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Bioactivity of phlorotannins in brown seaweed, *Fucus vesiculosus*

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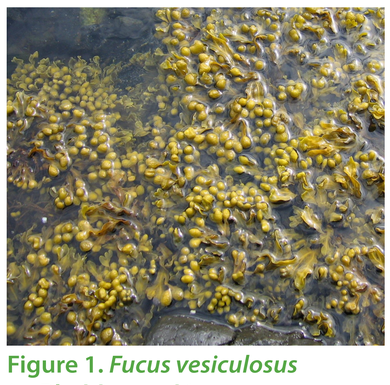

Figure 1. *Fucus vesiculosus* (e. Bladder wrak)

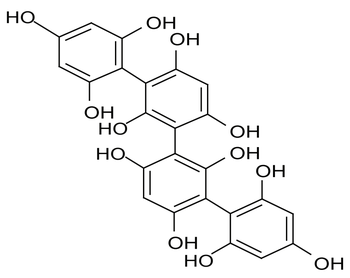

Figure 2. Example of common algal phlorotannins (Tetrafucol A)

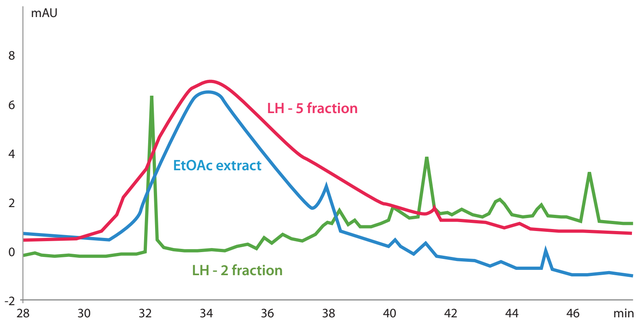

Figure 3. Capillary electropherogram of three different fractions obtained from *F. vesiculosus* extract of 80% aqueous ethanol.

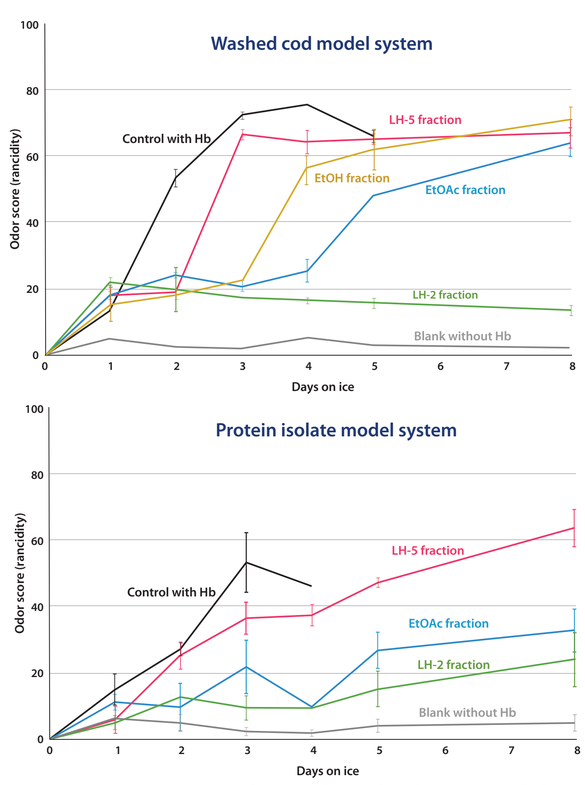

Figure 4. Sensory analysis of washed cod model system (top) and protein isolate model system.

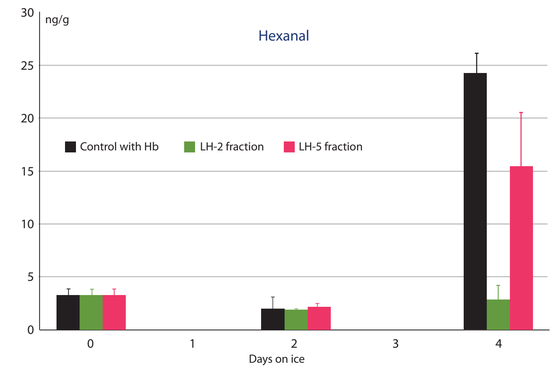

Figure 5. Formation of hexanal in protein isolate model system during storage.

Table 1. Total phenolic content (TPC) and antioxidant activity in EtOAc, LH-2 and LH-5 fractions from *F. vesiculosus*.

