Process Biochemistry 48 (2013) 1999–2008

Contents lists available at ScienceDirect

Process Biochemistry

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

Enzyme-assisted extraction of fucoxanthin and lipids containing polyunsaturated fatty acids from *Undaria pinnatifida* using dimethyl ether and ethanol

Jagan M. Billakanti*, Owen J. Catchpole, Tina A. Fenton, Kevin A. Mitchell, Andrew D. MacKenzie

Callaghan Innovation Research Limited, 69 Gracefield Road, PO Box 31310, Lower Hutt 5040, New Zealand

ARTICLE INFO

Article history:
Received 17 July 2013
Received in revised form 29 August 2013
Accepted 9 September 2013
Available online 25 September 2013

Keywords:
Fucoxanthin
PUFAs
Undaria pinnatifida
Dimethyl ether
Alginate lyase
Polyunsaturated fatty acids

ABSTRACT

A novel method for the efficient extraction of fucoxanthin and lipids containing polyunsaturated fatty acids (PUFAs) from the brown seaweed *Undaria pinnatifida* was developed and demonstrated at a laboratory scale. *U. pinnatifida*, also known as *Wakame*, contains a number of biologically active lipophilic compounds, particularly fucoxanthin, which has anti-oxidant, anti-cancer, anti-obesity and anti-inflammatory properties. The yield of fucoxanthin and lipids containing PUFAs was determined by extraction from wet and freeze-dried seaweed using dimethyl ether (DME) and ethanol and from enzyme-pretreated seaweed using the same solvents. The highest yields of fucoxanthin (94%) and lipids (94%) rich in PUFAs were obtained from fresh (wet) *U. pinnatifida* by enzyme pre-processing, followed by extraction using DME with ethanol as a co-solvent. In comparison, ethanol extraction resulted in lower extraction yields for both fucoxanthin (86%) and lipids (73%) under the conditions described. Enzyme pre-processing using alginate lyase resulted in the hydrolysis of cell wall polysaccharides, resulting in high extraction yields. The hydrolysis time, pH and temperature were found to be the most important parameters for the enzyme pre-processing step and for minimizing fucoxanthin losses due to oxidative degradation. The removal of water-soluble compounds (polysaccharides) following the enzyme pre-treatment prior to DME extractions doubled the throughput and maximized the yield. The residual biomass was colorless or a pale-brown color after the DME extraction, which indicated the highly effective extraction of fucoxanthin. The PUFA content and fucoxanthin levels were not affected by the enzyme or extraction using the described enzyme-assisted DME + ethanol co-solvent process.

1. Introduction

Undaria pinnatifida, also known as *Wakame*, is an edible brown seaweed native to South East Asia. As *U. pinnatifida* is readily available and fast growing, it is the most commonly consumed seaweed in Asia and other parts of world and is commonly used as an ingredient of soups and salads, and also as a garnish for sliced row fish "Sashimi". *Undaria* also has several health benefits [1–4]. *Undaria* is currently found in almost all marine regions of the world, including New Zealand coastal regions and harbors [5,6], particularly around greenshell mussel farms where it is regarded as an invasive pest species. Approximately 4000 tons per annum of *Undaria* are currently co-harvested as a waste stream [5] from mussel farm ropes [7] and could be utilized to produce added-value compounds. For example, *Undaria* contains high levels of ω-3 and ω-6 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA), arachidonic acid (ARA) and stearidonic acid (SDA), which are important for nutrition because humans and other vertebrates are unable to synthesize these compounds. *Undaria* seaweed also contains notable amounts of the following nutrients: glycolipids [8], including mono- and digalactosyldiacylglycerol (MGDG and DGDG) and sulfoquinovosyl diacylglycerol (SQDG); carotenoids (particularly fucoxanthin and zeaxanthin) and fucosterols; dietary fibers; proteins; vitamins; alginates and sulfated polysaccharides and several other mineral components [9]. *In vitro* and *in vivo* trials using fucoxanthin have demonstrated anti-acne, anti-obesity, anti-diabetic and anti-cancer properties [4,10]. Furthermore, *Undaria* contains 10–15% of proteinaceous matter on a dry basis [7], and peptides derived from these proteins have shown health-promoting benefits, such as anti-hypertensive activity [11]. The proteins are also rich in essential amino acids [7,9]. Additionally, the polysaccharides (alginates and fucoidans) derived from *Undaria* seaweed exhibit a wide range of physiological and biological activities [12,13]. Indeed,

* Corresponding author. Tel.: +64 4 931 3285.
E-mail address: jaganmohan.billakanti@callaghaninnovation.govt.nz (J.M. Billakanti).

1359-5113/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.procbio.2013.09.015

there is considerable research interest in *Undaria* because of the number of potential bioactive compounds and the potential to convert a waste stream into added-value products.

Brown seaweeds are complex biomaterials. Their cell wall components contain a mixture of sulfated and branched chain polysaccharides (40–50%) that are strongly associated with proteins, metal ions and various other cell wall compounds [14,15]. Alginates are a special class of polysaccharides found only in brown seaweeds and constitute >70% of the total *Undaria* polysaccharides. Alginates are chain-forming heteropolysaccharides composed of blocks of mannuronic acid (M) and guluronic acid (G) residues, and the ratio of M and G blocks varies greatly depending on the seaweed source and growing conditions. Poly-G or -M blocks bind to calcium ions to form strong gel structures, and the gel strength and structural properties vary with the M and G block ratio. Due to the high concentration of alginates and strong cell wall structure, the extraction of bioactive compounds from brown seaweed biomass is challenging, as the cell wall provides a significant barrier to extraction. To overcome this issue, the enzymatic digestion of algal cell wall polysaccharides prior to extraction is gaining increasing attention [8,16]. Brown seaweed cell walls are predominantly composed of alginates [16], which are not hydrolysable using such enzymes as cellulase, pectinase and hemicellulase. However, alginate lyase (EC 4.2.2.3, mannuronate lyase), catalyzes the hydrolysis of alginate or alginic acid polymers to lower molecular weight (<10 kDa) oligosaccharides. Although lyases have been isolated from various sources and characterized with regard to their specific activities [17,18], only a few are commercially available. The alginate lyase used in this work (alginate lyase from *Flavobacterium multivorum*, A1603 Sigma–Aldrich, New Zealand) hydrolyzes the glycosidic bonds specific to the β-D-mannuronate residues of alginate polysaccharides in brown seaweeds, partially degrading the cell walls and thus potentially improving the recovery of fucoxanthin and lipids [8]. The enzyme also reduces the viscosity of the reaction mixture.

The extraction of bioactive compounds, such as fucoxanthin, lipids and polyphenols, from *U. pinnatifida* seaweed has been previously reported [19–22], but the yields were poor. The areas in which improvements can be made include the drying step or an alternative to this step and in minimizing the degradation of fucoxanthin. Recent research into the recovery of bioactive compounds from seaweed using microwave-assisted [23,24] or enzyme-assisted extraction [8,16] techniques has reported improved yields in comparison to conventional methods. Furthermore, freeze-dried algae and air-dried seaweed result in lower amounts of fucoxanthin than fresh algae, indicating that either the processing conditions degrade fucoxanthin or that the processing conditions increase the resistance to mass transfer provided by the cell wall during solvent extraction. Fucoxanthin is a fat-soluble carotenoid that is vulnerable to oxidation and degradation when exposed to light, heat and acidic or alkaline conditions [19,20,25]. The most common laboratory-scale methods used for fucoxanthin extraction employ liquid organic solvents, such as toluene, hexane, acetone, methanol, ethanol and combinations thereof [21,26–28]. Acetone, methanol and ethanol are potentially suitable for extracting wet biomass because they are able to co-extract water. However, most of these solvents are unsuitable for the production of food ingredients or nutraceuticals, and the removal of solvents from the final extract at elevated temperatures could also cause partial degradation of fucoxanthin and other bioactive compounds [25,29]. In addition, the yields of fucoxanthin extracted using these methods vary widely. Nonetheless, ethanol is suitable for industrial-scale applications due to its wide acceptance as a food processing aid. Supercritical fluid extraction (SFE) using CO_2 provides an alternative food-suitable technology with potentially improved efficiency and recovery. However, conventional SFE using CO_2 requires that the seaweed be dry, and the solubility of fucoxanthin in supercritical CO_2 is low, requiring the use of co-solvents [22]. Liquid dimethyl ether (DME) is an alternative that can be used to process wet feed materials [30] for the extraction of neutral and polar lipids. DME is easily liquefiable at or slightly above room temperature, is miscible with polar solvents in its liquefied state and is a good solvent for extracting lipophilic compounds from both wet and dry feed materials. The extraction of naturally occurring bioactive compounds from complex feed materials and fermentation broths using DME has been extensively studied [30,31] and shows potential for the processing of such materials at a commercial scale. DME has also recently been approved for the processing of foods in New Zealand and Australia.

In this work, we investigate the use of alginate lyase enzyme-assisted DME, DME-EtOH co-solvent and ethanol extraction of lipids containing polyunsaturated fatty acids and fucoxanthin from *U. pinnatifida* and compare the results with those for ethanol and DME extraction of wet and dry seaweed.

2. Materials and methods

2.1. Materials

The *U. pinnatifida* seaweed used in this study was harvested from the Marlborough Sounds, New Zealand, by Tai Tipu (Marlborough, New Zealand), who supplied it partially cut/ground (1 cm^2) and frozen to Callaghan Innovation (formerly known as IRL). The freeze-dried seaweed was prepared using an FD24 model freeze-dryer, Cuddon Ltd., New Zealand. Food-grade azeotropic (95%) ethanol (EtOH) and absolute ethanol for laboratory-scale extractions were purchased from Anchor Ethanol, New Zealand. Aerosol-grade dimethyl ether (>99.5% DME by mass) was supplied by DAMAR Industries NZ Ltd. The methanol (MeOH) and chloroform ($CHCl_3$) used for the analytical-scale extractions were analytical grade, and the solvents used in the high-Performance liquid chromatography (HPLC) and gas chromatography (GC) analyses were HPLC grade. Unless otherwise stated, all other chemicals and solvents used in this work were analytical grade. Fucoxanthin and a mixture of carotenoid standards were purchased from DHI Lab, Denmark. Alginate lyase enzyme (A1603) was purchased from Sigma–Aldrich, Australia.

2.2. Methods

2.2.1. Solid content estimation

The frozen *Undaria* was separated into portions of 10 g and 20 g samples (triplicate samples for each weight) and transferred to 50-mL Falcon tubes (pre-weighed) and freeze-dried to complete dryness. The solid content of the frozen *U. pinnatifida* was measured as 10.3 ± 0.1% (w/w) by freeze drying a known mass of seaweed. The solid content of aqueous enzyme-digest extracts for the different treatments was determined in a similar manner.

2.2.2. Lipid content estimation

The total lipid (TL) content of *U. pinnatifida* pre- and post-extraction was determined using the Bligh and Dyer [32] method, with modifications necessary for handling seaweed. Typically, 10 g of *Undaria* seaweed (wet) was transferred into a 50-mL Falcon tube and mixed thoroughly for an hour with MeOH, double distilled H_2O and $CHCl_3$ (1:1:1). Phase separation was accomplished by centrifugation at 2500 × *g* for 5 min, and the chloroform layer containing the lipid compounds and fucoxanthin was then recovered. The residual biomass was re-extracted twice using the same procedure until maximum lipid extraction/complete color depletion was achieved. All of the solvent and extract solutions were pooled together, and the solvent was removed by rotary vacuum evaporation at ≤45 °C to complete dryness. The lipid obtained was analyzed for the fatty acid and carotenoid contents. Complete lipid extraction from dried seaweed was much more challenging and required 2–3 additional cycles of extraction to achieve maximum recovery.

2.2.3. Analysis of fatty acid profiles by gas chromatography

Fatty acid methyl esters (FAMEs) were prepared from the total lipid extracts using the method of Carreau and Dubacq [33]. The lipid samples (10–20 mg) were dissolved in 0.5 mL toluene containing methyl tricosanoate (1 mg/mL) as an internal standard. Sodium methoxide (0.5 mL, 1% in MeOH) was added, and the vial was vortexed to dissolve the sample. The sample was placed in an oven at 60 °C for 20 min. Methanolic HCl (1 mL: 10% (v/v) acetyl chloride in MeOH) was added, and the sample was then vortexed and placed into an oven at 60 °C for 20 min. Type 1 water obtained using a Barnstead Nanopure Diamond system (0.25 mL) and petroleum ether (boiling range 60–80 °C, 1 mL) were added, the mixture was shaken, and the top layer collected for GC analysis.

The fatty acid methyl esters (FAMEs) were analyzed using a Trace GC Ultra (Thermo Fisher Scientific) gas chromatograph equipped with a flame ionization