Autoxidation of Fish Oil Blended with Rice Bran Oil

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Abstract: Effects of rice bran oil on the oxidative and flavor stability of fish oil were investigated by the gas liquid chromatography-head space method. When fish oil blending with different ratio of rice bran oils was oxidized at room temperature in the dark, volatile compounds produced during autoxidation was measured by gas liquid chromatography. The amounts of volatile compounds were decreased with increased the ratio of blended rice bran oil as well as peroxide value. The level of propanal and acrolein which gave unpleasant flavor was also decreased with increased the ratio of blended rice bran oil. Especially, the level of propanal and acrolein and peroxide value were remarkably decreased when blending more than 75% of rice bran oil. Blending of rice bran oil improved the oxidative and flavor stabilities of fish oil.

Key words: acrolein, autoxidation, fish oil, polyunsaturated fatty acid, propanal, rice bran oil

1 Introduction

n-3 Polyunsaturated fatty acids (PUFAs) such as eicosa-pentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) contained in fish oil have important physiological functions such as antithrombotic, cholesterol depressing and anti-allergenic properties¹⁻⁴. Thus, fish oils such as sardine, tuna and mackerel oils are often used as dietary supplements and functional foods. However, n-3 PUFAs are susceptible to autoxidation and thermal oxidation. Oxidative degradation products of n-3 PUFAs, especially low molecular compounds give rise to off-flavor and reduce the quality of fish oil. This instability of n-3 PUFAs makes application of fish oil to dietary supplements and functional foods difficult. Therefore, stabilization of fish oil is necessary for them.

In general, antioxidants such as tocopherols are added to fish oils rich in n-3 PUFAs, to improve the oxidative stability. However, addition of antioxidants is not always satisfied for retarding the production of off-flavor from fish oils during storage. Blending other fats and oils to fish oils has been attempted to maintain the quality of fish oils during storage. Shiota *et al.*⁵ tried blending butter to fish oils to improve the oxidative stability of fish oils. When Saga *et al.*⁶ assessed the oxidative stability of fish oils blended with crude plant oils such as camellia oil and oat oil by the Schaal oven weight gain test and by the rancimat method, crude oat oil increased the oxidative stability of fish oil. However, these fats and oils may affect the physiological properties and give unpleasant flavor to fish oil.

Rice bran oil (RBO) which is a vegetable oil extracted

from the germ and bran of the rice grain is eaten and favorable throughout the world. RBO has specific characteristics that natural antioxidants such as vitamin E (tocopherols and tocotrienols) and γ -oryzanol are richer than other vegetable oil⁷. Thus, RBO is very stable for the oxidation and thermal degradation. Moreover, RBO may provide masking ability to give sweet and pleasant flavor based on vanillin derived from γ -oryzanol. Blending of RBO may improve the oxidative and flavor stabilities of fish oil rich in n-3 PUFAs. In this study, fish oil blended with different ratio of RBO was autoxidized and then volatile compounds produced during autoxidation were measured by gas liquid chromatography, to find the optimal blending ratio of RBO.

2 Materials and Methods

2.1 Rice bran oil and fish oil

Refined RBO were provided by Tsuno Food Industrial Co. Ltd (Wakayama, Japan). Refined fish oil was provided by Maruha Nichiro, Ltd. (Tokyo, Japan).

2.2 Fatty acid composition and tocopherol content

Gas liquid chromatographic method (2.4.2.3-2013)⁸ and high performance liquid chromatographic method (2.4.10.2013)⁹ described in the "Standard Methods for the Analysis of Fats, Oils and Related Materials" published by The Japan Oil Chemists' Society (2013) was employed to measure the fatty acid composition and tocopherol contents of fish oil and RBO, respectively.

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2.3 Autoxidation test

Sixty grams of fish oil blended with 0, 13, 25, 30, 50, 60, 75, 90 and 100% of RBO were put in a 140 mL-glass vessel and then they were stored at room temperature in the dark. Periodically, volatile compounds and hydroperoxides produced from fish oils blended with RBO were measured during autoxidation.

2.4 Peroxide value (PV)

The titration method (2.5.2.1₂₀₁₃)¹⁰⁾ to determine peroxide value (PV) described in the "Standard Methods for the Analysis of Fats, Oils and Related Materials" published by The Japan Oil Chemists' Society (2013) was employed.

2.5 Volatile compounds¹¹⁾

Two grams of oxidized oils was put in a 20 mL-glass vial, and it was sealed with a metal cap. The vial was heated at 55°C for 10 min, and then a 1.0 mL head space gas was injected into the gas chromatograph.

HERACLES II gas chromatograph (Alpha M.O.S Japan) equipped with a FID detector was used. For the columns, 18 m × 0.18 mm i.d. DB-5 or WAX fused silica capillary column was used. The injector temperature was 220°C and detector temperature was 250°C. The column was held at 40°C and then programmed to 250°C at 3°C/s after the injection of a head space gas. Hydrogen gas was used as a carrier gas.

