Antioxidative or prooxidative effects of seaweed fractions in oil emulsion model

YUMIKO YOSHIE-STARK AND TAKESHI SUZUKI
Department of Food Science and Technology, Tokyo University of Fisheries, Minato, Tokyo 108-8477, Japan (yumikoy@tokyo-u-fish.ac.jp)

SUMMARY: Some food products were known to contain many polyphenolic compounds which were reported to be antioxidant. However, there is little information available related to both antioxidative and prooxidative function of foods, especially edible seaweeds. We have analyzed polyphenolic component in seaweeds and tested their antioxidative or prooxidative activity in oil emulsion under ferrous or copper catalyzed oxidation. The anti- or pro-oxidative activity of seaweeds was compared to some teas and each polyphenolic compound alone was also tested for anti- or pro-oxidative activity. Peroxide value (POV), ferrous chelating effect, remained ferrous ion and remained soluble copper were analyzed. Methanolic fraction of seaweed contained some polyphenolic compounds. That fraction did not affect POV under ferrous catalyst, however they reduced POV under copper catalyst. Catechin showed prooxidative activity by increasing POV and without making ferrous chelate. Morin achieved strong chelating effect; however it did not significantly decrease POV. Some other compounds were not involved oxidation/reduction. Taken together, seaweed fractions might perform both of anti/pro-oxidative action depending on the catalyst and other conditions.

KEY WORDS: seaweeds, antioxidant, prooxidant, polyphenolic compounds

INTRODUCTION

Seaweeds have been taken in Japan from long time ago, and also they are important food in Japanese cuisine. There is great interest in plant polyphenolic compounds because of their potential role such as cancer chemopreventive agents and chronic disease protector. Their beneficial effect is considered to be mainly due to their antioxidant and chelating activities. The distribution of polyphenols in foods was reported mainly in vegetables, fruits, and some food products such as wine, tea and chocolate. However, there is little information on the distribution of polyphenols in edible seaweeds. There are several reports which explain antioxidative effect of polyphenolic compounds in different oxidation models. Huang and Frankel reported antioxidant activity in several lipid oxidation model, and Roedig-Penman and Gordon reported antioxidant properties of tea extracts in model food emulsions. In contrast, some antioxidative polyphenolic compounds were also reported to have prooxidative effects. Because food is a complex of many factors, a lot of model systems were considered depending on the concept and still there is a fertile research area left. In this research, we analyzed the content of polyphenolic compounds in edible seaweeds and compared their antioxidant activity in oil emulsion model to tea. Lipid oxidation models by iron or copper were selected. Peroxide value (POV) were used for evaluation of oxidative state of lipid. Crude extract of tea or seaweed was added to lipid emulsion and its change was determined. Also we tried to estimate the function of polyphenolics in oil oxidation system by using metal chelating or scavenging effects as markers. In addition, we have tested anti- or pro-oxidant activity of each polyphenolic compound alone.

MATERIALS AND METHODS

Edible seaweeds; Undaria pinnatifida (Japanese name; Wakame), Hizikia fusiformis (Japanese name; Hijiki) were collected in each growing district; Chiba in Japan. They were washed with tap water, minced by a food cutter (MK-K75, Matsushita Electric Co., Osaka, Japan), and stored at -20 °C until use. Green tea powder (Macha) and heated green tea powder (Hoji cha) were purchased from local supermarket.

Authentic catechin, epicatechin, epigallocatechin, catechin gallate, epicatechin gallate, epigallocatechin gallate, rutin, caffeic acid, catechol, quercitrin, hesperidin, myricetin, morin, luteolin, quercetin, apigenin, kaempferol, and baicalein were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were analytical grade and commercially available.

