Evaluation of antioxidant activity of enzymatic extracts from the red algae *Palmaria palmata*

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INTRODUCTION

- *P. palmata* (dulse, Rhodophyta) is the main edible seaweed species in Iceland for centuries. Nowadays, dulse is mainly consumed as snacks, but there is growing awareness that it should be utilized more as a potential source of new functional ingredients. Enzyme-assisted extraction has emerged as an alternative technology to overcome the drawbacks of conventional water and organic solvent extraction. However, no detailed studies have been performed on enzymatic extraction of antioxidant compounds from *P. palmata*.

OBJECTIVE

- The main objective of the present study was to investigate the potential application of commercial enzymes for enhanced extraction of hydrophilic antioxidant ingredients from *P. palmata*

METHODS

Materials

- Freeze-dried and powdered *P. palmata*
- Eleven commercial enzymes including five carbohydrases (Viscozyme, Celluclast, AMG, Termamyl and Ultraflo) and six proteases (Alcalase, Flavourzyme, Kojizyme, Neutrase, Protamex and Umamizyme) were used to prepare water soluble extracts

Enzymatic extraction and fractionation

- Enzymatic extraction was performed under optimal conditions of the particular enzyme for 24 h
- Umamizyme hydrolysate was further fractionated into crude polyphenol, crude polysaccharide and a low-molecular-weight aqueous fraction (MW < 5 KDa)

Measurements

- Proximate composition of freeze-dried *P. palmata*
- Antioxidant properties of enzymatic hydrolysates and fractions were evaluated by assaying for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, oxygen radical absorbance capacity (ORAC) and ferrous ion chelating ability
- Concentration of total phenolics, soluble carbohydrates, reducing sugars and soluble proteins in three subfractions from Umamizyme extract

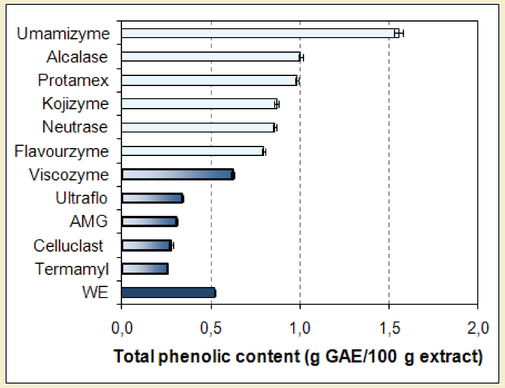

Figure 1. Total phenolic content of different enzymatic extracts from *P. palmata* compared to water extract (WE).

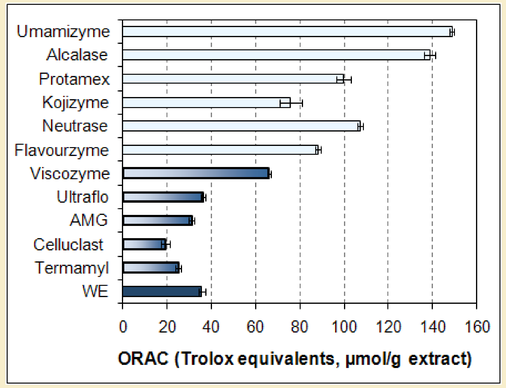

Figure 2. Oxygen radical absorbance capacity (ORAC) of different enzymatic extracts from *P. palmata* compared to water extract (WE).

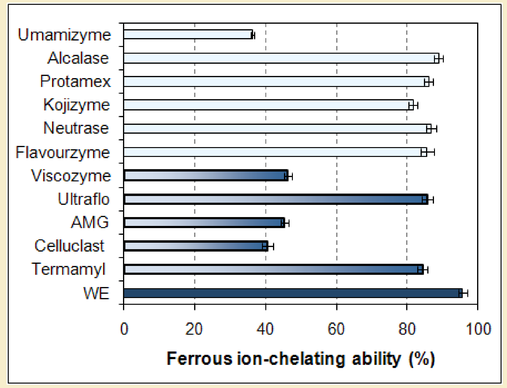

Figure 3. Ferrous ion-chelating activity of different enzymatic extracts from *P. palmata* compared to water extract (WE).

P. palmata (dulse) (Picture: Karl Gunnarsson)

Table 1. Antioxidant activities of crude polyphenol, polysaccharide and LMW aqueous fractions from Umamizyme extract of *P. palmata*

Sample	DPPH Scavenging [a] (ARP)	ORAC [b] (µmol TE/g extract)	Fe^{2+} chelating ability (%)
Crude polyphenol fraction	5.4 ± 0.3	629.5 ± 15.2	57.2 ± 2.9[c]
Crude polysaccharide fraction	4.8 ± 0.3	102.9 ± 2.8	94.3 ± 1.8[c]
LMW aq. fraction (<5 KDa)	2.4 ± 0.1	130.7 ± 3.4	20.5 ± 1.6[d]

Each value is expressed as means ± S.D. (n=3).
[a] ARP, antiradical power (ARP=1/EC_{50}), EC_{50}: concentration of extract (mg/ml) required to scavenge 50% of the DPPH• in the reaction mixture. [b] ORAC, oxygen radical absorbance capacity; TE, Trolox equivalents.
[c] was tested at concentration of 2.5 mg/ml. [d] was tested at concentration of 5.0 mg/ml.

RESULTS

- The dried *P. palmata* sample contained approximately 4.1 0.4 % moisture, 61.5 2.4 % carbohydrate, 20.5 0.4 % crude protein, 0.4 0.4 % crude fat and 13.5 0.5 % ash
- All the proteases tested were more effective than carbohydrases and cold water in enhancing the extraction of polyphenols (Figure 1) and other active components from *P. palmata*
- The Umamizyme extract had the highest TPC (Figure 1) and consequently exhibited the strongest scavenging capacity against DPPH (ARP = 1.8, EC_{50} = 0.6 mg/ml) and peroxyl radicals (148.6 µmol TE/g extract) (Figure 2). However, the ferrous ion-chelating capacity was relatively lower (Figure 3)
- Further fractionation of the Umamizyme extract revealed the combined antioxidant effects of different active constituents. Crude polyphenol fraction showed the highest peroxyl radical scavenging activity, whereas the crude polysaccharide fraction was more effective for chelating ferrous ions (Table 1)

CONCLUSION

- Enzyme-assisted extraction is an effective alternative to conventional methods for extraction of polyphenols and other hydrophilic antioxidant compounds from *P. palmata*.
- Protease treatment shows potential to improve value-added utilization of dulse extracts as antioxidants in functional foods and nutraceuticals

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