Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 87 (2004) 261–267
Food Chemistry
www.elsevier.com/locate/foodchem

Evaluation of quenching effects of non-water-soluble and water-soluble rosemary extracts against active oxygen species by chemiluminescent assay

M. Wada [a], H. Kido [b], K. Ohyama [a], N. Kishikawa [a], Y. Ohba [a], N. Kuroda [a], K. Nakashima [a,*]

[a] *Department of Clinical Pharmacy, Course of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan*
[b] *Mitsubishi Chemical Corporation, Specialty Chemicals Company, 1000 Kamoshida-cho, Aoba-ku, Yokohama 227-8502, Japan*

Received 23 May 2003; received in revised form 26 November 2003; accepted 26 November 2003

Abstract

The quenching effects of non-water-soluble (NWS) and water-soluble (WS) rosemary extracts against active oxygen species were investigated by a chemiluminescent assay. The EC_{50} values of the NWS extract for superoxide anion, singlet oxygen, hydroxy radical, hypochlorite ion and linolenic acid peroxide were 0.23 ± 0.02, 0.89 ± 0.06, 0.067 ± 0.005, 0.098 ± 0.009 and 0.020 ± 0.004 mg/ml ($n = 3$), respectively. The quenching effects of the NWS extract on superoxide anion, singlet oxygen, hydroxy radical and hypochlorite ion were significantly higher than those of the commercially-available hexane extracts of rosemary at 1.0 mg/ml ($p < 0.05$). The WS extract also showed higher quenching effects (except for singlet oxygen) and its EC_{50} values were 0.30 ± 0.02 for superoxide, 0.0048 ± 0.0005 for hydroxy radical, 0.58 ± 0.05 for hypochlorite ion and 0.13 ± 0.03 mg/ml for linolenic acid peroxide. The two extracts prepared might be available as antioxidants for foodstuffs.

Keywords: Rosemary; Quenching effect; Active oxygen species; Chemiluminescent assay

1. Introduction

The antioxidants have been widely used in processed foods to prevent or retard oxidation of fats or oils, because lipid oxidation can cause changes in colour, odour, and aroma, and loss of food quality. Lipid oxidation has been considered to be initiated by active oxygen species such as superoxide anion, singlet oxygen, hydroxy radical and hypochlorite ion. Thus, it is very important to study the quenching effects of antioxidants on each active oxygen species.

Synthetic antioxidants, such as *tert*-butyl-4-hydroxyanisol (BHA) and *tert*-butyl-4-hydroxytoluene (BHT), have been widely used. However, special attention has been recently focussed on natural antioxidants in food as alternatives to synthetic antioxidants (Kamil, Joen, & Shahidi, 2002; Karkonen et al., 1999; Sidduhuraju, Mohan, & Becker, 2002).

Rosemary (*Rosmarinus officinalis* L.), of the Labiatae family, shows strong antioxidative properties in ground form or as an extract. Therefore, it has been used, not only as a food flavouring but also as medicine, in foods containing animal fats, for centuries. Many researchers have reported the antioxidative activity of rosemary (Chang, Ostric-Matijasevic, Hsieh, & Huang, 1977), in which phenolic diterpenes and flavonoids have been identified as antioxidative constituents (Tada, 2000). Recently, existence of significant amounts of α-tocopherol in rosemary leaves was reported by Torre, Lorenzo, Martinez-Alcazar, and Barbas (2001). Rosemary extracts (with organic solvents) showed higher antioxidative activity in lard than the synthetic antioxidants (Inatani, Nakatani, & Fuwa, 1983). The antioxidative activities of commercially-available rosemary extracts

* Corresponding author. Tel./fax: +81-95-819-2450.
E-mail address: naka-ken@net.nagasaki-u.ac.jp (K. Nakashima).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.11.017

were evaluated and phenolic diterpenes were detected by high-performance liquid chromatography (Cuvelier, Richard, & Berset, 1996; Masuda, Inaba, & Takeda, 2001; Richheimer, Bernart, King, Kent, & Bailey, 1996). The antioxidant fractions obtained by supercritical-fluid extraction were exhaustively studied in terms of antioxidant activity and chemical composition (Ibanez et al., 1999; Senorans, Ibanez, Cavero, Tabara, & Reglero, 2000). All of these studies concluded that the most active antioxidative constituents of rosemary are lipid-soluble phenolic diterpenes including carnosic acid, carnosol, rosmanol, and epi- and iso-rosmanol. In fact, most commercially-available rosemary products are liquid or powder forms that are designed to be mixed with oils, or dispersions in oils. However, a study of the antioxidative activity in a water-soluble extract has not yet been reported.

