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Enzyme-enhanced extraction of antioxidant ingredients from red algae *Palmaria palmata*

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ABSTRACT

The effect of various protease and carbohydrase treatments on the extraction of polyphenols and other antioxidant ingredients from the red algae *Palmaria palmata* (dulse) was investigated. In addition, the relative contribution of different fractions to the overall antioxidant capacity of the hydrolysate was evaluated. Considerable differences were observed both in total phenolic content (TPC) and antioxidant activities of the hydrolysates evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, oxygen radical absorbance capacity (ORAC) and ferrous ion-chelating ability assays. All the proteases tested had significant enhancing effect on the extraction of polyphenols and other active components compared to carbohydrases and cold water extraction (control). The Umamizyme extract had the highest TPC and consequently exhibited the strongest scavenging capacity against DPPH and peroxyl radicals. Further fractionation of the Umamizyme extract revealed that the crude polyphenol fraction possessed the highest peroxyl radical scavenging activity, whereas the crude polysaccharide fraction was more effective for chelating ferrous ions. The data from this study suggest the potential of protease treatment to improve value-added utilization of dulse extracts as antioxidants in functional foods and nutraceuticals.

1. Introduction

Marine macroalgae have a long history of use as food and folk medicine in Asia, but are much less common in Europe and North America, as a part of the diet. Only a few species are harvested for direct human consumption in limited coastal regions. *Palmaria palmata* (dulse) is one of the most widely distributed edible seaweeds in Iceland and was an important source of food supply when food variety was scarce in earlier times according to documentaries (Kristjánsson, 1980). Nowadays, dulse is mainly consumed as snacks and sold in health stores, but there is growing awareness that it should be utilized more as a source of important nutrients and functional ingredients (Plaza, Cifuentes, & Ibanez, 2008). It has been reported that dulse has the second highest protein content of all common seaweeds, after *Porphyra tenera* (Nori) (Galland-Irmouli et al., 1999). The essential amino acids (EAAs) can represent between 26 and 50% of the total amino acids (Galland-Irmouli et al., 1999; Morgan, Wright, & Simpson, 1980). Dulse is also high in iron and many other easily assimilated minerals and trace elements as well as a good source of dietary fibers and vitamins (MacArtain, Gill, Brooks, Campbell, & Rowland, 2007; Morgan et al., 1980).

In recent years, there is increasing interest in the search for naturally occurring compounds with antioxidant activity as alternatives to synthetic products. Aquatic plants are also a rich source of natural antioxidants (Duan, Zhang, Li, & Wang, 2006). Previous studies have shown that *P. palmata* contains several classes of hydrophilic antioxidant components including L-ascorbic acid, glutathione (GSH), polyphenols as well as MAAs (Yuan, Carrington, & Walsh, 2005b; Yuan, Westcott, Hu, & Kitts, 2009). However, the

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high degree of structural complexity and rigidity of the algal cell wall is a major obstacle to the efficient extraction of the intracellular bioactive constituents (Deniaud, Quemener, Fleurence, & Lahaye, 2003). Conventional water and solvent extraction have several drawbacks such as low selectivity, low extraction efficiency, solvent residue and environmental pollution (Herrero, Cifuentes, & Ibanez, 2006). As an alternative technology, enzyme-assisted extraction has attracted considerable interest. The application of hydrolytic enzymes has shown a great potential to improve the extraction yield, enhance the release of secondary plant metabolites and preserve the bioactive properties of the extracts (Li, Smith, & Hossain, 2006). Enzymatic extraction has also been reported to increase the extractability of bioactive compounds from several brown algae (Heo, Park, Lee, & Jeon, 2005; Siriwardhana et al., 2008). These cell wall-degrading enzymes help to weaken or disrupt the cell wall structure, break down complex interior storage materials, thereby facilitating the release of the intracellular bioactive compounds from algal biomass. Furthermore, the hydrolytic breakdown of high-molecular-weight (HMW) polysaccharides and proteins may contribute to enhanced antioxidant activities (Siriwardhana et al., 2008).

P. palmata is one of the most abundant seaweed species along the coastline of Iceland, which has so far not been explored as a source of valuable antioxidant ingredients. Therefore, the aim of the present study was to investigate the efficiency of enzyme-assisted extraction of polyphenols and other antioxidant components from *P. palmata*. The potential of enzyme treatments to improve the antioxidant activity of the extract were assessed by three *in vitro* assays based upon different reaction mechanisms. The antioxidant tests were also carried out on three subfractions from Umamizyme extract, namely crude polyphenol, crude polysaccharide and an LMW aqueous fraction to estimate their relative contribution to the overall antioxidant capacity of the hydrolysate.

