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Comparison of antioxidant properties of persimmon vinegar and some other commercial vinegars in radical-scavenging assays and on lipid oxidation in tuna homogenates

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Abstract

Five kinds of vinegars (two kinds of persimmon vinegars, unpolished rice vinegar, polished rice vinegar, and apple vinegar) were evaluated for their scavenging activity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, superoxide radicals, and hydroxyl radicals, and for their antioxidant activity in tuna homogenate. The total phenolic content was also determined by the modified Folin-Ciocalteu method. Markedly high phenolic contents and radical-scavenging activities were found for vinegar made from persimmon Saijyo varieties, and unpolished rice vinegar. When incorporated into fatty tuna homogenates, persimmon vinegar effectively inhibited lipid oxidation in the homogenates. Persimmon vinegar may be as useful in fish processing as are other naturally occurring antioxidants, helping to prevent the formation of off-flavour in fish and their products and to increase shelf life.

Keywords: Vinegar; Persimmon; Radical-scavenging activity; Tuna homogenates

1. Introduction

Lipid oxidation in foods is a serious problem for the food industry because it results in subsequent development of undesirable off-flavours, odours, dark colours and potentially toxic reaction products (Lin & Liang, 2002; Wang, Pace, Dessai, Bovel-Benjamin, & Philips, 2002). Therefore, the control of lipid oxidation in food products is desirable, and the benefits of antioxidants in food storage have been studied by many researchers (Kikuzaki & Nakatani, 1993; Kim & Godber, 2001; Shin & Daigle, 2003). Synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate, have been commonly added to food products to retard lipid oxidation. However, the demand for natural antioxidants has recently increased because of questions and negative perception of consumers about the long-term safety of synthetic antioxidants (Yu et al., 2002).

Many antioxidative substances have been and are being isolated from natural materials, including foods. The antioxidative action and the structure of these compounds have been reported by many researchers and several antioxidants have already been developed (Bishov & Henick, 1975; Nagai, Inoue, Inoue, & Suzuki, 2003; Shahidi & Wanasundara, 1992). Fruit and cereal constituents have been reported to have antioxidative actions (Garcia-Alonso, Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2004; Madhujith, Izydorczyk, & Shahidi, 2006). Vinegar is a fermented food produced from a variety of cereals and fruits. Vinegar has been used for various foods for preservation and often used for flavouring food and for pickling. Moreover, diluted unpolished rice vinegar has been drunk as a health food in Japan and its antioxidant activity has been reported (Nishidai et al., 2000; Shimoji et al., 2002). Compounds in vinegars vary with raw materials of the vinegar production. Persimmon vinegar is made from only

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persimmon fruit, and it contains various polyphenolic compounds, such as kaki-tannin. Antioxidant activity of persimmon leaf tea was reported previously (Sakanaka, Tachibana, & Okada, 2005). Persimmon fruit has high contents of polyphenolic compounds; so many functions of persimmon vinegar can be expected (Matsuo & Ito, 1978). Two kinds of persimmon vinegars made from different persimmon fruits were compared with the other commercial vinegars.

In the present study, we evaluated the action of persimmon vinegar and some other commercial vinegars against free radicals, such as superoxide radicals, DPPH radicals, and hydroxyl radicals. In addition, we investigated effects of vinegars on lipid oxidation in tuna homogenates, as vinegar is often used in fish dishes and sushi in Japanese food.

2. Materials and methods

2.1. Samples and chemicals

Two kinds of persimmon vinegars (Kakisu), made from different persimmon astringent varieties (*Diospyros kaki* Thunb.), Saijo and Hiratanenashi, were used in this study. Rice (*Oryza sativa* subsp. *japonica*) vinegar (Komesu), unpolished rice vinegar (Kurosu) and apple (*Malus domestica*) vinegar (Ringosu) were obtained from the supermarket in Shobara City, Hiroshima Prefecture, Japan. Acetic acid contents of these vinegars were 4.3%, 4.3%, 4.5%, 4.5%, and 5.0%, and solid contents of them were 16.4%, 11.3%, 12.7%, 13.3%, and 8.0%, respectively.

