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Antimicrobial browning-inhibitory effect of flavor compounds in seaweeds

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Abstract

Since ancient times, the antimicrobial properties of seaweeds have been recognized. However, antimicrobial activities of volatile compounds in seaweeds have not been explored so far. Here, essential oils from seaweeds including green, brown and red algae such as *Laminaria japonica*, *Kjellmaniella crassifolia*, *Gracilaria verrucosa* and *Ulva pertusa* were prepared by using SDE (simultaneous distillation and extraction) apparatus. Volatile compounds in the essential oils were identified as aldehydes, ketones, carboxylic acids, alcohols and hydrocarbons by comparison of GC-retention times and MS data with those of authentic specimens. Flavor compounds such as (3*Z*)-hexenal, (2*E*)-hexenal and (2*E*)-nonenal in some essential oils showed strong antimicrobial activities against *Escherichia coli* TG-1, and *Erwinia carotovora*. Inhibition of browning can be achieved during either of two stages, namely, oxidation reaction by tyrosinase or subsequent non-enzymatic polymerization. Tyrosinase activity was measured by monitoring absorbance at 475 nm originating from dopachrome formed from L-DOPA. Many kinds of aliphatic carboxylic acids, aldehydes and alcohols were used as inhibitors for PPO activity. The results indicated that the α,β-unsaturated carbonyl compounds strongly inhibit tyrosinase activity. When seaweeds are damaged or macerated, the α,β-unsaturated aldehydes such as (2*E*)-hexenal and (2*E*)-nonenal are biosynthesized *via* the corresponding (3*Z*)-unsaturated aldehydes from linolenic acid and arachidonic acid. The flavor compounds that are formed could be valuable as safe antimicrobial browning-inhibitory agents of edible seaweed origin.

Introduction

Since ancient times, antimicrobial properties of herbs and spices have been used for food preservation (Zaika, 1988; Conner, 1993). Naturally occurring antimicrobial agents reported date back to more than a century (Maruzzella & Sicurella, 1960). Antimicrobials in extracts from seaweeds have been explored since the 1950s (Glombitza, 1979). A renewed interest in natural preservation appears to be stimulated by present food safety concerns, growing problems with microbial resistance, and a rise in production of minimally processed food together with "green image" policies of food industries (Suhr & Nielsen, 2003).

In recent years, cut vegetables have proven convenient, and also to reduce the amount of domestic garbage produced. These benefits are welcomed by consumers, and the demand for cut vegetables has increased. However, because the cut surface is exposed to air they are more prone to browning, which decreases their nutritional and market values (Friedman, 1996). The growing need for new and safe antimicrobial agents from edible plants, combined with recent vegetable-poisoning incidents in Japan due to *Escherichia coli* O157: H7, led us to study antimicrobial browning inhibitory effects of flavor compounds in essential oils from edible seaweeds.

Generally, polyphenol oxidase (PPO) occurs in most vegetables and fruits, together with polyphenols (Mayer, 1987). Polyphenols and PPO react in the presence of oxygen, to cause browning, when the tissues of vegetables and fruits are damaged. Tyrosinase, a known PPO, is a copper-containing enzyme that is widely distributed in plants, animals and microorganisms (Whitaker, 1995). Efficient inhibition of the browning process would be useful for improving the processing of cut vegetables. The aim of this study was to find safe and efficient antimicrobial inhibitors

of PPO from flavor compounds in edible seaweed essential oils.

Materials and methods

Materials

Antimicrobial activities of algal flavor compounds were tested against *Escherichia coli* TG-1, and *Erwinia carotovora*. Fresh seaweeds were collected along the Aio coast and Hikoshima beach in Yamaguchi, southern Japan, and along Charatsunai coast in Muroran, northern Japan. L-DOPA and DMSO (dimethyl sulfoxide) were purchased from Wako Pure Chemicals (Osaka, Japan). The mushroom tyrosinase (EC: 1.14.18.1) used for the bioassay was purchased from Sigma (St. Louis, MO, USA).

