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Effect of Natural Antioxidant Sources on Oxidation of Olive Flounder (*Paralichthys olivaceus*) and Fish Feed during Storage

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The effects of various natural antioxidant sources on oxidation of olive flounder (*Paralichthys olivaceus*) and fish diet during storage was determined. Juvenile fish were distributed among 18 flow-through tanks (40 fish per tank). Six experimental diets were prepared in triplicate: control (CT), antitox (AT), green tea extract (GE), fig extract (FE), Haeroc product (HP) and by-product of green tea (BG). The experimental diets were stored at two temperatures: room temperature (26.8°C) for 14 days and frozen (-30°C) for 16 weeks. Thirty fish were sampled from each tank at the end of the 8-week feeding trial. Whole bodies of fish were homogenized and stored in a home freezer (-9.6°C) for 24 weeks. Acid values (AVs) and peroxide values (POVs) of the diets and frozen fish during storage were monitored. AVs of the experimental diets tended to increase with the storage period except for that of the HP diet at room temperature. POVs from FE, CT, and BG diets peaked at day 7 and then decreased through the remainder of the experiment. AVs of the experimental diets and fish increased with time at -30°C and -9.6°C. Results of this study show that by-products of green tea and Haeroc product seem to have potential as antioxidants in fish feed to inhibit oxidation of both the feed and fish during storage.

Key words: Olive flounder, *Paralichthys olivaceus*, Shelf-life, Acid value, Peroxide value

Introduction

A high-lipid diet is commonly supplied to fish to lower feed cost and water pollution at fish farms (Company *et al*., 1999; McGoogan and Gatlin, 1999). However, fish fed on the high lipid diets are likely to produce high body lipid content because excess dietary lipids are stored as body fat (Vergara et al., 1996; Company et al., 1999; McGoogan and Gatlin, 1999). Additionally, fish with high lipid content is susceptible to lipid oxidation during storage.

Various synthetic antioxidants have been used to retard lipid oxidation in fish diet and flesh (Gonzalez et al., 1992; Kaitaranta, 1992). However, animals fed diets containing high concentrations of synthetic antioxidants exhibit suppressed growth performance, histopathology, and hepatomegaly (Branen, 1975; Surak et al., 1976). Additionally, some synthetic antioxidants have been banned in several countries due to their toxic properties and carcinogenic risk to humans (Tang et al., 2001). Therefore, development of new natural sources of antioxidants that can be included in fish feed without negatively affecting the growth of the fish or the final product for human consumption is highly desired.

Green tea has been shown to have antioxidant effects (Banerjee, 2006; Fukushimav et al., 2009). Crude tea containing catechins was more effective in reducing lipid oxidation in marine oils than vitamin E or BHA (Wanasundara and Shahibi, 1998). However, a few studies have applied green tea to fisheries sciences (Park et al., 1999; Cho et al., 2007; Cho and Kim, 2009). Another possible natural source for antioxidants is the fig (*Ficus carica*) (Jeong et al., 2002; Lim et al., 2005). Additionally, a commercially available product for aquaculture, which is a mixture of green tea and fig extracts and green tea powder, has been developed.

This study investigated the effects of various sources of antioxidants on oxidation of the feed and whole body of olive flounder (*Paralichthys olivaceus*) during storage.