Nitro-blue tetrazolium salt, xanthine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-deoxy-D-ribose, xanthine oxidase (from buttermilk, 0.049 U/ml), sodium dodecyl sulfate (SDS), trichloroacetic acid, 2-thiobarbituric acid, HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid) and 1,1,3,3-tetramethoxypropane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Folin-Ciocalteu reagent was obtained from Kanto Chemical Co. (Tokyo, Japan). All other reagents were of analytical grade.

Tuna (fatty portion of blufin tuna, *Thunnus thynnus*) were purchased from a local supermarket. Lipid content of tuna was measured by the solvent extraction method (AOAC, 2003).

2.2. Determination of total polyphenolic content

Samples were analyzed spectrophotometrically for the contents of total phenolics, using a modified Folin-Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventós, 1999; Wolfe, Wu, & Liu, 2003). A volume of 0.5 ml of distilled water and 0.125 ml of an appropriately diluted sample were added to a test tube, followed by an addition of 0.125 ml of Folin-Ciocalteu reagent. They were mixed well and then allowed to stand for 6 min before 1.25 ml of a 7% sodium carbonate solution were added. The mixture was brought to 3 ml with distilled water. The colour was developed for 90 min at room temperature, and the absorbance was measured at 760 nm using a spectrophotometer (UVIDEC-50, JASCO Corporation, Tokyo, Japan). The standard curve of gallic acid was prepared in the same manner and total polyphenol concentration was expressed as mean ± SD mg of gallic acid equivalents per millilitre of vinegar for the triplicate extracts.

2.3. Superoxide radical-scavenging activity

Superoxide radicals were generated *in vitro* by xanthine oxidase. The scavenging activity of the vinegars was determined using the nitro-blue tetrazolium (NBT) reduction method. In this method, O_2^- reduces the yellow dye (NBT^{2+}) to produce the blue formazan, which is measured spectrophotometrically at 560 nm. Antioxidants are able to inhibit the blue NBT formation (Cos et al., 1998; Parejo et al., 2002). The capacity of the samples to scavenge the superoxide radicals was assayed as follows: the reaction mixture contained 0.5 ml of 0.8 mM xanthine in 0.1 M phosphate buffer (pH 8.0), 0.5 ml of 0.48 mM NBT in 0.1 M phosphate buffer (pH 8.0) and 0.1 ml of the sample solution. After heating to 37 °C for 5 min, the reaction was initiated by adding 1.0 ml of XOD (0.049 U/ml) and carried out at 37 °C for 20 min, the reaction was stopped by adding 2.0 ml of 69 mM SDS. The absorbance of the reaction mixture was measured at 560 nm. The results were calculated as the percentage inhibition according to the following formula:

$$\% \text{ inhibition} = [\{(C - \text{CB}) - (S - \text{SB})\}/(C - \text{CB})] \times 100,$$

where S, SB, C, and CB are the absorbances of the sample, the blank sample, the control, and the blank control, respectively. SB and CB were prepared by the replacement of XOD solution by 0.1 M phosphate buffer.

2.4. DPPH radical-scavenging activity

The assay mixture contained 0.3 ml of 1.0 mM DPPH radical solution, 2.4 ml of ethanol, and 0.3 ml of the sample solution. The solution was rapidly mixed and after standing for 30 min at 25 °C, the absorbance of the mixture was measured at 517 nm (Nagai et al., 2003). The results were calculated as the percentage inhibition according to the following formula:

$$\% \text{ inhibition} = [\{(C - \text{CB}) - (S - \text{SB})\}/(C - \text{CB})] \times 100,$$

where S, SB, C, and CB are the absorbances of the sample, the blank sample, the control, and the blank control, respectively.

2.5. Hydroxyl radical-scavenging activity

Hydroxyl radical-scavenging activity was assayed using the 2-deoxyribose oxidation method (Chung, Osawa, & Kawakishi, 1997). 2-Deoxyribose is oxidized by hydroxyl

radicals formed by the Fenton reaction and degrades to malondialdehyde (Gutteridge, 1984, 1987). The reaction mixture contained 0.45 ml of 0.2 M sodium phosphate buffer (pH 7.4), 0.15 ml of 10 mM 2-deoxyribose, 0.15 ml of 10 mM $FeSO_4$–EDTA, 0.15 ml of 10 mM hydrogen peroxide, 0.525 ml of distilled water, and 0.075 ml of the sample solution in a tube. The reaction was started by the addition of hydrogen peroxide. After incubation at 37 °C for 4 h, the reaction was stopped by adding 0.75 ml of 2.8% trichloroacetic acid and 0.75 ml of 1.0% thiobarbituric acid. The mixture was boiled for 10 min, cooled in ice, and then measured at 520 nm. Hydroxyl radical-scavenging ability was evaluated as the inhibition rate of 2-deoxyribose oxidation by hydroxyl radicals. The results were calculated as the percentage inhibition according to the following formula:

$$\%\ \text{inhibition} = [\{(C - \text{CB}) - (S - \text{SB})\}/(C - \text{CB})] \times 100,$$

where S, SB, C, and CB are the absorbances of the sample, the blank sample, the control, and the blank control, respectively.

2.6. Antioxidant activity against lipid peroxidation in tuna homogenates

Tuna (containing 10.4% fat) was used in this experiment. The compositions of fatty acids (saturated fatty acids/monounsaturated fatty acids/polyunsaturated fatty acids) of tuna were 25/45/30. Fatty tuna is known to have high contents of polyunsaturated fatty acids, such as docosahexaenoic acid and eicosapentaenoic acid (Kagawa, 2004).

Tuna samples were homogenized (10% w/v) in 50 mM HEPES buffer (pH 7.0), using a homogenizer (Nihon Seiki Seisakusyo Co., Tokyo, Japan) at 8000 rpm for 5 min. Buffered systems have been widely used to study oxidation reduction reactions in food model systems (Lee & Hendricks, 1997). The mixture containing 0.8 ml of tuna homogenates and 0.2 ml of either the HEPES buffer or one of the test solutions was incubated at 37 °C for 60 min. After incubation, the mixture was tested for the formation of thiobarbituric acid-reactive substances (TBARS).

TBARS were determined by modifying the procedure as shown below (Buege & Aust, 1978; Lee & Hendricks, 1997; Macdonald & Hultin, 1987). On the day of use, a trichloroacetic acid/thiobarbituric acid (TCA/TBA) stock solution was prepared, consisting of 15% TCA (w/v) and 0.375% TBA (w/v) in 0.25 M HCl. After mild heating and agitation to dissolve the components, 3 ml of 2% butylated hydroxytoluene (BHT) in absolute ethanol were added per 100 ml of the TCA/TBA stock solution. At appropriate intervals, 1.0 ml of aliquot of the sample homogenate was added to 2 ml of the TCA/TBA stock solution in a test tube and immediately mixed, thoroughly, with a vortex mixer. The sample was then heated in a boiling water bath for 10 min and cooled to room temperature, and it was centrifuged at 1710 × g for 10 min. The absorbance of the supernatant was measured at 532 nm using a UNIDEC-50 spectrophotometer (JASCO Corporation, Tokyo, Japan). TBARS were calculated from a standard curve of malonaldehyde (MDA), a breakdown product of tetraethoxypropane (TEP).

2.7. Statistical analysis

Values represent means of triplicate analyses and are given with standard deviations. Differences among experimental data were analyzed by Tukey's studentized range test, and those at $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Total phenolics in vinegars

The total phenolic contents of five kinds of vinegars were determined (Fig. 1). Of the vinegars, the total phenolic content of the persimmon vinegar made from persimmon Saijyo varieties was highest at 799 ± 11.3 μg of gallic acid equivalents/ml, followed by 733 ± 10.2 for unpolished rice vinegar and 452 ± 14.1 for persimmon vinegar made from Hiratanenashi varieties. Total phenolic contents of persimmon vinegar and unpolished vinegar were significant higher ($p < 0.05$) than those of polished rice vinegar and apple vinegar. As apple vinegar used in this experiment was made from apple juice, the fact that total polyphenolic content of this vinegar was lower than those of other vinegars may be due to the raw material. Unpolished rice vinegar, which was made from unpolished rice containing rice bran, has phenolic compounds, such as dihydroferulic acid and dihydrosinapic acid (Shimoji et al., 2002), and hence phenolic compounds in rice bran may contribute to the content of total polyphenolic compounds in vinegar.