Preparation of essential oils

Cleaned fresh fronds (100 g wet wt) were homogenized in distilled water (100 mL). The homogenates were extracted by a simultaneous distillation extraction (SDE) apparatus for 15 min with pentane-CH_2Cl_2 (7:3, 20 ml) (Schultz et al., 1977). The extracts were dried over Na_2SO_4 and concentrated *in vacuo* to leave essential oils.

Identification of volatile compounds in essential oils

Volatile compounds in the essential oils were identified by comparison of Kovats indices and GC-MS data of synthetic compounds. The GC (a Hewlett Packard 5840 A) was equipped with a FID and a fused silica capillary column (Durabond column DB-1, 0.25 mm i.d. $\times$ 60 m). The column temperature was held at 50 °C for 5 min and programmed to increase at 3 °C min^{-1} from 50–240 °C. GC-MS was recorded on a Hitachi H-80A instrument equipped with a fused silica capillary column (Durabond column DB-1, 0.25 mm i.d. $\times$ 50 m). The column temperature was held at 75 °C for 5 min and programmed to increase at 3 °C min^{-1} from 75–240 °C. The ionization voltage was 20 eV.

Growth inhibitory effects of essential oils and flavor compounds

Each bacterial strain was incubated in nutrient broth No. 2 at 37 °C overnight (14 h), and test bacterial solutions were prepared with thesame broth to give a concentration of 10^6 cells mL^{-1} by using a hemacytometer. A serial 20-fold dilution of oils and flavor compounds (100, 50, 25, 12.5, 6.25 μg mL^{-1}) was prepared using 50% DMSO, which showed no effects against any bacterial strain tested. Twenty μL of each was added to 160 μl of nutrient broth No. 2 in a 96-well plate with 300 μl volume wells (Millipore, Tokyo, Japan). Finally, 20 μl aliquots of 10^6 cells mL^{-1} bacterial solution were inoculated into the wells and incubated at 37 °C for 24 h. Bacteriostatic activities of oils and flavor compounds were examined by turbidity (OD at 660 nm).

Bactericidal effects of essential oils and flavor compounds

Bactericidal effects of essential oils and flavor compounds were assessed on *E. coli* TG-1 and *Erwinia carotovora*. The test strains were harvested from cultures held overnight in nutrient broth No. 2 by centrifugation and were re-suspended in sterile 50 mM potassium phosphate buffer (pH 7.0) after being washed twice with the same buffer. Washed cell preparations were diluted to 10^6 cells mL^{-1} by using a hemacytometer. The essential oils and flavor compounds were diluted with 50% DMSO to prepare 100, 20, 10, 1, and 0.1 μg mL^{-1} solutions. Twenty μL of the diluted chemicals was mixed well with 160 μL of phosphate buffer (pH 7.0) in a sterile microtube with a volume of 1.5 mL. Next, 20 μL of the 10^6 cells mL^{-1} bacterial cell suspension was added into the tube and subsequently incubated at 37 °C for 1 h (Nakamura et al., 2002). After incubation, decimal dilutions of the sample were carried out up to $\times$ 10^4 using physiological saline adjusted to pH 7.2. One hundred μL of diluted cell suspension was spread onto a Mannitol-salt agar plate. All plates were incubated at 37 °C for 24 h, and surviving cells counted according to the colonies appearing on the plate. The percentage of survivors was presented with respect to the control mixture. The experiment was performed in triplicate.

Tyrosinase inhibitory assay by the spectrometer method

The flavor compounds (except those that are water soluble) were first dissolved in DMSO, to concentrations of 500 mM. The enzyme activity was measured by the spectrometric method by reading at A475 nm to detect dopachrome formation (Kubo et al., 1999). First, 33 μL of 1380 units/mL tyrosinase in 0.1 M K-Pi buffer