Fraction	TPC (g PGE/100 g)	DPPH EC50 (µg/ml)	Fe2+ chelating ability (%)	Reducing power (mg ASE/g)
EtOAc fraction	88.3 ± 2.2	3.76 ± 0.22	34.2 ± 1.3	757.7 ± 38.2
LH-2 fraction	96.6 ± 0.9	2.79 ± 0.05	28.5 ± 0.4	790.3 ± 61.3
LH-5 fraction	91.3 ± 2.4	3.50 ± 0.13	38.0 ± 0.5	785.8 ± 77.1

Introduction

Phlorotannins are the largest group of phenolic compounds in brown algae (Phaeophyceae). Phlorotannins have shown to exhibit many bioactive characteristics, like antioxidant, anti-inflammatory and anti-allergic activities. Our earlier research has shown that *Fucus vesiculosus* (Fig.1) have the highest total polyphenol content, the greatest scavenging activities against DPPH and peroxyl radicals as well as moderate ferrous ion-chelating ability (Wang and others, 2009). The aim of this work was to characterize further the antioxidant activity of phlorotannins in *F. vesiculosus* extracts and fractions and to study their inhibitory effect in washed cod model system and cod protein isolate system.

Materials and Methods

Seaweed extracts

Freeze-dried and powdered *F. vesiculosus* was extracted with 80% aqueous ethanol and partitioned successively with n-hexane, EtOAc, and n-BuOH.

Additional fractionation of the EtOAc fraction was done using Sephadex LH-20 column chromatography with solvent systems of decreasing polarity. Only two subfractions are shown here; LH-2 (75% MeOH) and LH-5 (MeOH:acetone 3:1).

Oxidation systems

A washed cod model system and cod protein isolate model system with added hemoglobin was prepared and used to induce lipid oxidation.

The level of added *F. vesiculosus* fractions to the models was 300 mg/kg.

Analyses

The concentration of total phenolics (TPC) was measured by F olin-Ciocalteu method.

Antioxidant properties of fractions were evaluated by assaying for DPPH radical scavenging activity, Fe^{2+} chelating ability, and reducing power.

EtOAc extract of *F. vesiculosus* was analyzed using LC-ESI/MS. HPLC separation was performed on a Hypercarb column (5 µm 4.6 x150 mm).

The fractions were analyzed using a capillary electrophoresis (CE) using tetra borate separation buffer, UV detector at 210 nm and uncoated silica capillary (Truus, K. and others, 2004).

Lipid oxidation in models was measured using sensory analysis, color, TBARS and GC analysis (GC-FID, GC-Olfactometry and GC-MS) (Jónsdóttir and others, 2007).

Results

TPC and antioxidant activity

LH-2 fraction exhibited the highest TPC and strongest DPPH radical scavenging activity and reducing power (Table 1).

LC-ESI/MS and Capillary Electrophoresis

The EtOAc fraction was composed of units of phloroglucinol (benzene-1,3,5-triol) from 3 to 6 units, thereof 73% with 4 and 5 units (Fig. 2). The presence of monomers was not detected. Most of the compounds present were polymers in which the units can be bonded through a C-C or C-O bond (ether bridges).

The LH-5 fraction showed one broad peak while the LH-2 fraction showed more successful separation of three possible active polar compounds (Fig.3). These compounds appear to have an effective antioxidant activity in the model systems.

Model systems

The LH-2 fraction had an effective lipid oxidation inhibitory effect in both model systems measured by rancid odor (Fig. 4) color and TBARS (results not shown). The LH-5 fraction did not show high antioxidant activity and only increased the lag phase for about one day in the washed cod model and less in the isolate model.

Addition of EtOAc fraction increased the lag phase, especially in the protein isolate system (Fig. 4).

The most intense oxidized odors were potato odor (*cis*-4-heptenal and heptanal), mushroom odor (1-octen-3-ol), grass-like odor (hexanal), fatty, rancid-like odor (2,4-heptadienal) and cucumber-like (2,6-nonadienal).

Formation of hexanal showed a similar pattern as the rancid odor (Fig. 5).

The oxidation was slower in the protein isolate model compared to the washed cod muscle model which can be related to lower content of phospholipids in the isolate model.

Conclusion

The EtOAc fraction of *F. vesiculosus* showed antioxidant ability but the LH-2 fraction showed the highest antioxidant potential. The LH-5 did not notably delay oxidation.

Further studies are needed to identify the phlorotannin compounds with the high antioxidant ability in the LH-2 fraction.

The outcome of this study will support the development of seaweed based functional ingredients for application in functional foods and nutraceuticals

Acknowledgement

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