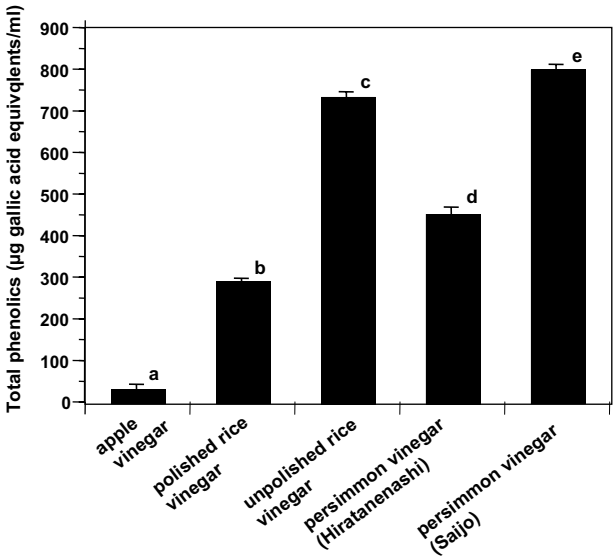

Fig. 1. Total phenolics in vinegars. Data represent the means ± SD of three determinations. Bars with different letters are significantly different ($p < 0.05$).

3.2. Superoxide radical-scavenging activity

Superoxide radicals have been observed to kill cells, inactivate enzymes and degrade DNA, cell membranes and polysaccharides (Fridovich, 1978). These radicals may also play an important role in the peroxidation of unsaturated fatty acids and possibly other susceptible substances (Nice & Robinson, 1992). Therefore, studying the scavenging effects of vinegars on superoxide radicals is one of the most important ways to clarify the mechanism of antioxidant activity.

The superoxide-scavenging activities of various vinegars were measured using the xanthine–xanthine oxidase system, and the results were indicated as the inhibition rate of superoxide activity. All of the tested vinegars exhibited superoxide-scavenging activity and the activity of persimmon vinegar made from persimmon Saijo was significant higher than those of other vinegars (Fig. 2). These results show that vinegars have superoxide radical-scavenging effects and especially persimmon vinegar and unpolished vinegar showed higher activities than did polished rice vinegar and apple vinegar.

3.3. DPPH radical-scavenging activity

DPPH· is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples (Benvenuti, Pellati, Melegari, & Bertelli, 2004; Hatano, Takagi, Ito, & Yoshida, 1997; Kang & Saltveit, 2004). Fig. 3 shows the scavenging activity of vinegars on DPPH radicals at 4-fold dilutions of vinegars. The persimmon vinegars made from persimmon Saijo showed the highest radical-scavenging activity on DPPH radicals (84.2 ± 1.2%).

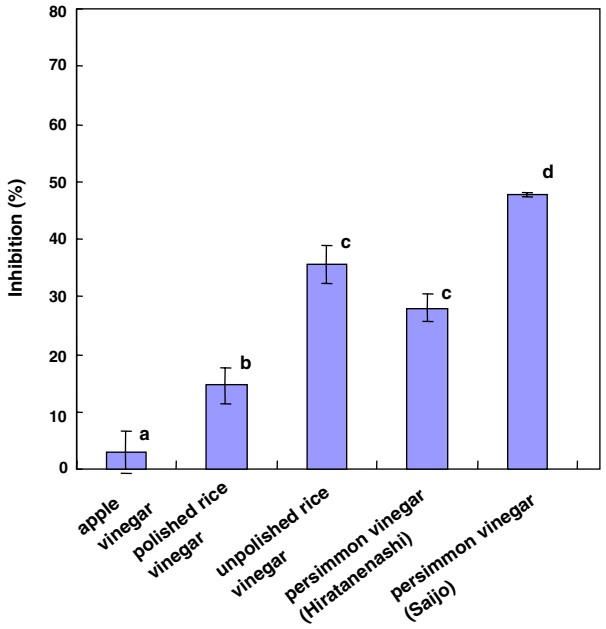

Fig. 2. Superoxide radical-scavenging activity of vinegars. Data represent the means ± SD of three determinations. Bars with different letters are significantly different ($p < 0.05$).

