COMPARISON OF ANTIOXIDATIVE PROPERTIES OF DIFFERENT MUSHROOM EXTRACTS ON THE OXIDATION OF COD LIVER OIL IN EMULSION

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ABSTRACT

Antioxidative properties of different edible mushroom 'enokitake' *Falmmulina velutipes,* 'Shitake' *Lentinula edodes*, and 'Tamogitake' *Pleurotus cornucpiae* extracts were compared with each other and catechin in oil-in-water (o/w) emulsions of purified cod liver oil. Lyophilized mushroom was homogenized using 70% acetone, and acetone was then evaporated. Residue was dissolved in water to obtained 'enokitake', 'Shitake' and 'Tamogitake' crude extract respectively (ECE, SCE, TCE) and further lyophilized to get dry powder. Oxygen uptake, 2-thiobarbituric acid value, hydroperoxide and hexanal formation of the oils were measured as indices of lipid oxidation. Cod liver oil in o/w emulsions with added ECE, SCE and TCE was significantly more stable against lipid oxidation than the control emulsions without the extract. ECE, SCE and TCE of 1 mg/mL emulsion demonstrated much better antioxidative properties than catechin. These observations clearly showed that ECE, SCE and TCE contains higher amount of ergothioneine, which is a hydrophilic compound could effectively prevents oxidation of fish oil in emulsion system.

Key*words: Ergothioneine, mushroom, emulsion, cod liver oil, catechin, antioxidant*

INTRODUCTION

Lipid oxidation is a major cause of quality deterioration in many natural and processed foods (McClements and Decker, 2000). It can alter the flavour and nutritional quality of foods and produce toxic compounds (Min and Boff, 2002). Polyunsaturated fatty acids (PUFA) are easily oxidized due to large contents of eicosapentanoic acid and docosahexaenoic acid, which proceed by a free radical chain mechanism (Aruoma, 1998). Lipid peroxidation, which involves a series of free radical-mediated chain reaction processes, is also associated with several types of biological damage. Using antioxidants to prevent lipid oxidation is a popular and common practice. The butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroxyquinone (TBHQ) were authorized as synthetic antioxidants for use in food (Abdalla *et al.,* 1999). The main concern about the safety of these synthetic compounds is related to their metabolism and possible absorption and accumulation in body organs and tissues. There are some serious problems concerning the toxicity of these compounds (Hayashi *et al.,* 1993; Linderschmidt *et al.,* 1986). Natural antioxidants are preferable to synthetic ones in terms of consumers' concern for safety. Although tocopherols are widely used as safe antioxidants but compare to synthetic antioxidant, they are not so strong, in addition to high manufacturing cost involved (Addis and Hassel, 1992). Antioxidants can be briefly classified as water- and lipid-soluble types based on their solubility in these media. Since water is the continuous phase of an o/w emulsion, water-soluble antioxidants can be directly and easily added into the emulsion system with 1-step preparation. In contrast, lipid-soluble antioxidants need to be dissolved in the oil phase first, which may require organic solvents for dilution

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and dispersion. In this case, it becomes necessary to remove the solvents subsequently. Hence, it is laborious and uneconomic for large-scale sample preparation, such as that for industrial process. Moreover, the problem of residual solvent may not be neglected.

Recently, focus on certain edible mushrooms, including 'maitake' *Grifola frondosa* and lion's mane *Hericium erinaceus*, for their therapeutic effects such as antitumor, inmmunomodulating (Waser and Weis, 1999) and antioxidant properties has been paid. However, no report is available on antioxidative properties of different mushroom extracts on polyunsaturated oils in oil-in-water (o/w) emulsions so far. Therefore, in the present study, the antioxidative properties of different mushroom crude extracts including catechin in emulsion system were investigated and compared.

MATERIALS AND METHODS

Materials and chemicals: Fresh 'enokitake', 'Shitake' and 'Tamogitake' were purchased from local retailers in Tokyo. 1-Myristoyl-2-(12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl)- *sn*-glycero-3-phosphocholine (NBD-labeled PC) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). HPLC grade methanol, chloroform, and 1-butanol were purchased from Kokusan Chemical Co. Ltd. (Tokyo, Japan). All other chemicals of analytical grade were obtained from Aldrich Chemical Co. (Milwaukee, WI) and Sigma- Aldrich (St. Louis, MO).