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Materials and Methods

Rearing conditions and preparation of the experimental diets

Juvenile olive flounders were purchased from a private hatchery and acclimated to the experimental conditions for a week. Juveniles with an initial body weight of 12.9 g were distributed into 18 (180 L) flow-through tanks (40 fish per tank). Water temperature ranged from 14.8°C to 24.0°C (mean ± SD: 18.6 ± 2.89°C). Fish were hand-fed with one of the experimental diets to apparent satiation twice a day (09:00 and 17:00) for 8 weeks. Six experimental diets were prepared in triplicate: control (CT), antitox (AT) containing 14% ethoxyquin (Dongsun Industrial Inc., Gyeonggi-do, Korea), green tea extract (GE), fig extract (FE), Haeroc product (HP), and a by-product of green tea (BG) produced in the processing of green tea. Antitox was supplied by Jeilfeed Co. Ltd. (Ham-an gun, Gyeongsangnam-do, Korea), and green tea extract, fig extract, and Haeroc product were purchased from Haeroc Co. Ltd. (He-nam gun, Jeollanam-do, Korea). The by-product of green tea was supplied by Hanbattea Co. Ltd. (Hadong-gun, Gyeongsangnam-do, Korea). Other conditions of the feeding trial and feed manufacturing process were described in Cho and Kim (2009) in detail.

Storage conditions of the experimental diets and whole body of olive flounder

Experimental diets were stored at two temperatures: room temperature (mean ± SD: 26.8 ± 2.52°C) for 14 days and frozen (-30°C) for 16 weeks. Thirty fish were sampled from each tank at the end of the 8-week feeding trial; the whole bodies of the fish were homogenized and stored in a home freezer (mean ± SD: -9.6 ± 4.55°C) for 24 weeks to monitor changes in fish quality during storage. Moisture contents of the CT, AT, GE, FE, HP, and BG diets were 19.6, 20.5, 18.6, 19.3, 18.5, and 18.6%, respectively. Moisture contents of the whole bodies of fish fed the CT, AT, GE, FE, HP, and BG diets were 75.1, 75.4, 75.1, 75.2, 75.4, and 75.3%, respectively.

Analytical procedures

The acid value (AV) and peroxide value (POV) of the experimental diets and whole body of olive flounders during storage were determined according to AOAC (1990) methods.

Statistical analysis

One-way analysis of variance (ANOVA) and Duncan`s multiple range test (Duncan, 1955) were used to analyze the significance of the differences among the means of treatments using SAS version 9.1 (SAS Institute, Cary, NC, USA). Additionally, differences in the AV and POV of the experimental diets and whole body of fish were tested with analysis of covariance (ANCOVA) using with body weight as covariate.

Results and Discussion

Changes in AVs and POVs of the experimental diets stored at room temperature (26.8 ± 2.52°C) for 14 days are shown in Fig. 1. AVs of the experimental diets tended to increase with time of storage except for the HP diet. At day 14, FE diet had the highest AV (43.8 mg/g), followed by the CT (38.1 mg/g), AT (34.1 mg/g), BG (28.4 mg/g), GE (28.3 mg/g), and HP diets (19.8 mg/g). Similarly, AV of the extruded pellet tended to increase with time of storage at 5, 20, and 35°C (Jang et al., 2008).

However, POV of the FE, CT, and BG diets peaked at day 7 and decreased for the remainder of the experiment. POV of the extruded pellet, regardless of moisture content, increased with the time of storage at 5°C, but peaked at days 6 and 8 for the extruded pellet with 25% and 15% moisture content, respectively, at 35°C (Jang et al., 2008). Choi et al.

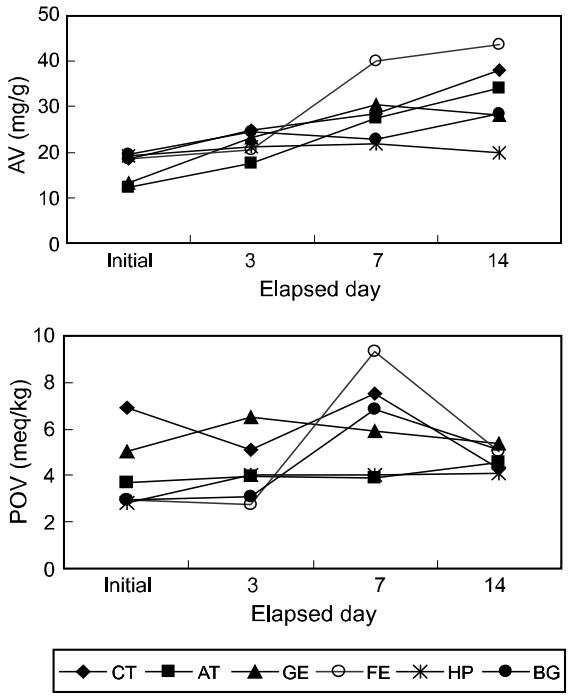

Fig. 1. Acid values (AV) and peroxide values (POV) of the experimental diets stored at room temperature (26.8°C) for 14 days.