Analysis of catechins

Catechins were extracted according to the method in our previous report. Catechins were determined by high-performance liquid chromatography (HPLC) according to the modified
methods of Suematsu et al.\textsuperscript{14}) and Terada et al.\textsuperscript{6})

HPLC analysis of catechins was performed with an ODS column (GL Science, Inertsil ODS-2, 5 μm, 250 x 4.6 mm ID) fitted with a guard column (10 x 4.0 mm ID), and UV detection at 280 nm with column oven at 40 °C. The mobile phase was consisted of CH3CN/CH3COOC2H5/0.1 % phosphoric acid (85/20/895) with a flow rate of 1 ml/min. Standard solutions for all catechins were prepared in methanol. Catechin concentrations in the seaweeds were calculated using standard curve at the concentration of 0-50 μg/mL.

**Analysis of flavonoids and related compounds**

Total flavonoids were extracted according to the method of Hertog et al.\textsuperscript{15}) as follows. Each minced fresh seaweed sample (5g) was homogenized with 40 ml of 75 % methanol with 2g /L TBHQ (t-butylhydroquinone) using a mixer (Ultra-Turrax T-25 Janke & Kunkel, GmbH Co. Staufen, Germany) at 5000-10000 rpm for 60 seconds. Ten milliliters of 6M hydrochloric acid was added and carefully mixed. The homogenate was refluxed at 90 °C for 2 hours. After cooling, the supernatant was filtered through Advantec filter paper No. 101 (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and transferred to the volumetric flask with methanol. After replacing air with nitrogen gas in order not to decompose flavonoids, the extracts were kept at -80°C until analysis.

Flavonoids were determined by HPLC analysis modified from the methods of Hertog et al.\textsuperscript{15}) and Vinson et al.\textsuperscript{5}) Flavonoids were separated by a C18 column ( Nova-Pak C18 , 4 μm, 150 x 3.9 mm ID, Waters Co., Milford, MA, USA) fitted with a guard column (20 x 3.9 mm ID), using 25 % acetonitrile in 0.25M KH2PO4 at pH 2.4 as mobile phase, flow rate at 0.9 ml/min and analyzed by a diode array detector SPD-M10Avp (Shimadzu Co., Kyoto, Japan). Methanolic standard solutions for all flavonoids were prepared. Flavonoid concentration in the seaweed was calculated using standard curve within concentration 0-200 μg/ml. Separation, identification, and linear curve of standard solution with concentration were made certain by retention time, spectra and peak area.

**Lipid oxidation model with iron**

Three percent Fish oil (from Menhandeln) emulsion with 0.3 % Tween 20 was incubated with polyphenolics at the presence of iron ion at 50 °C for 3 hours. Standard solutions of polyphenolic compounds or tea concentrate was added at the concentration 0-10 mg/ml. Lipid oxidation was analyzed by POV.\textsuperscript{16}) The chelating effect of Fe$^{2+}$ was analyzed following the method by Dinis et al.,\textsuperscript{17}) using Ferrozine solution and measuring chelate by spectrophotometer at 510 nm. Ferrous ion in the oil emulsion was analyzed following the method by Fukazawa et al.\textsuperscript{18}) using 2,2-dipyridyl in methanol/buthanol solution then measuring Fe$^{2+}$ by a spectrophotometer at 520 nm.

**Lipid oxidation model with copper**

Emulsions were prepared with fish oil (from Menhandeln, 3%) in 50mM phosphate buffer at pH 5 and 7.4 containing Tween 20 (0.3%). Cupric acetate was added at 500 μM to oxidate lipid. Tea powder was added to the emulsion at a final concentration of 600μg/ml, approximate final concentration of catechins was 150-200 mM. Oxidation was performed at 37 °C for 2 h. Instead of tea powder, methanol extract of Hizikia fusiformis was also tested as a polyphenolic source. The seaweed extract was evaporated, dried, and then dissolved into the oil emulsion. After homogenized the emulsion, cupric acetate was added to start the oxidation of the oil emulsion. After the reaction, POV was analyzed as an indicator of oxidation. Remained soluble copper was analyzed by an atomic absorption spectrophotometer.

**RESULTS AND DISCUSSION**

**Polyphenolic and related compounds of seaweeds and teas**

There are no reports to show flavonoids in seaweeds, however in this method, both brown algae showed several peaks with spectra like flavonoids and catechins. In this report, we calculated these UV-absorbing peaks as flavonoids or catechins.