To evaluate the antioxidant properties of rosemary extracts, various methods have been used, such as a free radical method with 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) (Ibanez et al., 1999; Senorans et al., 2000), a Rancimat method (Richheimer et al., 1996), a TBA method and a ferric thiocyanate method (Inatani et al., 1983). Although many studies of the antioxidative constituents of rosemary have been performed, few studies have reported its antioxidant activity. A chemiluminescent (CL) assay with luminol or 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (MCLA) can evaluate the quenching effect on each active oxygen species. For instance, phenolic antioxidants, such as probucol (Cynshi, Takashima, Katoh, & Tamura, 1995) and medicines, such as flavastain and its metabolites (Nakashima et al., 2001), were evaluated by a CL method.

In this report, water-soluble (WS) and non-water-soluble (NWS) rosemary extracts were prepared and studied for their quenching effects against each active oxygen species (i.e. superoxide anion, singlet oxygen, hydroxy radical, hypochlorite ion and linolenic acid peroxide) by a CL assay. The applicability of such extracts as antioxidants was evaluated by comparison with commercially available rosemary hexane extracts. Also, the contents of phenolic diterpene in the NWS and hexane extracts are described.

Furthermore, the pattern of the quenching effect of the WS extract was compared with those of rosmarinic acid and 1-methoxyluteolin-2-glucoside (MLG), which are major components possessing quenching effects.

2. Materials and methods

2.1. Materials and chemicals

Luminol, hypoxanthine, NaBr, diethylenetriaminepentaacetic acid (DETAPAC), lactoperoxidase, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (Hepes) and linolenic acid were purchased from Sigma Chemical Corporation (St Louis, MO, USA). MCLA was from Tokyo Kasei (Tokyo, Japan). Xanthine oxidase from buttermilk, H_2O_2 (30%), $FeCl_2$ and NaClO solution from Wako Pure Chemicals (Osaka, Japan) were used. Rosmarinic acid ($\geqslant$98%) was obtained from Funakoshi Co. (Tokyo). MLG was prepared as follows: the WS extract of rosemary (5%, w/v) was applied to HP 20 adsorbent (800 × 17 mm, i.d., packed volume, 160 ml, Mitsubishi Chemical Co., Tokyo) and eluted with 20% ethanol aqueous solution. The structure of MLG was identified by ^{1}H-NMR and LC-MS (Kido, 2002). The fraction obtained was evaporated to dryness. Rosmanol ($\geqslant$99%), epirosmanol ($\geqslant$99%), carnosol ($\geqslant$99%), rosmadial ($\geqslant$99%) and carnosic acid ($\geqslant$95%) were purchased from Cayman Chemical Corporation (MI, USA). Distilled water was passed through a Pure Line WL21P system (Yamato Scientific Co., Tokyo, Japan). All other chemicals were of analytical reagent grade. The hexane extracts of rosemary used were all commercially-available.

2.2. Preparation of rosemary extracts

Dried rosemary leaves and stem (1 kg) were added to 10 l of 50% aqueous ethanol solution and refluxed for 3 h. After filtration, the residue was further refluxed twice with 6 l of 50% aqueous ethanol solution. To the combined ethanol aqueous solution 5 l of water was added to form precipitates. After adding active carbon, the filtrate was dried to obtain a WS extract. On the other hand, the precipitate-active carbon mixture was refluxed three times with 4 l of ethanol for 3 h. The filtrates were mixed and dried to obtain a NWS extract.

2.3. Assay procedure of quenching effects for active oxygen species

2.3.1. Extract preparation

All extracts were dissolved in DMSO or DMF to prepare 0.02–2 mg/ml of solutions (except for 0.002–0.67 mg/ml of the WS and NWS extracts for hydroxy radical) and used for the measurement of quenching activity. The quenching activity of the rosemary extracts for active oxygen species was measured according to the previous method (Nakashima et al., 2001).