Preparation of crude extract: Mushroom extract was prepared following a procedure of Bao *et al*. (2008) with slight modifications. Briefly, 102 g of lyophilized powder of the mushroom was obtained from 1 kg of fresh fruiting body. A portion (5 g) of the mushroom lyophilized powder was homogenized in 40 ml of 70% (v/v) aqueous acetone at 10000 rpm for 4 min. The homogenate was centrifuged at 900g for 15 min at 4 °C, and the supernatant was collected. The precipitate thus obtained was homogenized again in 40 ml of 70% (v/v) aqueous acetone and centrifuged. The combined supernatant was evaporated at 40 °C in vacuo. The acetone free residue obtained was dissolved in 5 ml of distilled water to get crude extract and further lyophilized in order to get powder.

Preparations of oil-in-water emulsion: Purification of oils: Cod liver oil was purified by column chromatography on silica gel 60 (6 cm i.d x 72 cm, spherical, 40-50 μm, Kanto Chemical Co. Inc, Tokyo, Japan) to remove non polar compounds and tocopherols. The oil (35 g) was charged on the column and eluted with n-hexane, followed by 1%, 3%, 5% and 10% (v/v) diethyl ether in n-hexane.

Preparations of oil-in –water emulsions: The o/w emulsions (60 ml) were prepared by mixing purified cod liver oil (6 g) and 25 mM citric acid: 25 mM disodium hydrogenphosphate (1:5) at pH 6.6 with added 100 μM EDTA. 2 Na. 1% sucrose lauryl ester was used as an emulsifier. SPG technique was used to prepare the emulsion. Three o/w emulsions with different mushroom extracts (1 mg/ml emulsions) were prepared as test samples. For cod liver oil studies, emulsion containing catechin (500 ppm) and control without extract was prepared for comparison.

Determination of oxygen absorption: Headspace air (0.1 ml portion) in the vial was withdrawn with a gas-tight micro syringe using a 24 –G needle and immediately subjected to gas chromatography using a Shimadzu gas chromatograph GC3BT equipped with a glass column (2.5 mm i.d x 1.7 m) packed with molecular sieve 5A (80-100 mesh, Nihon Chromato Co.Ltd., Tokyo, Japan) and a thermal conductivity detector. Helium was used as a carrier gas at an inlet pressure of 1.2 kg/cm2.

Determination of Total Lipid Hydroperoxides (HPO): Hydroperoxide contents were determined by a slightly modified flow injection analysis (FIA) system equipped with a fluorescent detection instruments as described by Sohn *et al.* (2005). Briefly, a mixture of 1-butanol: methanol (2:1, v/v) was used as the mobile phase at 0.5 ml/min. A mixture of DPPP solution (5 mg DPPP and 100 mg

BHT in a 200 mL mixture of methanol) and 1- butanol (1:2, v/v) was pumped into a reaction coil (0.25 mm i.d. x 40 mm) at 0.3 mL/min. Hydroperoxide in the samples reacted with DPPP to form DPPP oxide in the coil, which was subsequently   detected with a fluorescence HPLC monitor RF 535 (Shimadzu Co., Kyoto, Japan) at excitation (Ex) and emission (Em) wavelengths of 535 and 380 nm, respectively, with a Shimadzu spectroflurometric detector RF 10AxL set in the flow line behind the front fluorescent detector.
 A standard curve was prepared by using Cumene hydroperoxide for the quantification.

Determination of Thiobarbituric acid reactive substances (TBARS): Thiobarbituric acid-reactive substances (TBARS; McDonald and Hultin, 1987) were determined by mixing 0.1 ml of emulsion with 0.9 ml water and 2.0 ml of TBA reagent (15% w/v tricholoracetic acid and 0.375% w/v thiobarbituric acid in 0.25 M HCl) in test tubes followed by heating in a boiling water bath for 15 min. The tubes were cooled to room temperature for 10 min and then centrifuged at 3000 rpm for 20 min. Absorbance was measured at 532 nm. Concentrations of TBARS were determined from a standard curve prepared using 1,1,3,3-tetraethoxypropane.

Determination of volatiles: Conditions of gas chromatography and identification of volatile compounds: 2 ml samples were weighed into special 22 ml headspace vials (Perkin Elmer) sealed with silicone rubber teflon caps by using a crimper and equilibrated by auto headspace sampler Perkin Elmer 40XL. After equilibration, the vial was thermo- stated for 10 minutes and an aliquot of the headspace was transferred with a valve in to a sample loop heated in an oven controlled at 60^0C, pressurized with carrier gas for 50 s and injected directly into the gas chromatograph through a automated system. Volatiles were determined on a Shimadzu model 14A gas chromatograph equipped with a Supelcowax TM-10 fused silica open tubular capillary polar column (0.25 mm i.d. x *30 m*) and a flame ionization detector. Helium was used as a carrier gas with a column inlet pressure of 3 kg/cm^2. The column temperature was programmed from 40^0C (holding time 3 min) to 65^0 C at the rate of 3^0C per min and rise to 220^0C at a rate of 15^0 C/min with a final hold of 10 min. Injector and detector temperatures were 100^0 and 250^0C. Volatile compounds were identified by comparison of retention times with those of authentic reference compounds.

Statistical analysis: For all of the experiments, triplicate samples (*n*= 3) were conducted from same raw materials under similar conditions, Microsoft Excel 2003 was used to calculate means and standard deviations for all multiple measurements and to generate graphs. Analysis of variance (ANOVA) was applied to the data using R software version 2.4.1 (http://cran.R-project.org). Significant differences were determined by one-way ANOVA, and Tukey's multiple comparisons of means were used to determine the statistical difference between samples.

RESULTS

Changes in oxygen absorption Changes in oxygen absorption of cod liver oil o/w emulsion with different mushroom extracts (TCE, ECE and SCE) were compared against the control and catechin during oxidation at 60^0C (Fig. 1). Emulsions with added TCE, ECE and SCE showed lower oxygen absorption significantly (p<0.05) after 120 hours compared to the control sample without any additives. However, oxygen absorption rate with added catechin was faster than that of control. Similar results were found by Frankel *et al* 1994, who reported that hydrophilic antioxidant ascorbic acid was more effective in bulk oil than in oil-in-water emulsion system. In the o/w emulsions, the hydrophilic antioxidants are dissolved and become diluted during the water phase (Frankel *et al,* 1994). The Tamogitake crude extract showed highest residual oxygen content compare to ECE and TCE due to high content of ergothioneine as obtained by Bao *et al,* (2010) who also found that TCE has high radical scavenging activity compare to ECE and SCE. Jang *et al* (2004).
an inhibitor of •OH generation from hydrogen peroxide, which is catalyzed by iron or copper ion .
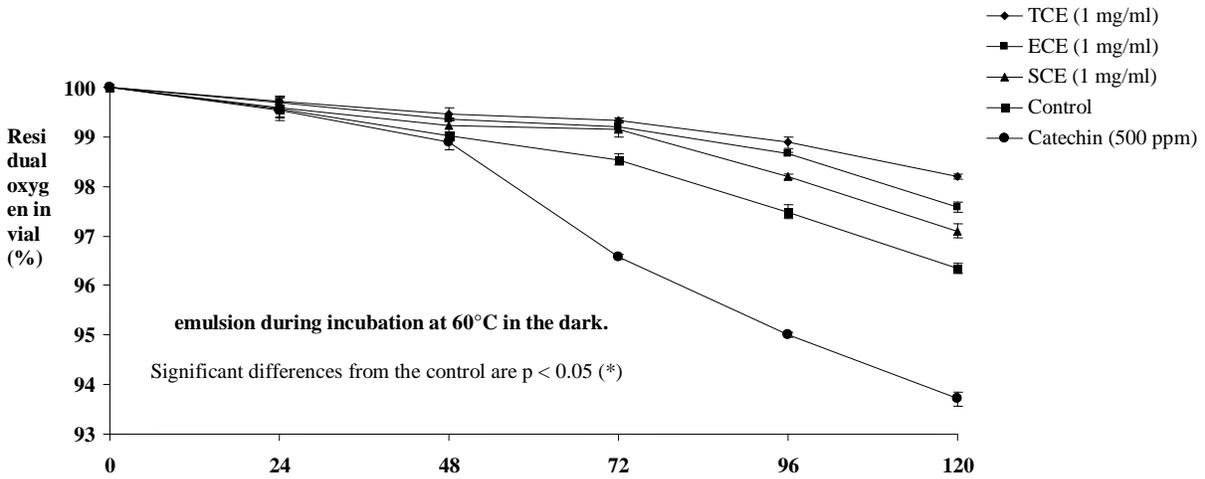
Figure 1. Effect of different mushroom crude extracts on the residual amount of oxygen absorbed of cod liver oil in o/w
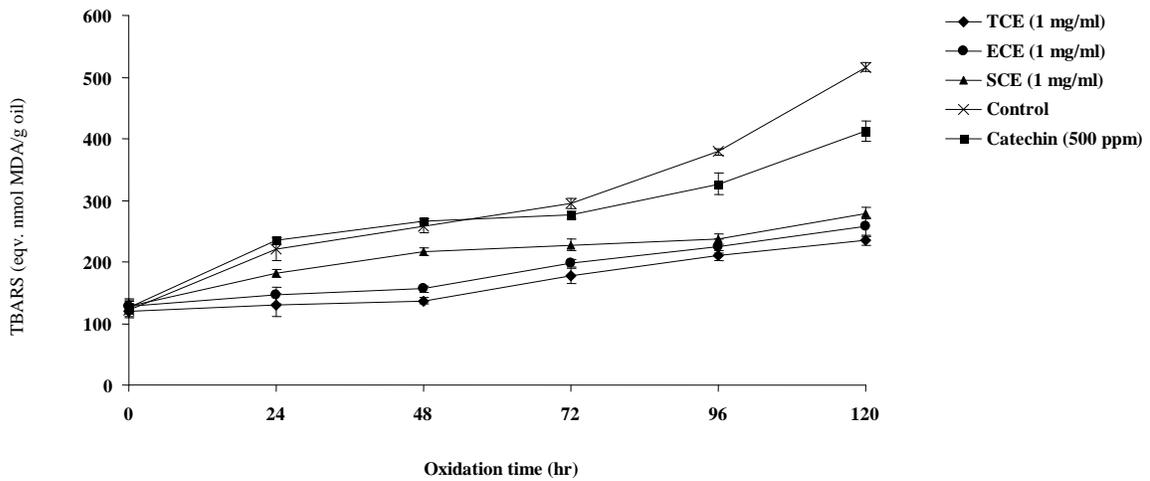
emulson during incubation at 60°C in the dark.