Undaria pinnatifida is popular edible brown alga cultivated in Japan; it is not containing catechins, but containing rutin, caffeic acid, catechol, quercitrin, and morin at concentrations of 457, 53.6, 1830, 202, 1020 μg/g dry weight, respectively. Hizikia fusiformis had 3770 μg/g of epigallocatechin, 750μg/g of catechol, and 1010 μg/g of morin. Seaweeds did not contain catechin, epicatechin, epicatechin gallate, epigallocatechin gallate, hesperidin, luteolin, quercetin, myricetin, apigenin, kaempferol, and baicalein.

Green tea (Macha) contained catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate at concentrations of 590, 400, 3000, 210, and 2650 μg/g dry weight, respectively. From these results, seaweeds showed different composition of polyphenolic compounds from tea. It has been reported that epigallocatechin gallate, epigallocatechin, epicatechin gallate, and epicatechin, quercetin, kaempferol, apigenin, luteolin and myricetin were most important components in...
terms of antioxidant ability among all the polyphenolic compounds. They were detected from some vegetables, coffee, tea, beer, and wine. From this result, "anticarcinogenic" flavonoids were not detected.

**Anti/pro-oxidant activity under Fe catalyst**

Changes of POV and chelating effect by tea or seaweed extract were shown in Fig. 1 and Fig. 2, respectively. Green tea (Macha) including catechin, epicatechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate did not affect POV and made stronger chelating effect with Fe$^{2+}$ than heated green tea (Hoji-cha). Heated green tea (Hoji-cha) showed pro-oxidant activity by increasing POV. Both seaweed extracts did not affect POV, but *Hizikia fusiformis* showed stronger chelating activity of Fe$^{2+}$ than Hoji-cha. From this result, there was no clear relationship between chelating effect of Fe$^{2+}$ and reduction of POV. After reaction, percentage of remaind Fe$^{2+}$ with Macha, Hoji-cha, Hijiki and Wakame were 4, 54, 48, 0, and 0 %, respectively. Taking all results of POV, chelating effect, and remained ferrous ion together, the mechanism to affect oxidation is different between teas and seaweeds.

The changes of POV and chelating effect by pure polyphenolic and related compound alone were shown in Fig. 3 and Fig. 4, respectively. Morin and Rutin showed stronger chelating effect than other polyphenolic compounds. Catechin and quercetin did not show chelating effect. Catechin increased POV, rutin and morin did not increase POV value. After reaction, percentage of remaind Fe$^{2+}$ with catechin, rutin, quercetin, gallic acid, and morin were 4, 94, 82, 70, 70, 94 %, respectively. In this condition, catechin acted as a pro-oxidant.

**Anti/pro-oxidant activity under Cu catalyst**

Changes of POV by tea or seaweed extract at pH 5 and at pH 7.4 were shown in Fig. 5 and Fig. 6, respectively. Green tea (Macha) and heated green tea (Hoji-cha) reduced POV at pH 5, however it did not affect POV at pH 7.4. In contrast, *Hizikia fusiformis* reduced POV at both pHs. After reaction, percentage of remaind soluble Cu with Macha, Hoji-cha, and Hijiki at pH 5 were 100, 95, 100, and 80 %, respectively. Remained soluble Cu with
Macha, Hoji-cha, and Hijiki at pH 7.4 were 100, 100, 100, and 78%, respectively.

From these results, we assume that antioxidant activity of Hizikia fusiformis might be depending on the chelating effect of copper.

From both anti or pro oxidant studies, we expected that polyphenolic compounds from certain food may act as an effective antioxidant but from another food may not act effectively. Since diet consists of many compounds, we should carry on the research to explain what kind of polyphenolic compounds in the diet can express benefit on our health.

Fig. 5. Changes of POV by tea or seaweed extracts at pH 5.

Fig. 6. Changes of POV by tea or seaweed extracts at pH 7.4.

REFERENCE