2.3.2. Superoxide anion

To 6 μl of sample in DMSO in a test tube, 600 μl of 0.8 mM hypoxanthine in 100 mM Hepes buffer (pH 7.4) and 300 μl of 1.6 mM luminol in Hepes buffer were added. After incubation at 37 °C for 10 min, 300 μl of 1.0 unit/ml of xanthine oxidase in Hepes buffer were added to the mixture and the CL intensity was immediately measured.

2.3.3. Singlet oxygen

To 6 μl of sample in DMSO, 300 μl of 0.4% H_2O_2 in 100 mM acetate buffer (pH 4.5), 300 μl of 80 mM NaBr in acetate buffer and 0.8 mM luminol in acetate buffer were added. The mixture was incubated at 37 °C for 10 min. The CL intensity of the mixture was immediately measured after adding 300 μl of 10 μg/ml of lactoperoxidase in the acetate buffer.

2.3.4. Hydroxy radical

To 6 μl of sample in DMF, 150 μl of 1.6% H_2O_2 in 100 mM Hepes buffer (pH 7.4), 150 μl of 0.8 mM DETAPAC in Hepes buffer and 0.8 mM luminol in Hepes buffer were added. After incubating at 37 °C for 10 min, 300 μl of 200 μM $FeCl_2$ in Hepes buffer were added to the mixture, followed by immediate measurement of the CL intensity.

2.3.5. Hypochlorite ion

900 μl of 0.53 mM luminol in 50 mM borate buffer (pH 9.5) were added to 6 μl of sample in DMSO. After incubation at 37 °C for 10 min, 300 μl of 40 μM NaClO in borate buffer were added to the mixture and then the CL intensity was measured.

2.3.6. Linolenic acid peroxide

5 mM of linolenic acid in *n*-BuOH were aerobically oxidized in a water bath at 37 °C for 60 min. To 6 μl of sample in DMSO, 900 μl of the oxidized solution were added and then incubated at 37 °C for 3 min. After adding 300 μl of 8 μM MCLA in *n*-BuOH, the CL intensity was measured.

The CL measurements were performed at room temperature for 1 min by a Lumatag Analyzer Auto-250 (Berthold, UK). Triplicate measurements for every extract were performed. The CL intensities for samples are indicated as percentages of relative CL intensity (RCI) against the value (RCI_0) for blank (DMSO or DMF). Quenching effect against the active oxygen species is calculated by the following equation: quenching effect $\% = 100 - (RCI/RCI_0) \times 100$. Increased value means an increased quenching effect. The sample concentration that causes 50% quenching (EC_{50}) against the active oxygen species was calculated by a concentration-quenching effect curve ($n = 3$). A linear equation, given by a semi-log plot of quenching effect against concentration, was used.

2.4. Analysis of rosemary extract components

The total concentrations of diterpenes in rosemary extracts were determined spectrophotometrically by measuring the absorbance at 280 nm. After the samples were diluted to 0.1% (w/v) with water, the absorbance was measured. Measurements were duplicated and the data expressed as means.

Analysis of each antioxidant component in rosemary extract was performed by an HPLC-UV system (Schwarz, Ternes, & Schmauderer, 1992). The separation was achieved by an Intersil ODS 80A column (250 × 4.6 mm, i.d., particle size 5 μm, GL Sciences Inc., Tokyo) with a stepwise gradient elution programme, using a mixture of (A) 4% (w/v) phosphoric acid solution and (B) CH_3CN. The elution programmes were as follows: (1) 10% B (0–30 min), 40% B (30–35 min) and 10% B (35–40 min) for WS extract; (2) 10% B (0–30 min) 80% B (30–38 min) and 10% (38–40 min) for NWS and hexane extracts. The total flow rate of eluent was set at 1.0 ml/min. The absorbance at 280 nm was measured with a UV 8020 detector (Tosoh, Tokyo). The concentrations of rosmarinic acid, MLG and diterpenes (rosmanol, epirosmanol, carnosol, rosmadial and carnosic acid) were determined in duplicate by a standard addition method.