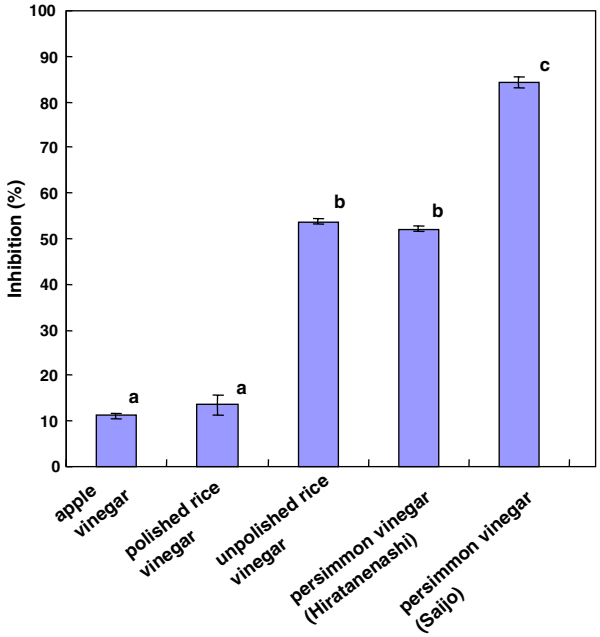

Fig. 3. DPPH radical-scavenging activity of vinegars. Data represent the means ± SD of three determinations. Bars with different letters are significantly different ($p < 0.05$).

According to Fig. 3, vinegars from persimmon and unpolished rice exhibited 52.1–84.2% scavenging activity, whereas scavenging activity of vinegars from polished rice and apple were 13.5% and 11.2%, respectively. The result shows that vinegars are also free radical-scavengers, particularly of the peroxyl radicals, which are the major propagators of the oxidation chain of fat, thereby terminating the chain reaction (Frankel, 1991; Yen, Chang, & Chen, 2002).

3.4. Hydroxyl radical-scavenging activity

The oxygen radicals induce some oxidative damage to biomolecules, e.g. carbohydrates, proteins, lipids and nucleic acids, and this damage causes aging, cancer, and several diseases (Suematsu, Kamada, Abe, Kikuchi, & Yagi, 1977). Among the oxygen radicals, the hydroxyl radical is the most reactive and severely damages adjacent biomolecules. Therefore, it is important and urgent to search for hydroxyl radical-scavengers from food materials to enable the prevention of several diseases. The scavenging effect against hydroxyl radicals was investigated by using the Fenton reaction. Fig. 4 shows the hydroxyl radical-scavenging effects of 4-fold dilutions of vinegars using the 2-deoxyribose oxidation method. The results are shown as the inhibition rate. Tested vinegars showed hydroxyl radical-scavenging activity (32.7–67.1%) and persimmon vinegar made from persimmon Saijo showed the highest scavenging activity.

3.5. Antioxidant effects in tuna homogenates

As mentioned above, tested vinegars showed radical-scavenging activities with three different *in vitro* methods.

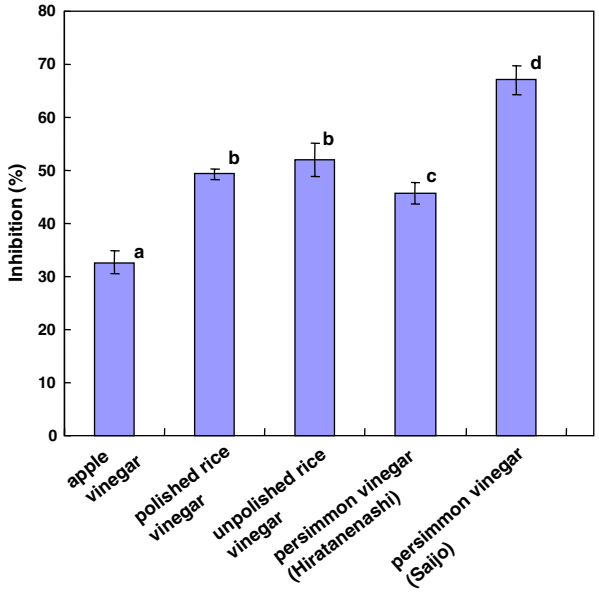

Fig. 4. Hydroxyl radical-scavenging activity of vinegars. Data represent the means ± SD of three determinations. Bars with different letters are significantly different ($p < 0.05$).

In addition, their antioxidant activities were tested using a food model system. The lipid content of the raw tuna used in this experiment was 10.4%. Persimmon vinegar was added to the tuna homogenates at different concentrations, and lipid oxidation in the homogenates was evaluated. The reaction was measured by monitoring TBARS, and the results are shown in Fig. 5. In tuna homogenized with 50 mM HEPES buffer at pH 7.0, persimmon vinegar effectively inhibited the formation of TBARS in a dose-dependent manner (Fig. 5). With 2-fold dilutions of persimmon vinegar, the inhibition rate was 98.7%.