Volatile compounds were identified by the retention time of authentic standards and gas chromatography-mass spectrometry.

Sensory evaluation was also performed for stored fish oils blended with RBO during storage by 4 to 5 panelists. Panelists compared the intensity of off-flavor of fish oils blended with RBO after autoxidation.

Experiments were performed in triplicate. All data described the average, because the relative standard deviation was below 10%. Data were statistically analyzed by analysis of variance (ANOVA). Duncan's multiple range test was used to determine the differences among samples.

3 Results and Discussion

3.1 Fatty acid composition and vitamin E content

Table 1 shows the fatty acid composition of fish oil and RBO. Fish oils contained 6% EPA and 27% DHA as n-3 PUFAs. On the other hand, RBO contained 43% oleic acid and 36% linoleic acid as major fatty acids. Table 2 shows the vitamin E (tocopherols and tocotrienols) contents of fish oil and RBO. Fish oil contained high amounts (123 mg/100 g) of tocopherols, because tocopherols were added as food additives. RBO contained 75 mg/100 g tocopherols and 55 mg/100 g tocotrienols. Total vitamin E content in fish oil was same as that in RBO. RBO also contained 1.5% γ -oryzanol as a natural antioxidant. γ -Oryzanol consisted of 36% cycloartenyl ferulate, 45% 24-methylenecycloartenyl ferulate, 14% campesteryl ferulate and 4% β -sitosterol ferulate.

3.2 Peroxide value

Figure 1 shows the peroxide value (PV) of fish oils blended with RBO during autoxidation. PV of fish oils blended with RBO was increased during autoxidation at room temperature, although unoxidized oils had very low PV (less than 1 meq/kg). Increase rate of PV was higher with the ratio of fish oil, because fish oil had considerable amounts of PUFAs. PV of fish oil and fish oil blended with 13% RBO exceeded 30 meq/kg after 21 days, while those of fish oils blended with 75% and 90% RBO besides RBO were less than 5 meq/kg after 28 days. When fish oils were blended with more than 75% RBO, the production of hydroperoxides was remarkably retarded during autoxidation at room temperature. Fish oils blended with 75% and 90% RBO were oxidatively stable, although they contained 1.5% EPA and 6.8% DHA, and 0.6% EPA and 2.7% DHA, respectively. From these results, blending of more than 75% RBO to fish oil was effective for improving the oxidative stability of fish oil rich in n-3 PUFAs.

3.3 Volatile compounds

Volatile compounds in head space gas from fish oil blended with RBO were measured by gas liquid chromatog-

Table 1 Major fatty acids of fish oil and rice bran oil (RBO).

Fatty acid (%)	16:0	16:1	18:0	18:1	18:2	18:3	20:5	22:6
Fish oil	20	11	5	25	3	3	6	27
Rice bran oil	17	—	2	43	36	1	—	—

Table 2 Tocopherols and tocotrienols contents of fish oil and rice bran oil (RBO).

Vitamin E (mg/100g)	α -Toc	β -Toc	γ -Toc	δ -Toc	Total Toc	α -Tri	β -Tri	γ -Tri	δ -Tri	Total Tri	Total VE
Fish oil	24.1	1.6	74.8	22.3	122.9	—	—	—	—	—	122.9
Rice bran oil	47.7	1.8	19.7	5.4	74.6	25.4	1.8	27.2	0.8	55.1	129.7

VE, vitamin E; Toc, tocopherol; Tri, tocotrienol

Fig. 1 Peroxide value (PV) of fish oils blended with RBO during autoxidation at room temperature. Values with different letters are significantly different ($p < 0.05$).

raphy (GLC) during autoxidation at room temperature. Gas chromatograms of volatile compounds from fish oil and fish oil blended with 75% of RBO after autoxidation for 28 days are shown in Figs. 2 and 3, respectively. As shown in Fig. 2, many varieties of volatile compounds were detected in oxidized fish oil. Major volatile compounds included carbonyl compounds such as acetaldehyde, propanal, acrolein, butanal besides ethanol as an alcohol. These volatile compounds could be derived from n-3 PUFAs such as EPA and DHA besides α -linolenic acid (18:3) contained in vegetable oils¹². Among volatile compounds, carbonyl compounds could be responsible for off-flavor of oxidized fish oil, because they had very low threshold (ppb level).

As shown in Fig. 3, several volatile compounds were also detected in oxidized fish oil blended with 75% of RBO

after autoxidation for 28 days, although their peak intensities were low. A gas chromatogram of volatile compounds from oxidized fish oil blended with 75% RBO was quite different from that from oxidized fish oil. Peaks due to propanal and acrolein which were major volatile compounds from oxidized fish oil, as shown in Fig. 2 were very small. That is, volatile compounds derived from n-3 PUFAs such as EPA and DHA were not almost produced during autoxidation of fish oil blended with 75% RBO. Major volatile compounds included acetone, acetaldehyde, butanal and pentanal in oxidized fish oil blended with 75% RBO, although their peak intensities were very low. Probably, they were mainly produced from linoleic acid (18:2) contained in RBO during autoxidation.

Among volatile compounds, propanal and acrolein had very low threshold and irritating odor. Moreover, their levels were much higher among volatile compounds produced from n-3 PUFAs in fish oil and they gave rise to off-flavor. Therefore, the levels of propanal and acrolein were estimated during autoxidation of fish oils blended with RBO. Figures 4 and 5 show the levels of propanal and acrolein produced during autoxidation of fish oils blended with RBO at room temperature in the dark, respectively. As shown in Fig. 4, the level of propanal from fish oil was increased during autoxidation, although that from unoxidized fish oil was 4.6 ppb. The propanal level was increased to 18.6 ppm in fish oil oxidized for 28 days. On the other hand, fish oils blended with 75% and 90% RBO showed approximately 5 ppb propanal after 28 days. Propanal was not almost produced from fish oil blended with more than 75% RBO during autoxidation at room temperature.

As shown in Fig. 5, the level of acrolein from fish oil was also increased during autoxidation, although that from un-

Fig. 2 Gas chromatogram of volatile compounds from fish oil after autoxidation at room temperature for 28 days.

Fig. 3 Gas chromatogram of volatile compounds from fish oil blended with 75% RBO after autoxidation at room temperature for 28 days.

Fig. 4 Propanal produced from fish oils blended with RBO during autoxidation at room temperature. Values with different letters are significantly different ($p < 0.05$).

Fig. 5 Acrolein produced from fish oils blended with RBO during autoxidation at room temperature. Values with different letters are significantly different ($p < 0.05$).

oxidized fish oil was 4.3 ppb. The acrolein level was increased to 61.7 ppb in fish oil oxidized for 28 days. On the other hand, fish oils blended with 75% and 90% RBO showed approximately 6 ppb acrolein after 28 days. Acrolein was not almost produced from fish oil blended with more than 75% RBO during autoxidation at room temperature. The low levels of propanal and acrolein in fish oil blended with more than 75% RBO could be based on the low level of PV (hydroperoxides), because these carbonyl compounds were produced from hydroperoxides of n-3 PUFAs such as EPA and DHA.

As for the sensory evaluation, fish oil showed unpleasant off-flavor during autoxidation at room temperature, while the intensity of off-flavor from fish oil blended with 75% or

90% RBO was very weak. The intensity of off-flavor from fish oil was related with the level of volatile compounds such as propanal and acrolein. Most of off-flavor from fish oil during autoxidation could be due to propanal and acrolein, because they are known to be off-flavor compounds from vegetable oils rich in α -linolenic acid (18:3) during thermal oxidation¹¹⁾. Reduced propanal and acrolein in fish oil by blending RBO could not give off-flavor. Especially, blending with more than 75% RBO was effective for improving the flavor stability of fish oil.

Our group tested blending effects of other vegetable oils. However, remarkable stabilization effects which RBO provided were not observed for other vegetable oils from the point view of sensory evaluation. Canola oil produced more

than 10 ppb acrolein and propanal at least after autoxidation for 28 days (data not shown). One of stabilization effects of RBO is due to diluting effects of RBO. Blending of RBO to fish oil reduced the n-3 PUFA content. The n-3 PUFA content was reduced to 25% of original fish oil by blending of 75% RBO. Another one of stabilization effects of RBO may be due to high level of vitamin E. Vitamin E level of RBO was 130 mg/100 g and much higher than other vegetable oils. Especially, RBO had high level of tocotrienols (55 mg/100 g). It is well known that antioxidant activity of tocotrienols is higher than that of corresponding tocopherols¹³⁾. Therefore, tocotrienols contained in RBO could improve the oxidative and flavor stability of fish oil besides tocopherols. γ -Oryzanol as an antioxidant¹⁴⁾ also could contribute to the stabilization effects of RBO. Moreover, our group found that oleic acid located in triacylglycerol could reduce the oxidation rate of sardine oil rich in n-3 PUFAs¹⁵⁾. RBO consisted of 43% oleic acid and 36% linoleic acid as major fatty acids. Oleic acid content of fish oil blended with 75% RBO was 38% of total fatty acids. Considerable amounts of oleic acid in fish oils blended with more than 75% RBO may also contribute to their oxidative and flavor stability. From these reasons, blending of RBO may be effective for maintaining the quality of other fish oils during storage.

Even if the contents of n-3 PUFAs such as EPA and DHA in fish oil are reduced by blending with 75% RBO, residual n-3 PUFAs in blended fish oil will show physiological and nutritional effects to humans.

4 Conclusions

When fish oil was blended with RBO and stored at room temperature in the dark, the production of hydroperoxides and volatile compounds during its autoxidation were retarded. Blending of RBO improved the oxidative and flavor stabilities of fish oil. Especially, blending with more than 75% RBO was effective for maintaining the flavor quality of fish oil during storage.

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