Significant differences from the control are $p < 0.05$ (*)

Oxidation time (hr)

Figure 2. Effect of different mushroom crude extracts on the TBARS value of cod liver oil in o/w emulsion during incubation at 60°C in the darkSignificant differences from the control are $p < 0.05$ (*)
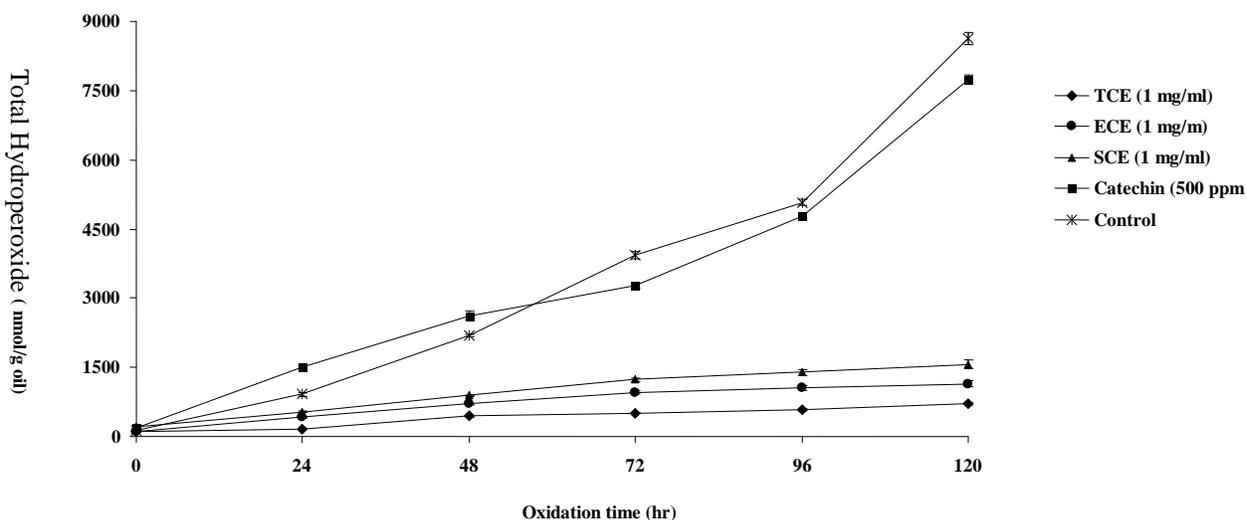
Figure 3. Effect of added different mushroom crude extracts on the hydroperoxide formation of cod liver oil in o/w emulsion during incubation at 60°C in the dark. Significant differences from the control are p < 0.05 (*)