The quenching effect is expressed as the mean ± SD ($n = 3$). Statistical analysis was performed using the Student's *t* test.

3. Results and discussion

3.1. Quenching activities of active oxygen species

3.1.1. General

The WS, NWS and commercially-available hexane extracts (extract A, B and C), MLG and rosmarinic acid were used for the experiment. The quenching effect of 1.0 mg/ml (= 50 μg/ml as final concentration) of each sample is summarized in Table 1.

3.1.2. Superoxide anion

The WS and NWS extracts showed higher quenching effects than the other extracts (Fig. 1). EC_{50} values for the WS and NWS extracts were 0.30 ± 0.02 and 0.23 ± 0.02 mg/ml, respectively. The quenching effect of the extract A at 1.0 mg/ml ($EC_{50} = 0.69 \pm 0.06$ mg/ml) was significantly less than those of the WS and NWS extracts ($p < 0.05$). The extract B and C had little quenching effect on superoxide anion.

3.1.3. Singlet oxygen

The NWS extract ($EC_{50} = 0.89 \pm 0.06$ mg/ml) showed significantly higher quenching effect than those of extracts B and C ($p < 0.05$). Slight quenching effects of extracts B and C were indicated. The WS extract and extract A had no quenching effect on singlet oxygen.

3.1.4. Hydroxy radical

All samples showed relatively high quenching effects compared to the other active oxygen species. In Fig. 2(a), the quenching effects of the WS and NWS extracts were also superior to those of the hexane

Table 1
Quenching effects of rosemary extracts against active oxygen species

Sample[a]	Quenching effect, mean ± SD%[b]				
	Superoxide anion	Singlet oxygen	Hydroxy radical	Hypochlorite ion	Linolenic acid peroxide
WS extract	81.2 ± 0.7	n.d.[c]	95.0 ± 1.2[d]	44.8 ± 3.6	73.7 ± 4.4
NWS extract	83.0 ± 1.4	48.5 ± 1.6	93.1 ± 1.7[d]	53.5 ± 5.3	79.0 ± 1.8
Extract					
A	61.9 ± 3.4	n.d.[c]	84.5 ± 4.5	31.7 ± 3.9	74.3 ± 7.9
B	19.2 ± 1.9	19.0 ± 0.7	75.8 ± 3.9	23.1 ± 2.1	57.4 ± 1.4
C	33.2 ± 1.1	21.5 ± 2.8	68.4 ± 5.2	28.5 ± 0.5	61.7 ± 2.6

[a] Sample concentration = 1.0 mg/ml (50 μg/ml final concentration).
[b] $n = 3$.
[c] n.d. means that significant difference was not detected against the value for blank.
[d] Quenching effect at 0.67 mg/ml (33.5 μg/ml final concentration).

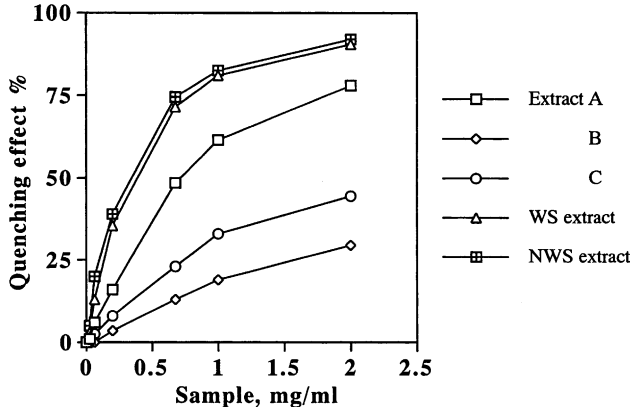

Fig. 1. Concentration-effect curve of rosemary extracts on quenching effect against superoxide anion. Experimental details are shown in Section 2.

extracts ($p < 0.05$). EC_{50} of the WS extract (0.0048 ± 0.0005 μg/ml) was ca. 100 times lower than those of the hexane extracts (sub-milligramme level), and EC_{50} for the NWS extract (0.067 ± 0.003 μg/ml) was ca. 10 times lower than those of the hexane extracts (Fig. 2(b)).

3.1.5. Hypochlorite ion

The NWS extract showed the highest quenching effect ($EC_{50} = 0.098 \pm 0.009$ mg/ml). Although the quenching effect of the WS extract at 1.0 mg/ml was less than that of the NWS extract, it was as high as that of extract A which showed the highest quenching effect among all the extracts with hexane. ClO^- is produced by a neutrophile-derived enzyme at inflammation sites when activated neutrophiles infiltrate reoxygenated tissues. One of the important targets attacked by ClO^- in vivo is α-antiproteinase (Aruoma, Halliwell, Hoey, & Butler, 1989). The scavenging of an alcoholic rosemary extract on ClO^- with elastase assay was reported by Martinez-Tome et al. (2001). The relatively high scavenging of ClO^- shown in the previous report agreed well with the result obtained in the proposed study.

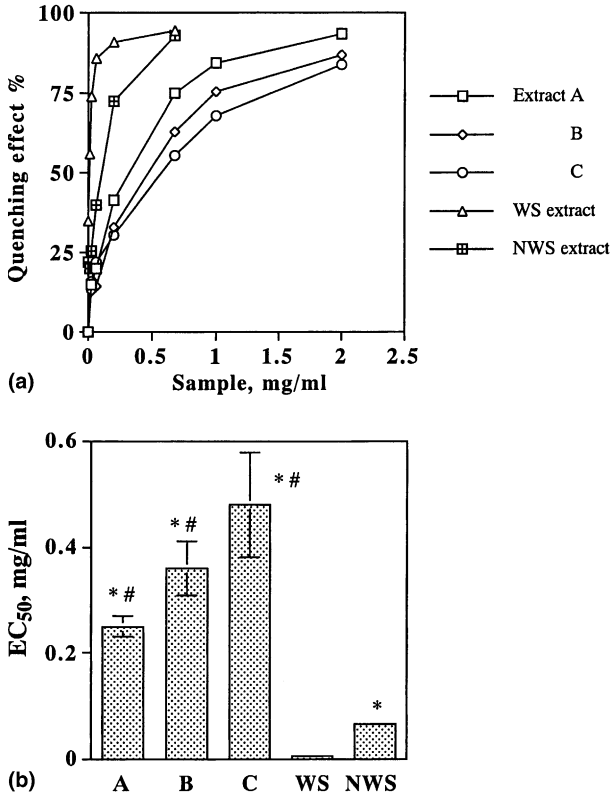

Fig. 2. Concentration-effect curve (a) and EC_{50} (b) of rosemary extracts on quenching effect against hydroxyl radical. (*) $p < 0.05$, compared with WS extract; (#) $p < 0.05$, compared with NWS extract. Experimental details are shown in Section 2.

3.1.6. Linolenic acid peroxide

In this test, the NWS extract also showed the highest quenching effect ($EC_{50} = 0.020 \pm 0.004$ mg/ml) among the extracts tested. Fig. 3 shows that EC_{50} for the WS extract ($EC_{50} = 0.13 \pm 0.03$ mg/ml) is comparable to that of extract A ($ED_{50} = 0.15 \pm 0.02$ mg/ml) on linolenic acid peroxide quenching, and the extracts B and C are moderate.

In this study, as the evaluation of quenching effect on the most of active oxygen species was performed in an

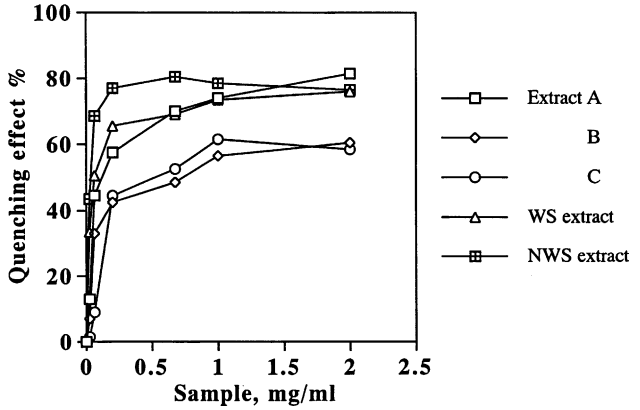

Fig. 3. Concentration-effect curve of rosemary extracts on quenching effect against linolenic acid peroxides. Experimental details are shown in Section 2.

aqueous solution, the hexane extracts might be less effective than the WS and NWS extracts. However, the quenching effects on linolenic acid peroxide by both of the WS and NWS extracts were as high as those with the hexane extracts.

3.2. Analysis of rosemary extract components

The total contents of diterpene derivatives in the NWS and hexane extracts were measured. The results (A_{280}/g sample) obtained were as follows: the NWS extract (183), extract A (92), extract B (46) and extract C (73). The NWS extract showed the highest total content compared to the other extracts. This decreasing order of the total diterpene concentration corresponds well to the decreasing order of the quenching effects on superoxide anion, hypochlorite ion and linolenic acid peroxides. Especially, good relationships between the quenching effect and the total diterpene content were observed for superoxide anion and hypochlorite ion ($r \geqslant 0.925$).

The contents of rosmarinic acid, MLG and each diterpene in the rosemary extracts are summarized in Table 2. Higher contents of rosmanol and carnosol were observed in the NWS extract and extract A than in extracts B and C. On the other hand, large amounts of rosmadial and carnosic acid were observed in extracts B and C. The NWS extract and the hexane extracts rarely contained rosmarinic acid and MLG. The diterpene contents in the NWS extract and the commercially-available extracts could be characterized into two groups: the first group, the NWS extract and the extract A, had high contents of rosmanol, epirosmanol, carnosol and rosmadial, which showed relatively high quenching effect on active oxygen species. The second group, extracts B and C, contained small amount of diterpenes except for carnosic acid. The main compound responsible for antioxidative activity was carnosic acid, which was measured by accelerated autoxidation of methyl linoleate (Cuvelier et al., 1996) and a free radical method with DPPH (Senorans et al., 2000). Carnosic acid could be converted to less effective compounds, such as carnosol and rosmanol by a bleaching or a deodorization procedure (Chen, Shi, & Ho, 1992). These results are different from ours; although the extract contains relatively high amounts of carnosic acid, it showed low quenching effect. This might be caused by a difference in the measuring methods for antioxidative activity. In our study, the quenching effects on most active oxygen species were examined in an aqueous solution, whereas hydrophobic conditions had been employed in the previous reports. The results obtained suggest that the quenching effect in an aqueous solution depends on the total concentration of diterpene derivatives and that the constituents participating in the quenching of active oxygen species might be rosmanol, epirosmanol, carnosol and rosmadial. The fraction extracted with hexane showed higher antioxidative activity than those with dichloromethane or ethanol in lard (Inatani et al., 1983). Thus the rosemary extract with hexane was selected as the reference.

For preparation of the extract A, hexane-alcohol mixture was used as an extractant. On the other hand, antioxidative activity varies, depending on parameters such as quality of original plant, its geographic origin, harvesting date, its storage, and extraction conditions (Chen et al., 1992; Cuvelier et al., 1996; Ibanez et al., 1999). Thus the large differences among the hexane extracts observed might be due to the differences of extractants.

The quenching profile of WS extract against active oxygen species, compared with those of hexane extract,

Table 2
Concentrations of quenching components in rosemary extracts

Sample	Quenching composition, mmol/g extract						
	Rosmanol	Epirosmanol	Carnosol	Rosmadial	Carnosic acid	Rosmarinic acid	MLG
WS extract	n.d.	n.d.	n.d.	n.d.	n.d.	4.17	2.18
NWS extract	0.80	0.36	4.56	0.57	0.74	n.d.	n.d.
Extract							
A	0.47	0.22	1.87	0.28	0.18	n.d.	n.d.
B	1.15×10^{-3}	n.d.	0.18	0.15	0.80	3.60×10^{-2}	n.d.
C	0.58×10^{-3}	0.87×10^{-3}	0.77	0.08	0.88	n.d.	n.d.

n.d. = not detected.

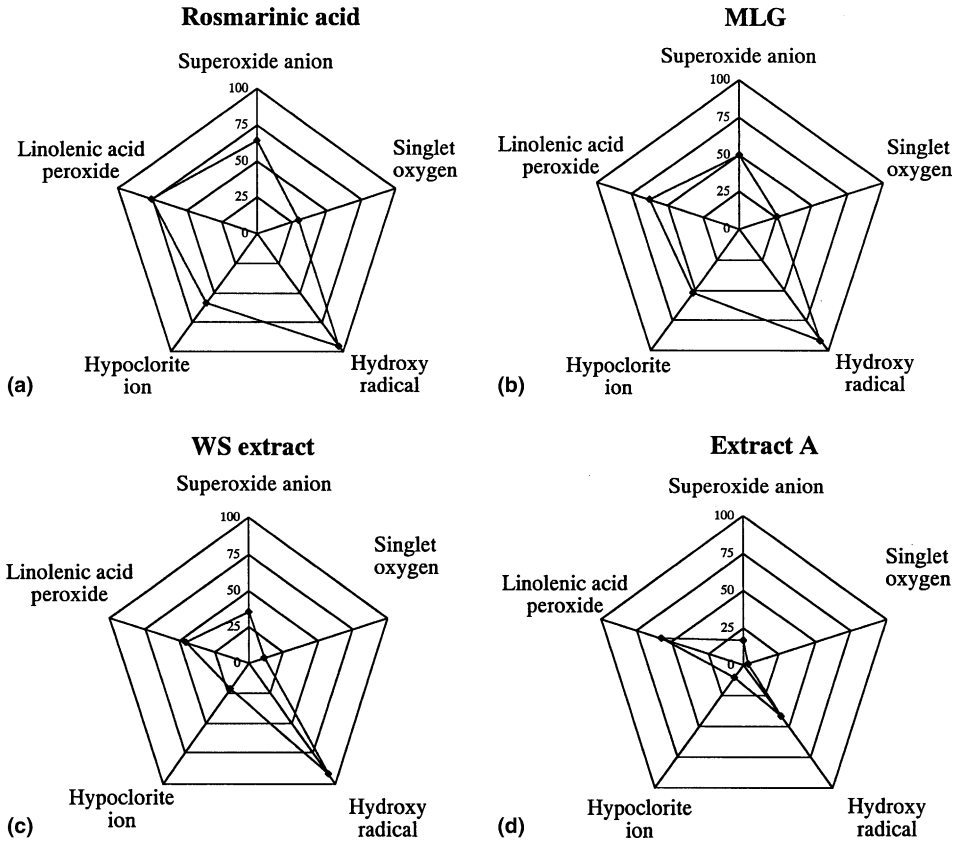

Fig. 4. Quenching profile of rosmarinic acid (a), MLG (b) WS extract (c), and extracts with hexane (d) on active oxygen species. Sample concentration, 0.2 mg/ml (10 μg/ml in final concentration).

rosmarinic acid and MLG is shown in Fig. 4. Rosmarinic acid and MLG had high quenching effects. It has been reported that rosmarinic acid has a higher antioxidative effect than the synthetic acid phenols and γ-tocopherol (Cuvelier, Richard, & Berset, 1992). The WS extract profile (c) was more comparable to rosmarinic acid and MLG profiles (a and b) than was extract A (d). This result indicates that the major antioxidative substrate in the WS extract is different from that of the hexane extracts. The contents of rosmarinic acid and MLG in the WS extract by HPLC analysis were 4.17 and 2.18 mmol/g extract (Table 2), respectively, while, in the hydrophobic extracts, both rosmarinic acid and MLG could not be detected. These results support the above results. Quenching effect of the WS extract against singlet oxygen was not observed; on the other hand, the major constituents, rosmarinic acid and MLG showed high activity (see Fig. 4). Although the reason for this phenomenon could not be elucidated, an inhibition by cofactors in the extracts might be one of the possible explanations.

4. Conclusions

An exhaustive study of the quenching effect of rosemary extracts against active oxygen species could be performed by the CL assay. The NWS extract had stronger effects on most of the active oxygen species examined than did commercially-available hexane extracts. Except for singlet oxygen, relatively strong quenching effects of the WS extracts were also observed. Both of the extracts prepared in this study might be useful as antioxidants for foods or drinks.

References

Aruoma, O. I., Halliwell, B., Hoey, B., & Butler, B. (1989). The antioxidant action of *N*-acetylcysteine: Its reaction with hydrogen peroxide, hydroxyl radicals, superoxide, and hypochlorous acid. *Free Radicals Biology and Medicine, 6*, 593–597.