(2004) explained that using a single parameter from peroxide, anisidine, total oxidation, AV, iodine value, and fatty acids to evaluate the quality of fish oil would be difficult.

This is the first study to report changes in the quality of the diets and whole bodies of fish fed the diets simultaneously during storage. Generally, AVs indicate the content of free fatty acid (FFA) in the diet. Determination of FFA values is often used as a general indication of the condition and edibility of oil (Low and Ng, 1987). Lipid availability of land animals was suppressed by high FFA in the diet (Wiseman and Salvador, 1991; Powles et al., 1995). However, since the experimental diets were stored frozen (-20°C) before use throughout the feeding trial, this was not a serious concern to be considered in this study.

Since peroxides, primarily hydroperoxides, are thought of as the early toxic products of autoxidation (Hung and Slinger, 1981; Oberbach et al., 1989), any effect on fish performance in a trial is usually assumed to be likely due to hydroperoxides, although many animals cope with peroxidized lipids at the intestinal wall. Koshio et al. (1994) showed that weight gain of Atlantic salmon (*Salmo salar*) fed a diet containing mildly oxidized oil (POV of 40 meq/kg of oil) was less than that of fish fed the diet containing fresh oil (POV of <1 meq/kg of oil). However, no sensory difference was found from fillets of fish fed the different diets.

Changes in AV and POV of the experimental diets stored frozen (-30°C) for 16 weeks are shown in Fig. 2. AVs of the experimental diets increased with time. At 16 weeks of storage, AVs of all experimental diets ranged between 19.3 and 22.0 mg/g (CT > FE > HP > AT > GE > BG). The POVs of the experimental diets peaked at 1 week of storage and dropped thereafter. The relatively high AVs of all experimental diets stored at room temperature (26.8°C) for 14 days compared to diets stored frozen (-30°C) for 16 weeks showed the necessity of storing the feed at low temperatures to retain quality. Additionally, relatively low AVs of the HP, BG, and GE diets compared to that of CT diet occurred at both room temperature and when frozen (-30°C), probably indicating that the by-product of green tea, Haeroc product, or green tea extract suppressed FFA formation during feed storage. Similarly, Park *et al.* (2001) reported that supplementation of 0.5% freeze-dried green tea powder suppressed AVs in soybean oil during storage at 60°C.

However, inclusion of the commercial synthetic antioxidant (AT) into the diet did not distinctively affect its AV when stored at room temperature (Fig. 1) or below freezing (Fig. 2) in this study. Bautista and Subosa (1999) reported that diets with and without 0.05% BHT stored at 10, 20, and 28-30°C had FFA values that were not significantly different from each other, but the FFA value in the diet without BHT stored at 40°C was higher than that in the other diets with BHT stored at 10, 20, 28-30, and 40°C, probably indicating that storage temperature and/or anti-oxidants affected the rate of lipolysis and the FFA values of the diets.