Table 1. Effect of added mushroom extracts (1mg/ml) on volatile aldehydes formation after 120 hour during incubation at 60^0C

Major Volatile aldehydes	Retention time (min)	Concentration of aldehyes (ppm) (mean ±SD, n=3) (GC-Static Headspace Analysis)			
		Control	TCE	ECE	SCE
Hexanal	11.2	237.6[a]±17.8	84.2[b]±17.8	97.6[c]±17.4	102.6[c]±17.3
2- Heptanal	17.0	255.1[a]±23.1	168.4[b]±17.2	174.6[c]±17.1	184.6[c]±17.2
2, 6 nonadinal	24.6	86.9[a]±17.2	79.6[b]±17.3	85.6[c]±17.6	86.7[c]±17.8

Values having a different letter in the same row are significantly different (p < 0.05)

Under the experimental conditions used in this study some decomposition of hydroperoxide also occurred. The main methods used to analyze the volatile fraction of foods in recent years are: static headspace analysis and purge-and-trap dynamic headspace analysis (van Ruth *et al*, 2002a, 2002b). The results obtained from this study demonstrate that headspace GC analysis of volatiles can be employed to evaluate the oxidative stability of oils. Volatile compounds such as hexanal, 2-heptenal, 2, 6 nonadinal could be used **as** "markers" for evaluating the rate and extent of oxidation in marine oils (Boyd *et al*, 1998). These compounds vary during oxidation and have been implicated in rancid off-flavors, off-odors and fishy odor resulting from lipid oxidation (Lindsay, 1985; Nawar, 1985). In the current work, it was found that hexanal, 2-heptenal and 2,6-nonadienal were the major volatiles present in the oxidized emulsion samples (table 1). The finding generally agrees with similar studies identifying these certain aldehydes among the main products of linoleic acid hydroperoxide decomposition (Snyder, Frankel, & Selke, 1985). Interestingly, all the tested extracts (added at 1 mg/ml) inhibited markedly the formation of all the volatile aldehydes when compared with the control as indicated in table 1. TCE and ECE were generally found to be more effective than SCE, a finding which is consistent with their efficiency in inhibiting the formation of hydroperoxides and TBARS. In the literature, there is very limited data about the effect of extracts on the formation of volatile oxidation products.

DISCUSSION

Now a days mushroom was treated as one of the useful candidates of antioxidants due to its high radical scavenging activity (Liu F *et al*, 1997). Mushroom extract can be used as a potential colour stabilizer and as well as can prevent shrimp melanosis (Bao *et al*, 2008 and Encarnacion *et al,* 2010). Moreover, fruiting body of *Hypsizigus marmoreus* was found to have slight activity for trapping peroxyl and alkoxyl radicals (Matsuzawa *et al,* 1997). It was also observed that water and ethanolic extracts of oyster mushroom, which have β – glucan as a major component, served as potent antioxidants (Filipek, 1992). However no results have yet been reported on the comparison of antioxidative properties of different mushroom extracts on fish oils in o/w emulsions. Lipid oxidation affects the colour, flavour, texture and nutritive value of foods (Frankel, 1996). In recent years, the cultivation of mushrooms has become increasingly popular, as several functionalities of mushrooms have been demonstrated. It has been reported that and extract prepared from the fruiting body of *F. velutipes* has antioxidant properties (Fu *et al*, 2002), especially in polyunsaturated lipids, which are present in fish muscle (Jang *et al*., 2004). Bao *et al*, 2008 have established that ergothioneine (ESH, 2-mercaptohistidine trimethylbetaine) exists in the fresh fruiting body of *F. velutipes* at a level of 300 µg/g and the mushroom extract containing ESH can prevent lipid oxidation and stabilize the colour of beef and fish meats during low-temperature storage. In the present study, the crude extracts of Tamogitake, Enokitake and Shitake clearly showed antioxidative properties in o/w emulsion system compared to control and catechin during oxidation in terms of oxygen absorption, TBARS, hydroperoxide and volatiles formation. This observation clearly indicated that the crude extract of those mushroom contain ergothioneine, which have potential antioxidative property. So, it is suggested that the mushroom extracts, which contain more ergothioneine can be used as an alternative source of antioxidant instead of synthetic antioxidant.

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