supernatant was evaporated using vacuum rotary evaporator with the water bath temperature at 65 °C and inert gas (nitrogen) stream.

2.5.2. Cyclooxygenase (COX-2) inhibition assay

The prepared extracts were dissolved in 100% DMSO to prepare a stock with a concentration of 10 mg/ml. The extract was tested at 1 mg/ml and 100 μg/ml using a commercial cyclooxygenase (COX-2) inhibitory screening assay kit, Cayman test kit-560,131 (Cayman Chemical Company, Ann Arbor, MI, USA). The COX-2 inhibitor screening assay directly measures the amount of Prostaglandin F2α generated from arachidonic acid (AA, 20:4 ω6) in the cyclooxygenase reaction. A volume of 10 μl each of test extract or DMSO was used. The reaction was initiated by addition of 10 μl 10 mM AA and each reaction tube was incubated at 37 °C for 2 min. The reaction was terminated by addition of 50 μl 1 N HCl and saturated stannous chloride. Assays were performed using 100 units of human recombinant COX-2. An aliquot was removed and the prostanoid produced was quantified spectrophotometrically (412 nm) *via* enzyme immunoassay (ELISA) after 18 h incubation, washing, addition of Ellman's reagent, and further 90 min incubation. Interference by solutions and digestive enzymes used in the bioaccessibility method was taken into account by subtracting COX-2 inhibition of the bioaccessibility blank from the COX-2 inhibition measured with the bioaccessible fraction samples.

2.6. In vitro digestion model

An *in vitro* digestion model was chosen for the determination of bioaccessibility in freeze-dried *I. galbana* microalgae. The model was comprised of three sections, which enable the simulation of digestion in three different parts of the GI tract: mouth, stomach, and small intestine. The solutions and enzymes used in this model followed Afonso et al. [12]. Briefly, approximately 1.5 g freeze-dried *I. galbana* was weighed taking into account the assumptions defined by Versantvoort et al. [20]. The sample was mixed with 4 ml of artificial saliva at a pH 6.8 ± 0.2 for 5 min, then 8 ml of artificial gastric juice (pH 1.3 ± 0.02 at 37 ± 2 °C) was added, and pH was lowered to 2.0 ± 0.1. The mixing lasted 2 h in a “head-over-heels” movement (37 rpm at 37 ± 2 °C). Finally, 8 ml of artificial duodenal juice (pH 8.1 ± 0.2 at 37 ± 2 °C), 4 ml of bile (pH 8.2 ± 0.2 at 37 ± 2 °C), and 1.33 ml of HCO_3^- solution (1 M) was added. The pH of the mixture was set at 6.5 ± 0.5 and agitation for 2 h was identical to gastric conditions. The mixture generated in the *in vitro* model was subjected to centrifugation at 2750 × *g* for 5 min, thus yielding a non-digested portion and the bioaccessible fraction. While chemicals were supplied by Merck (Darmstadt, Germany), enzymes were attained from Sigma (St. Louis, MO, USA).

2.6.1. Calculation of bioaccessibility

The percentage (%) of each *I. galbana* microalgae constituent (C) in the bioaccessible fraction was estimated as follows: