Journal of Functional Foods 24 (2016) 221–230

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Impact of extraction processes on prebiotic potential of the brown seaweed *Ecklonia radiata* by *in vitro* human gut bacteria fermentation

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ARTICLE INFO

Article history:
Received 15 December 2015
Received in revised form 6 April 2016
Accepted 18 April 2016
Available online 26 April 2016

Keywords:
Carbohydrate
Enzyme-assisted extraction
Gut microbe
Macroalgae
Prebiotic activity
Short chain fatty acid

ABSTRACT

The prebiotic potential of the extracts of a South Australian brown seaweed *Ecklonia radiata* obtained using different processes was investigated. Six extracts of this seaweed were prepared by enzymatic, acidic, and water extraction processes. The extracts were added to an *in vitro* anaerobic fermentation system containing human faecal inocula to assess their ability to generate short chain fatty acids (SCFAs) and to promote the growth of selected bacterial genera (as assessed by quantitative PCR). Following 24 h fermentation, all seaweed extracts significantly increased ($P < 0.05$) total SCFA production (50.7–72.7 μmol/mL) and the total number of bacteria ($\log_{10}$ 10.2–10.4 cells/mL) when compared to controls (blank and cellulose). The extracts prepared using Celluclast-assisted extraction showed the greatest potential for improving gut health as these induced significantly higher production of butyrate (9.2 μmol/mL), and the growth of bacteria regarded as beneficial, including *Bifidobacterium* ($\log_{10}$ 6.6 cells/mL) and *Lactobacillus* ($\log_{10}$ 5.3 cells/mL).

1. Introduction

The relationship between diet and health is being widely recognised by consumers, and this leads to the demand for food ingredients with functionality and health benefits. There is growing evidence that indigestible dietary polysaccharides can have a significant benefit to health through their ability to stimulate the growth of beneficial microbes in the large bowel (Conlon & Bird, 2015), and hence may fulfil the definition of a prebiotic. Examples of prebiotic polysaccharides include inulin, fructooligosaccharides, galactooligosaccharides and lactulose (Al-Sheraji et al., 2013; Kolida & Gibson, 2008).Other dietary components, including polyphenols and proteins, can also reach the large bowel and potentially exert a health benefit via activities of the gut microbiota (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; Windey, De Preter, & Verbeke, 2012).

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http://dx.doi.org/10.1016/j.jff.2016.04.016
1756-4646/

Brown seaweeds (Phaeophyceae) have a relatively high diversity among all the macroalgae. Of the 231 species reported in Southern Australia, 57% are considered endemic (Womersley, 1990). Recent research on seaweed-derived functional food ingredients has shown that brown seaweeds have high nutraceutical potential, containing a variety of bioactive compounds such as phloroglucinol-based polyphenolic compounds (phlorotannins), carotenoids (fucoxanthin), polyunsaturated fatty acids (PUFAs), and bioactive peptides (Holdt & Kraan, 2011). In addition, brown seaweeds are a rich source of polysaccharides possessing various biological functions (Synytsya, Čopíková, Kim, & Park, 2015). The complex structure of brown seaweed polysaccharides, mainly alginate, fucoidan, and laminarin, makes them resistant to degradation by human digestive enzymes, and as a consequence can be regarded as dietary fibres. Most of these can be fermented by gut microbiota, which may provide a health benefit to humans through a prebiotic effect (O'Sullivan et al., 2010; Zaporozhets et al., 2014). Recent studies report that polysaccharides and oligosaccharides derived from seaweeds are potential modulators of intestinal metabolism, including fermentation, inhibitors of pathogen adhesion and evasion, and may even treat inflammatory bowel disease (Devillé, Gharbi, Dandrifosse, & Peulen, 2007; Kuda et al., 2015; Lean, Eri, Fitton, Patel, & Gueven, 2015; Ramnani et al., 2012). In addition, the wide variety of phlorotannins (polyphenols) present in brown seaweed (Li, Wijesekara, Li, & Kim, 2011) may not be readily absorbed in the small intestine, and would reach the large intestine where they can be converted by microbial activity into beneficial bioactive phenolic metabolites (Cardona et al., 2013).