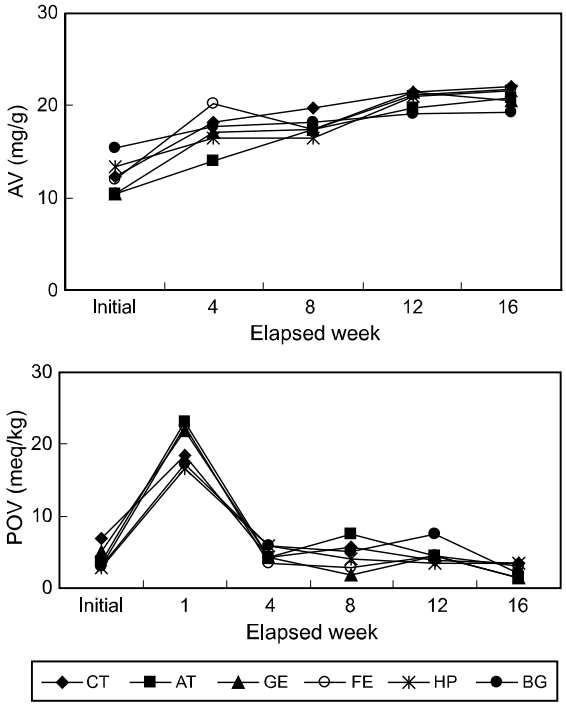

Fig. 2. Acid values (AV) and peroxide values (POV) of the experimental diets stored at frozen (-30°C) for 16 weeks.

Fish lipids are susceptible to lipid oxidation, even in frozen storage, due to high levels of highly unsaturated fatty acid, which could affect the flavor, texture, taste, aroma, and shelf life of fish (Ke and Ackman, 1976). Changes in AVs and POVs of the whole body of olive flounder fed the experimental diets stored at frozen (-9.6°C) for 24 weeks are depicted in Fig. 3. AVs of fish increased over time, but no significant (P>0.05) difference was observed among the experimental diets over time. This probably indicates that various sources of antioxidants in the experimental diets did not distinctively affect changes in FFA in fish. POVs of fish slowly increased at week 8, peaked at week 12, and then decreased. At week 12, the POVs of

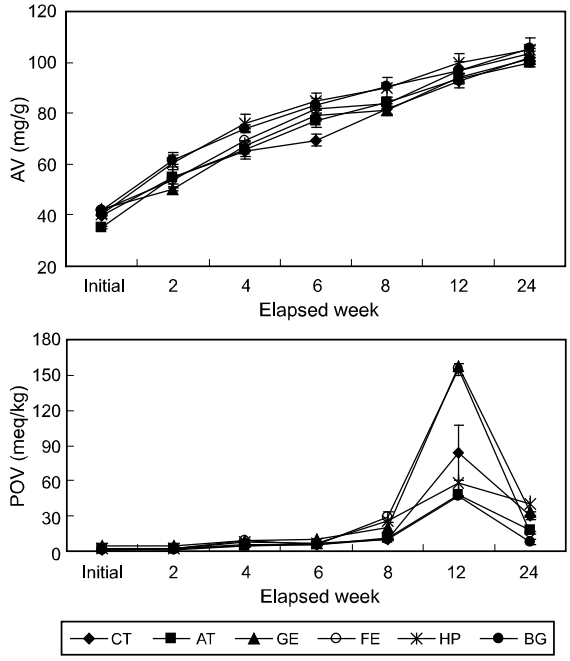

Fig. 3. Acid values (AV) and peroxide values (POV) of the whole body of olive flounder fed the experimental diets stored at frozen (-9.6°C) for 24 weeks (mean of triplicate ± SE).

fish fed the GE, FE, CT, HP, AT, and BG diets were 157.3, 155.0, 83.5, 57.6, 48.0, and 47.2 meq/kg, respectively. The increased POV at week 12 in this study was probably due to the generation of hydroperoxides resulting from an addition of oxygen at the broken double bond in the carbon skeleton of a fatty acid, and a decreased value at week 24 probably indicates that hydroperoxides were decomposed to secondary products, such as aldehydes, ketones, alcohols, acids, and esters when exposed to prolonged autoxidation (Flankel, 1998). A similar trend was observed in other fish stored for a prolonged time (Reddy et al., 1992; Simeonidou et al., 1997).

Results of this study showed that dietary inclusion of the by-product of green tea and Haeroc product seem to have potential as natural antioxidants in fish feed to reduce oxidation of both the feed and whole body of the fish during storage.

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