Chang, S., Ostric-Matijasevic, B., Hsieh, O., & Huang, C.-L. (1977). Natural antioxidants from rosemary and sage. *Journal of Food Science, 42*, 1102–1106.

Chen, Q., Shi, H., & Ho, C. T. (1992). Effects of rosemary extracts and major constitutes on lipid oxidation and soybean lipoxygenase activity. *Journal of American Oil Chemists Society, 69*, 999–1002.

Cuvelier, M. E., Richard, H., & Berset, C. (1992). Comparison of the antioxidative activity of some acid-phenols: Structure-activity relationship. *Bioscience Biotechnology and Biochemistry, 56*, 324–325.

Cuvelier, M. E., Richard, H., & Berset, C. (1996). Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *Journal of American Oil Chemists Society, 73*, 645–652.

Cynshi, O., Takashima, Y., Katoh, Y., & Tamura, K. (1995). Action of phenolic antioxidants on various active oxygen species. *Journal of Bioluminescence and Chemiluminescence, 10*, 261–269.

Ibanez, E., Oca, A., Murga, G., Lopez-Sebastian, S., Tabera, J., & Reglero, G. (1999). Supercritical fluid extraction and fractionation of different preprocessed rosemary plant. *Journal of Agricultural Food Chemistry, 47*, 1400–1404.

Inatani, R., Nakatani, N., & Fuwa, H. (1983). Antioxidant effect of the constituents of rosemary (*Rosmarinus officianalis* L.) and their derivatives. *Agricultural and Biological Chemistry, 47*, 521–528.

Kamil, J. Y. V. A., Joen, Y. J., & Shahidi, F. (2002). Antioxidative activity of chitosans of different viscosity in cooked comminuted flesh of herring (*Clupea harengus*). *Food Chemistry, 79*, 69–77.

Karkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural Food Chemistry, 47*, 3954–3962.

Kido, H., JP patent P2002-363557A.

Martinez-Tome, M., Jimenez, A. M., Ruggieri, S., Frega, N., Strabbioli, R., & Murcia, M. A. (2001). Antioxidant properties of Mediterranean spices compared with common food additives. *Journal of Food Protection, 64*, 1412–1419.

Masuda, T., Inaba, Y., & Takeda, Y. (2001). Antioxidant mechanism of carnosic acid: Structural identification of two oxidation products. *Journal of Agricultural Food Chemistry, 49*, 5560–5565.

Nakashima, A., Ohtawa, M., Iwasaki, K., Wada, M., Kuroda, N., & Nakashima, K. (2001). Inhibitory effects of flavastain and its metabolite on the formation of several reactive oxygen species. *Life Sciences, 69*, 1381–1389.

Richheimer, S. L., Bernart, M. W., King, G. A., Kent, M. C., & Bailey, D. T. (1996). Antioxidant activity of lipid-soluble phenolic diterpenes from rosemary. *Journal of American Oil Chemistry, 73*, 507–514.

Schwarz, K., Ternes, W., & Schmauderer, E. (1992). Antioxidative constituents of Rosmarinus officinalis and Salvia officinalis. II. Isolation of carnosic acid and formation of other phenolic diterpenes. *Zeitschrift fur Lebensmittel-untersuchung Und-forschung, 195*, 99–103.

Senorans, F. J., Ibanez, E., Cavero, S., Tabara, J., & Reglero, G. (2000). Liquid chromatographic-mass spectrometric analysis of supercritical-fluid extracts of rosemary plants. *Journal of Chromatography A, 870*, 491–499.

Sidduhuraju, P., Mohan, P. S., & Becker, K. (2002). Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chemistry, 79*, 61–67.

Tada, M. (2000). Biological activities of antioxidants from herb in Libiatae. *Food and Food Ingredients Journal of Japan, 184*, 33–39.

Torre, J., Lorenzo, M. P., Martinez-Alcazar, M. P., & Barbas, C. (2001). Simple high-performance liquid chromatography method for α-tocopherol measurement in Rosmarinus officinalis leaves new data on α-tocopherol content. *Journal of Chromatography A, 919*, 305–311.