Antioxidant effects of several kinds of vinegars produced from different raw materials were evaluated in tuna homogenates. All the vinegars inhibited the formation of TBARS in the tuna homogenates (Fig. 6). With 4-fold dilutions of vinegars, persimmon vinegar, produced from Saijo, showed the highest activity in this test and inhibition rate was 89.7%.

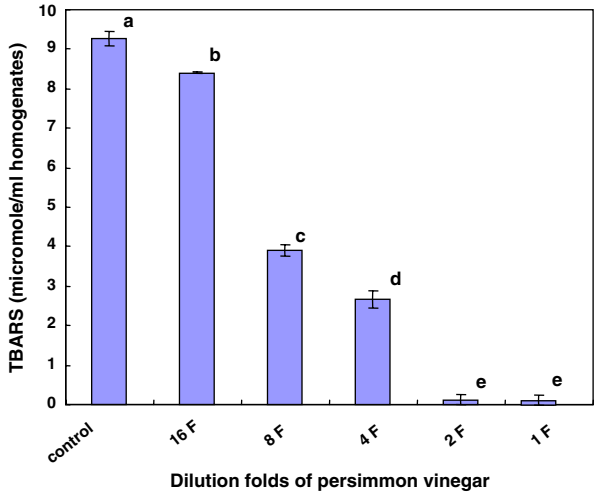

Fig. 5. Effect of persimmon vinegar on the formation of TBARS in tuna homogenates. Data represent the means ± SD of three determinations. Bars with different letters are significantly different ($p < 0.05$).

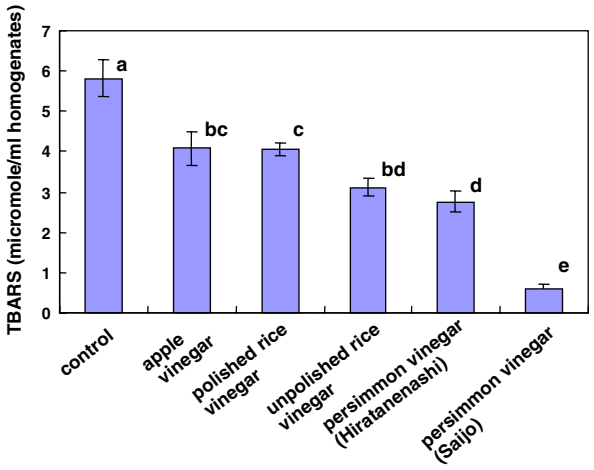

Fig. 6. Effect of vinegars on the formation of TBARS in tuna homogenates. Data represent the means ± SD of three determinations. Bars with different letters are significantly different ($p < 0.05$).

The antioxidant activity was assayed using several different test systems. Recent investigations show differences between the test systems in determining antioxidant activity (Gahler, Otto, & Böhm, 2003; Schlesier, Harwat, Böhm, & Bitsch, 2002). Use of at least two methods is recommended. In this study, we used several methods showing different sensitivities and using different systems. Vinegars tested in this experiment showed antioxidant activity in all of the different *in vitro* assay systems. The persimmon vinegar showed especially high antioxidant activity. These results suggest that persimmon vinegar is a good source of a natural antioxidant.

Persimmon fruit has a high content of polyphenolic compounds, and persimmon vinegar, produced from persimmon fruit by microorganism's fermentation, was shown to have also a high content of polyphenols. Persimmon fruit also contains a well-known antioxidant, ascorbic acid, but it was not detected in the persimmon vinegar. Further works on the characterization of antioxidant compounds in the persimmon vinegar are in progress to establish the connection between antioxidant activity and chemical composition.

4. Conclusions

All of the tested vinegars were found to be effective antioxidants in different *in vitro* assay systems. The persimmon vinegar was especially most effective on antioxidant activity in comparison with other tested vinegars. When incorporated into fatty tuna homogenates, persimmon vinegar effectively inhibited lipid oxidation in the homogenates. Persimmon vinegar may be as useful in fish processing as are other naturally occurring antioxidants, helping to

prevent the formation of off-flavour in fish and their products and to increase shelf life.

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