South Australia has one of the world's highest diversity of brown seaweed, and *Ecklonia radiata* (C. Agardh) J. Agardh is one of the most abundant species (Lorbeer, Tham, & Zhang, 2013). The aim of this present study was to examine whether *E. radiata* can be utilised as a source of prebiotic components, and how different extraction processes can impact on their prebiotic potential. Given the high degree of structural complexity and rigidity of seaweed cell walls (Deniaud-Bouët et al., 2014) that present a major obstacle to the efficient extraction of polysaccharides and other bioactive compounds, enzyme-assisted extraction (EAE) was used. EAE has attracted considerable interest in the release of high molecular weight intracellular compounds such as polysaccharides, polyphenols, and proteins (Kadam, Tiwari, & O'Donnell, 2013; Wijesinghe & Jeon, 2012); however, only a few studies have examined the prebiotic potential of enzyme-assisted extracts from seaweeds (Michel, Lahaye, Bonnet, Mabeau, & Barry, 1996; Rodrigues et al., 2015; Wu, Kang, Kazlowski, Wu, & Pan, 2012; Wu, Wang, & Pan, 2007). There is no study on the impact of different enzyme-assisted extraction processes on the prebiotic potential of seaweed extracts using a human gut fermentation model; hence, this study is valuable for the development of an efficient process for seaweed derived marketable functional foods. Fractions containing isolated components of the seaweed were tested for their prebiotic potential by including them in an *in vitro* anaerobic fermentation containing human faecal inocula that mimics the microflora of the human large bowel. The production of key beneficial gut fermentation products, namely short chain fatty acids (SCFAs), and the growth of selected microorganisms are used as indicators of potential benefit.

Table 1 – Characteristics of seaweed samples prepared by different processes used in the batch fermentation.

Seaweed sample/substrate	Process	Characteristics
Celluclast	Enzyme-assisted extraction by Celluclast or	Low molecular weight polysaccharides
Alcalase	Alcalase	Low molecular weight proteins
Acid Water	Conventional extraction by acid or water	High molecular weight polysaccharides and proteins
Free sugar fraction	Water extraction and fractionation	Unbound small sugars
Polysaccharide fraction		Bound high molecular weight polysaccharides
Seaweed residue	Residues from water extraction	Non-digestible polysaccharides
Seaweed powder	Seaweed raw material	Complex structure polysaccharides

2. Materials and methods

2.1. *Seaweed*

Brown seaweed (*Ecklonia radiata* – identification confirmed by the State Herbarium of South Australia) was collected from freshly deposited beach-cast seaweed in Rivoli Bay, Beachport, South Australia in March 2013. It was rinsed in fresh water to remove any visible surface contaminants, and placed on mesh racks to dry. The whole plants were collected at one time to provide a consistent sample for the whole studies. It was dry blended (Blendtec, Orem, UT, USA), then passed through a 0.25 mm sieve, and dried in an oven at 45 °C. The ground powder was stored at −20 °C prior to extraction and fermentation.

2.2. *Enzymes*

Two commercial enzymes used for the preparation of seaweed extracts, carbohydrases (Celluclast® 1.5 L) and proteases (Alcalase® 2.4 L FG,) were kindly provided by Novozymes (Bagsvaerd, Denmark).

2.3. *Chemicals and substrates*

All chemicals used are of analytical or gas chromatography grade from Merck and Sigma. The positive control substrates for comparison purposes in batch fermentation were glucose, resistant starch Hi-maize® 958 (Starch Australasia, Lane Cove, NSW, Australia), and inulin. α-Cellulose and blank were used as negative controls.

Eight seaweed samples used in the experiments were prepared by different extraction processes and summarised in Table 1. The criteria to select these samples were based on the key nutrients and potential fermentable components in the seaweed extracts, mainly carbohydrates and proteins. The indigestible dietary polysaccharides are the most important