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Natural Antioxidants to Improve Stability of Refined Anchovy Oil against Oxidation

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Abstract The oxidation stability of fish oil containing omega-3 polyunsaturated fatty acids (PUFAs), which is very susceptible to oxidative deterioration, needs to be improved before it can be successfully applied to functional foods. The antioxidant activities of 17 species of materials in anchovy oil (AO) were compared and a potent antioxidant was determined to improve the shelf-life of refined AO. Antioxidant activities of the 0.05% (w/w) materials in AO were compared against control during storage at 30°C for 10 days. While no antioxidant effect was shown in alpha tocopherol against control, 3 species of grapefruit seed extracts (GSEs), astaxanthin (AX), soybean lecithin, and green tea extract showed good antioxidant activities. Especially, GSE B, GSE C, and AX showed significantly high peroxide inhibitory activities (PIAs) of 16.2±2.1, 20.±3.5, and 17.7±3.5%, respectively, after the 4th day ($p<0.01$). Radical scavenging activities (RSAs) of GSE B, GSE C, and AX were 85.1±0.8, 95.3±0.3, and 85.9±0.8%, respectively. Correlation between PIAs and RSAs was high ($R^2=0.926$) in GSE B, GSE C, and AX. Therefore, we concluded that one of the main antioxidative mechanisms of GSEs and AX must operate through an RSA pathway. The RC_{50} (concentration required for 50% reduction of 1,1-diphenyl-2-picryl-hydrazyl, DPPH) of GSE C was 258 μg/mL.

Keywords: polyunsaturated fatty acid, antioxidants, anchovy oil, grapefruit seed extract

Introduction

Omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosapentaenoic acid (DHA, 22:6 n-3), are richly found in fish oil and improve the blood stream (1-3) and aid brain nutrition (4, 5). Though fish oil has been used as an ingredient of functional health food, it is very susceptible to oxidative deterioration because of the high PUFA content. Therefore, the oxidation stability of fish oil requires improvement for successful application to functional foods.

The use of antioxidants, which could prevent fish oil from oxidization, represents a good alternative to secure the oxidative stability of fish oil. The antioxidant effects of three kinds of commercial lecithin in fish oil were investigated (6). The antioxidant effects of phospholipid and phosphatidylcholine were evaluated on the oxidation of refined fish oil (7). The antioxidant activity and synergistic effect for tocopherol of spermine, which is one of the typical polyamines in nature, were studied using fish oil (8). The activity of green tea extracts on the oxidation of menhaden oil was examined under oven condition (9). Chung *et al.* (10) investigated the activity of sixty medicinal and food plants native to Korea. The effect of tocopherols (α, γ, δ) on the formation and decomposition of hydroperoxides in purified fish oil triacylglycerols was studied (11). White grapefruit contained high quantities of antioxidative compounds and had high antioxidative potential (12, 13).

Seventeen species were selected in terms of not only antioxidant activity but also convenience and economic basis for application as an antioxidant. The antioxidant activities of these materials in anchovy oil (AO) were compared and a potent antioxidant was determined to improve the shelf-life of refined AO.

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Materials and Methods

Materials Crude AO purchased from Han Jin Co., Ltd. (Yeosu, Korea) was refined by HIT Inc. The refined AO contained about 30% omega-3 fatty acids (EPA 15.7%, DHA 14.2%). Seventeen species of materials obtained in Korea such as alpha-tocopherol acetate (Won Poong Pharm. Co. Ltd., Seoul), sesame oil and perilla oil (Chung Yang Foods Co. Ltd., Anseong), walnut oil (Dae Yang association, Chungbuk), green tea extract A (Young Won Chem. Co., Ltd., Seoul), rosemary extract, and green tea extract B (Hyang Won Co., Ltd., Chungbuk), extract from each of *Achyranthes japonica*, *Cassia tora*, *Glycine max*, pine needles, and *Solanum nigrum* (Kyung-Dong market, Seoul), soybean lecithin (Dain Natural. Co., Ltd., Seoul), astaxanthin (Marine Products Tech. Co., Ltd., Seongnam), and grapefruit seed extracts (GSEs) A, B (Food Additives Bank Co., Ltd., Anyang), and GSE C (Pharmcle Co., Ltd., Ansan) were used for antioxidation test.

Preparation of extracts Five species of medicinal and

food plants were pulverized and screened with 60 mesh. Each powder was extracted with 10 volumes of ethanol (Food grade, Korea Ethanol Supplies Co., Seoul, Korea) 3 times for 48 hr and then concentrated by evaporation as a sample extract.

Analyses of antioxidant activities The refined AO with each additive material was incubated at 30°C and the peroxide value (POV) of AO was measured by Korean Functional Health Food Code (14). Total phenol contents and radical scavenging activities (RSAs) of the additive materials were determined by the methods of Park *et al.* (15). The antioxidant activity was calculated as follows:

Antioxidant activity (%) = [1 - (POV of sample / POV of control)] × 100

Results and Discussion

Screening of potent antioxidant The antioxidant activities of the 17 species of materials mixed with refined AO were determined by POVs of AO during storage at 30°C for 10 days. As tocopherols have been used as antioxidants of refined fish oils etc, POVs of the refined AO were determined according to the 0.05, 0.5, and 1.0% (w/w) of tocopherol and the results are shown in Fig. 1. Compared with control (no tocopherol) no significant antioxidant activity was shown in any of the concentrations of tocopherol until the 7th day but a little activity was shown at 0.5 and 1.0% on the 10th day ($p<0.05$). The antioxidant activity of alpha tocopherol used in this test was the lowest among the tocopherols and the variation of results was caused by differences of structure and purity (reagent vs. food additive) of tocopherols (11, 16). Furthermore, the prooxidative activity was found to be affected by its concentration and substrate (16).

Sesame oil contains antioxidant compounds, such as sesamol, sesamin, and sesamolin etc. It is therefore well known that the oxidative stability of sesame oil is very good (17) and the antioxidant effect of the solvent fraction of perilla seed cake was also found (18). The antioxidant effects of oleoresin of rosemary were increased with increasing oleoresin concentration (19). Therefore, POVs of the refined AO mixed with sesame oil, perilla oil, and walnut oil, and POVs with rosemary extract and green tea extract were determined, as shown in Fig. 2. Compared with control, no antioxidant activity was shown in sesame oil, perilla oil, or walnut oil (Fig. 2A). Rosemary extract was also rarely effective, whereas green tea extract showed about 10.7% of peroxide inhibitory activity (PIA) against control after the 4th day (Fig. 2B, $p<0.01$). Catechin of green tea extract was determined to be a strong antioxidant compound (20) and the high antioxidant activity of 0.1% green tea extract at 60°C was confirmed (21). In terms of the variations of storage condition (temperature and time), the activities of green tea extracts were similar with the results of the present study.

Five medicinal and food plants, *A. japonica*, *C. tora*, *G. max*, pine needles, and *S. nigrum*, were selected in terms of their antioxidant activity (10) and the activities of these extracts in refined AO were investigated with the results shown in Fig. 3. During storage, the antioxidant activity was rarely found in *A. japonica*, *C. tora*, pine needles or *S. nigrum*. Furthermore, *A. japonica* promoted oxidation of

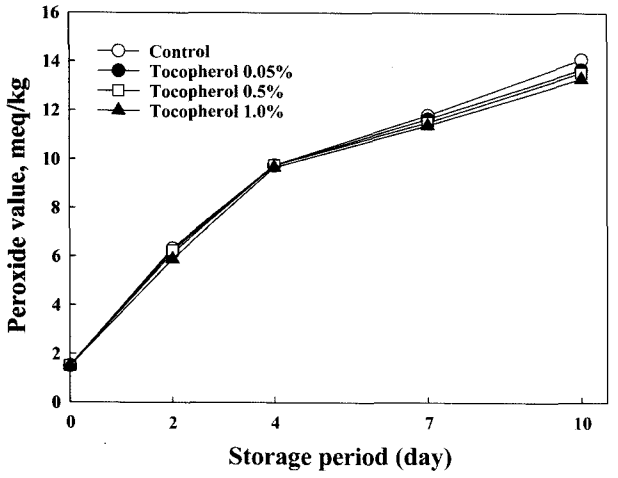

Fig. 1. Changes of peroxide values of refined anchovy oils as affected by concentration of tocopherol added during storage at 30°C.

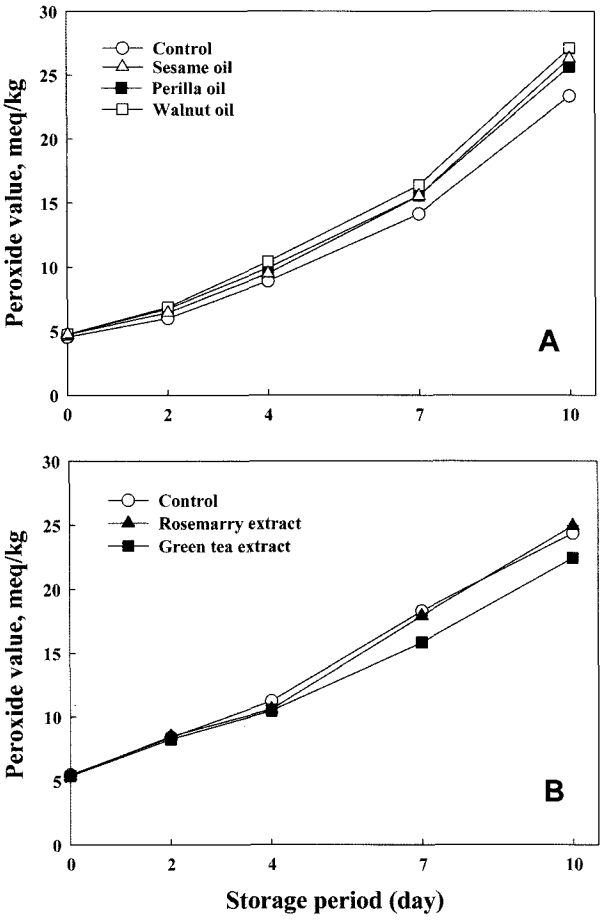

Fig. 2. Changes of peroxide values of refined anchovy oils as affected by addition of potent antioxidants during storage at 30°C.

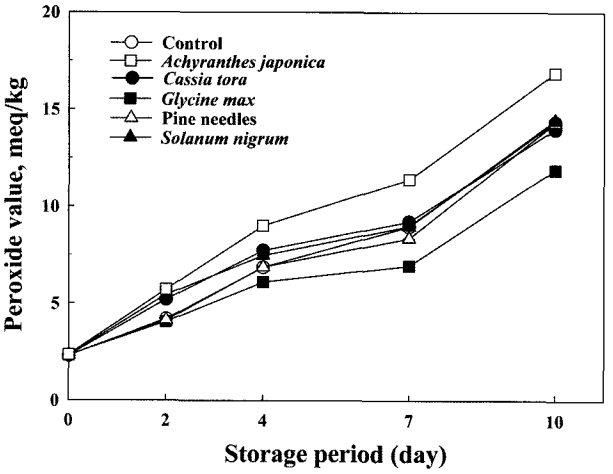

Fig. 3. Changes of peroxide values of refined anchovy oils as affected by addition of ethanol extracts from medicinal and food plants during storage at 30°C.

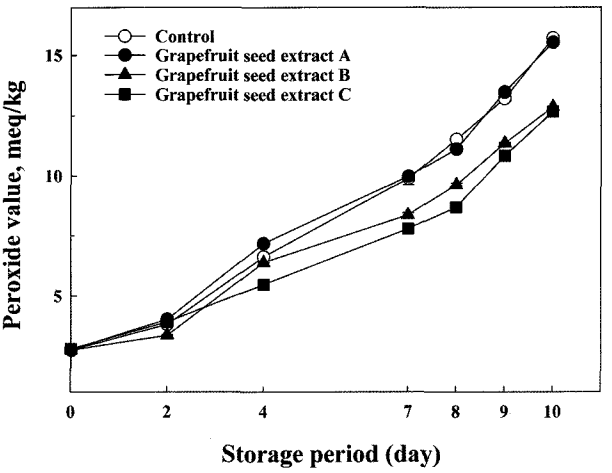

Fig. 5. Changes of peroxide values of refined anchovy oils as affected by addition of grapefruit seed extracts during storage at 30°C.

AO against control. Among the tested plants, only *G max* showed a significant antioxidant activity after the 4th day (16.9%, $p<0.01$).

Astaxanthin (AX) dose (12.5, 25.0, 50.0 μg/mL) significantly prolonged the oxidation lag time (31.5, 45.4, 65.0 min) compared with the control (19.9 min) in low-density lipoprotein-oxidation (22). Increasing lecithin concentration in egg yolk extended the oxidation induction period of the supplemented borage oil, while soybean lecithin was more effective in stabilizing the borage oil than egg lecithin (23). Therefore, POV of the refined AO mixed with each AX and soybean lecithin was determined, as shown in Fig. 4. Soybean lecithin rarely showed antioxidant effect until the 7th day, after which only a little antioxidant activity was found against control until the 10th day. On the other hand, AX showed significantly high and continuous activity throughout the storage period and the average activity after the 4th day against control was 19.2% ($p<0.01$). Ahn *et al.* (6) determined the antioxidant effects of three kinds of commercial lecithin in fish oil at 97.8°C and found that the antioxidant effects of the lecithins increased with increasing lecithin concentration. However, our result was a little different.

White grapefruit contain a high concentration of natural antioxidants that have not only a high activity, but also a good quality (12, 13). The antioxidant activities of 3 species of GSEs were determined (Fig. 5). Among the tested GSEs, no activity was found in GSE A (red GSE 50% and glycerol 50%) while significantly high activity was shown in GSEs B (white GSE 70% and glycerol 30 %) and C (GSE 51%, citric acid 27%, and malic acid 3%) ($p<0.01$). Especially, GSE C was the most efficient antioxidant with an antioxidant activity against control of about 20.7% after the 4th day. From this result, the

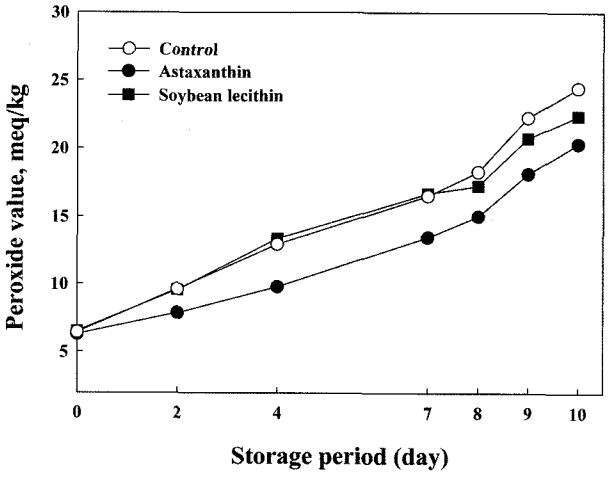

Fig. 4. Changes of peroxide values of refined anchovy oils as affected by addition of astaxanthin and soybean lecithin during storage at 30°C.

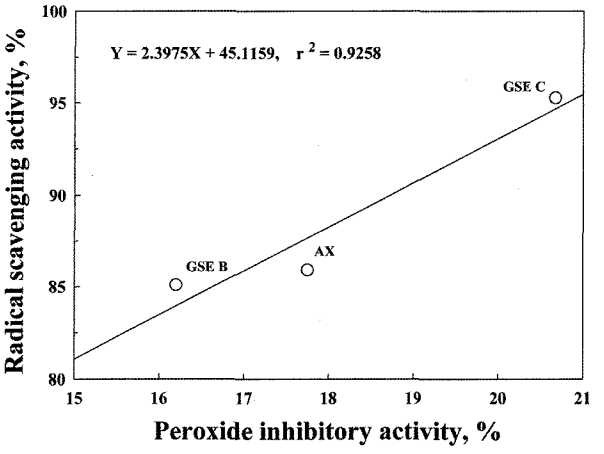

Fig. 6. Correlation between peroxide inhibitory activity and radical scavenging activity of grapefruit seed extracts (GSEs) B, C, and astaxanthin (AX). Peroxide inhibitory activity of each antioxidant is the average of storage on the 8, 9, and 10th days.

Table 1. Total phenol contents and antioxidant activities of grapefruit seed extracts (GSEs) and astaxanthin

Activities antioxidants	Total phenol contents (mg/100 mL)	Antioxidant activities		RC_{50}[3] (μg/mL)
		Peroxide inhibitory activity[1] (%)	Radical scavenging activity[2] (%)	
GSE B	36.9±0.8	16.2±2.1	85.1±0.8	494.4
GSE C	16.2±0.1	20.7±3.5	95.3±0.3	258.1
Astaxanthin	28.0±0.5	17.7±0.9	85.9±0.8	912.1

[1]The activity calculated from the reduction rate of peroxide value against control is the average of each peroxide inhibitory activity of storage on the 8, 9, and 10th days.
[2]Measurement at 1.0% (w/w) concentration.
[3]Concentration required for 50% reduction of DPPH.

antioxidant activities of the GSEs varied according to the species and composition. GSE has been shown to exert antibacterial, antifungal and antioxidant activity, possibly due to the presence of naringenin, the flavonoid (24).

Furthermore, we consider that the highest activity of GSE C is due to the synergistic effect by reducing the reaction of the organic acids. Ogawa *et al.* (8), studying the synergistic effect of spermine on the antioxidation of polyunsaturated oil, elucidated that this effect was caused by the hydrogen donation from amino-hydrogen of spermine, which regenerated tocopherol from tocopheroxyl radical and the resulting spermine radical could scavenge lipid or lipid peroxy radicals to form lipid complexes.

Antioxidant activity of GSE Figure 6 shows the correlation between PIAs and RSAs of GSEs B and C and AX. PIAs are averages of the inhibitory activities of storage on the 8, 9, and 10th days. Correlation between the PIAs and RSAs of GSEs B, C, and AX was high (R^2= 0.926). The antioxidant activity (PIA and RSA) and total phenol contents of GSEs B and C and AX are shown in Table 1, with significantly high PIAs of 16.2±2.1, 20.7± 3.5, and 17.7±0.9% (p<0.01) and RSAs of 85.1±0.8, 95.3± 0.3, 85.9±0.8%, being shown, respectively. Nevertheless, no significant relationship was found between the antioxidant activity and total phenol contents. Both high 1,1-diphenyl-2-picryl-hydrazyl (DPPH) RSA and reducing power were shown in *Callistemon citrinus* extract (25). Also, slower degradation of tocopherols and lignan compounds was thought to contribute partly to the high oxidative stability of the roasted and bleached sesame oil (26).

In conclusion, among the tested antioxidants, GSE C was the most efficient to improve the shelf-life of refined AO and we concluded that one of the main antioxidant mechanisms of GSE C must operate via RSA. The RC_{50} (concentration required for 50% reduction of DPPH) of GSE C was 258 μg/mL.

Acknowledgments

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