2. Materials and methods

2.1. Algal materials

The red algae (Rhodophyta) *P. palmata* (Linnaeus) Kuntze, was collected in Hvassahraun coastal area nearby Hafnarfjordur, southwestern Iceland on October 12th, 2007. The samples were carefully rinsed with tap water. The small cut pieces (about 2 cm × 2 cm) were freeze-dried, ground into powder and passed through a 1.0 mm sieve. The ground powders were stored in tightly sealed polystyrene containers at −20 °C prior to extraction.

2.2. Chemicals

Fluorescein sodium salt (FL), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid monosodium salt (Ferrozine), Folin–Ciocalteu's phenol reagent were obtained from Fluka (Buchs, Switzerland). 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH) was purchased from Sigma–Aldrich (Steinheim, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

The following eleven commercial enzymes were used for the preparation of hydrolysates, including six types of proteases (Umamizyme, Alacalase 2.4L FG, Protamex, Kojizyme 500 MG, Neutrase 0.8 L and Flavourzyme 500 MG) and five types of carbohydrases (Viscozyme L, Ultraflo L, AMG 300 L, Celluclast 1.5 L FG and Termamyl 120 L). Umamizyme was obtained from Amano Enzyme Inc. (Nagoya, Japan). The other ten enzymes were kindly provided by Novozymes A/S (Bagsvaerd, Denmark). The optimum hydrolysis conditions (Heo et al., 2005; Sato et al., 2002), characteristics and sources of these enzymes are summarized in Table 1.

2.3. Proximate composition analyses of dried P. palmata

Water content was determined gravimetrically by heating the sample in an oven at 103 ± 2 °C for 4 h (ISO, 1999). Total fat was determined by extraction with petroleum ether, boiling range 40 °C–60 °C using an extraction apparatus 2050 Soxtec Avanti Automatic System (AOCS 1998). Protein content was determined by the Kjeldahl method (ISO, 2005) and ash according to ISO 5984 (ISO, 2002). Percentage of total carbohydrates was determined by subtracting the sum percentage of moisture, crude protein, crude fat and ash.

2.4. Preparation of enzymatic extracts

The enzymatic extracts were prepared according to the method of Heo, Lee, Song, and Jeon (2003) with slight modifications. Briefly, 2 g of dried algal sample was dispersed in 50 mL of distilled water and incubated in a water bath shaker for 10 min. After adjusting pH, 100 mg of enzyme was added. The enzymatic hydrolysis was performed under optimal conditions (Table 1) of the particular enzyme for 24 h (Heo et al., 2005; Sato et al., 2002). The reaction was terminated by boiling the sample at 100 °C for 10 min and thereafter immediate cooling in an ice bath. The hydrolysate was centrifuged at 5000 *g* for 10 min at 4 °C, filtered with Whatman no. 4 filter paper and the pH of the filtrates was adjusted to pH 7.0 with

Table 1
Summary of the optimum hydrolysis conditions, characteristics and sources of specific enzyme.

Enzyme[a]	Optimum conditions[b]		Characteristics	Sources
	pH	Temperature (°C)		
Umamizyme	7.0	50	Endo- and exo-peptidase complex	*Aspergillus oryzae*
Alacalase 2.4L FG	8.0	50	Endo-peptidase	*Bacillus licheniformis*
Protamex	6.0	40	Protease complex	*Bacillus* sp.
Kojizyme 500 MG	6.0	40	Amino- and carboxy-peptidase	*Aspergillus oryzae*
Neutrase 0.8 L	6.0	50	Metallo-endoprotease	Bacillus amyloliquefaciens
Flavourzyme 500 MG	7.0	50	Endo-protease and exo-peptidase	*Aspergillus oryzae*
Viscozyme L	4.5	50	A multi-enzyme complex (containing arabanase, cellulase, β-glucanase, hemicellulase and xylanase)	*Aspergillus aculeatus*
Ultraflo L	7.0	60	Heat-stable multi-active β-glucanase	Humicola insolens
AMG 300 L	4.5	60	Exo-1,4-α-D-glucosidase	Aspergillus *niger*
Celluclast 1.5 L FG	4.5	50	Cellulase	*Trichoderma reesei* ATCC 26921
Termamyl 120 L	6.0	60	Heat-stable α-amylase	Bacillus licheniformis

[a] Umamizyme was obtained from Amano Enzyme Inc. (Nagoya, Japan). The other enzymes were from Novozymes A/S (Bagsvaerd, Denmark).
[b] Heo et al. (2005); Sato et al. (2002).