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Effects of combined antioxidants and packing on lipid oxidation of salted dried snakehead fish (*Channa striata*) during refrigerated storage

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Abstract

The objectives of this study were to determine the effects of combined antioxidants (Propyl gallate (PG) and sodium ascorbate) and packing on lipid oxidation in salted dried snakehead fish during storage at refrigerated temperature (4°C). The magnitude of oxidative changes was monitored by peroxide values (PV), thiobarbituric acid reactive substances (TBARS) values, moisture contents, water activity (A_w), color values (L^*, a^*, b^*) and fatty acid composition. The results showed that the moisture content and Aw remained unchanged in all samples during storage. Changes in a^* values of salted dried snakehead fish significantly decreased ($P < 0.05$) in all samples. However, salted dried fish using a combined 100 ppm PG and 100 ppm sodium ascorbate had significantly higher ($P < 0.05$) a^* values compared to the other samples. The PV and TBARS values of the treatment without PG and sodium ascorbate increased, while they remained unchanged when using of 100 ppm PG and 100 ppm and sodium ascorbate and packed under vacuum. Total polyenoic acids in the PG and ascorbate mixed-treated decreased at a slower rate than those in the other treatments. The most effective method in controlling of lipid oxidation was vacuum-packing. A combination of PG and sodium ascorbate were effective in delaying lipid oxidation and showed a greater antioxidant activity in terms of the lower PV and TBARS values than did not with antioxidants.

Introduction

Fish is one of the most important foodstuffs for people. Fish does not only being food but also provided livelihood activities to millions of people in the country. Snakehead fish is a very popular food fish in Thailand and it is one of the most important economic freshwater fish, constituting about 15.4% of the consumption of fish in the inland provinces of Thailand (FAO, 2009). Traditionally, snakehead fish has been processed into the salted dried products in many Asian countries. In Thailand, snakehead fish are commonly salted dried.

Fish is highly perishable, and is spoiled rapidly if improperly handled. Processing of fish foods and the cooking process involved during preparation of culinary items based on fish foods, generally, might result in degradation of lipid. Salted dried, one of the techniques for preserving fish has been practiced for long period and it is an alternative to lowering the water activity of flesh fish (Horner, 1997) and thus prevention of growth of many spoilage organisms along with the formation of a more membranous surface which further inhibits the growth of microorganism (Leroi and Joffraud, 2000). Salting as a method of preserving fish has been used for centuries and in many places around the world such as Asia, Europe, and Latin America. The simplicity of the salting process, the low cost of production and the ease with which it combines with other preservation methods, such as drying or smoking, has led to its popularity and extensive use (Berhimpon *et al*., 1991). Although salt allows a prolonged storage, its contact with fish has been reported to enhance lipid oxidation of the highly unsaturated lipids directly related to the production of off flavor and odors, protein denaturation and texture changes (Ackman, 1989; Hsieh and Kinsella, 1989; Davis *et al.*, 1993; Mackie, 1993). Thus, it becomes clear that salted dried step involved in preparation of fish foods could potentially result in oxidized lipids.

Fish lipids are characterized by a high degree of unsaturation in the form of multiple double bonds in the fatty acids and are generally susceptible to molecular oxygen (Olcott, 1962). Under most circumstances, production of off-flavor compounds constitutes the primary quality deterioration observed during lipid oxidation, although the process of lipid oxidation can also lower nutritional quality and modify texture and color (Hultin, 1992).

Antioxidants are important both from the perspective of food products and for their implication

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on human health. In food, antioxidants are added to improve the quality and sensory attributes such as color, flavor and texture. In human nutrition, antioxidants play an important role in promoting health and in preventing disease (Raghavan *et al.*, 2010). One preventive measure of oxidative rancidity is the use of single or combined antioxidant treatments. Specific uses for these additives are carefully regulated and may not be applicable to certain fish (Ladikos and Lougovois, 1990). There are two mechanistically distinct classes of antioxidants which can be used to retard lipid oxidation. One group of the antioxidants controls the radical chain-breaking mechanism by inactivating alkyl peroxyl and alkyl radicals which are important in the chain-propagating step. The other group involves the prevention of the introduction on chain-initiating radicals, and this includes α-tocopherol (vitamin E), propyl gallate, BHA, BHT, TBHQ, ascorbic acid (vitmin C), a transition metal chelator, phosphate and citrate (Harris and Tall, 1994; Ladikos and Lougovois, 1990). Ascorbic acid is known to preserve red meat color and possess antioxidative properties. However, the antioxidant activity of ascorbic acid in meat and meat products on lipid oxidation have been found to depend on concentration, the presence of transition metal ions, and the presence of other antioxidants (Okayama *et al*., 1987; Srinivasan *et al.*, 1996; Sanchez-Escalante *et al*., 2001).

This paper deals with the influences of addition of combined antioxidants (Propyl gallate and ascorbic acid) and packing on lipid oxidation in salted dried snakehead fish during storage at refrigerated temperature.

Materials and Methods

Chemicals

Trichloroacetic acid (TCA), methanol, chloroform and 14% boron trifluoride in methanol (BF_3-MeOH) were purchased from Merck (Darmstadt, Germany). n-Propyl gallate, 2-thiobarituric acid (TBA) and sodium ascorbate were purchased from Sigma (St. Louis, MO, USA). Authentic lipid standard compounds were obtained from Sigma-Aldrich Group (St. Louis, MO, USA). All chemicals were of analytical grade.

Preparation of sample

Snakehead fish (*Channa stiata*), caught from Phitsanulok Province and were placed in ice with a fish/ice of 1:2 (w/w) and transported to the Department of Agro-Industry, Naresuan University within 1 h. Upon arrival, fish were washed, scaled, gutted and filleted. The size of each fish was selected to be in the range of 300 - 350 g. Fish were divided into four groups, each group was dried at 60°C for 8 h, then incubated at 4°C for 49 days. Packing material was a film bag with 230 x 290 mm. Nylon 15/LLDPE 65. Before drying, the first group was immersed in 20% NaCl (w/v) without a combined 100 ppm PG and 100 ppm sodium ascorbate in plastic containers at room temperature for 75 min and packed under air and the second group was packed under vacuum. While the other 2 groups were immersed in 20% NaCl (w/v) with a combined 100 ppm PG and 100 ppm sodium ascorbate at room temperature for 75 min and packed under air and vacuum.

Samples were taken every 7 days of the storage time for analyses. Prior to analyses, the Nylon/polyethylene bags were removed. Samples were cut and ground in a meat grinder (model DPA1, Moulinex, France) for 2 min and kept for further analysis.

Determination of moisture contents

The moisture contents of sample were determined according to the AOAC (2002). Moisture content was determined by drying 3.0 - 5.0 g of sample at 100-102°C to a constant weight.

Lipid extraction

Lipid was extracted for total lipid content and fatty acid analysis determined following the procedures of Bligh and Dyer (1959).

Peroxide value measurement

Peroxide value (PV) of the extracted lipid was measured following a procedure of Buege and Aust (1978) and expressed as mmol/kg of lipid.

Determination of thiobarbituric acid reactive substances (TBARS)

TBARS was determined according to the method of Buege and Aust (1978). Sample (5 g) was homogenized with 25 ml of TBARS solution (0.375% TBA, 15% TCA and 0.25 N HCl). The mixture was heated for 10 min in boiling water (95 - 100°C) to develop a pink color. Then the mixture was cooled with running water and centrifuged at 5,500*g* for 25 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. TBARS value was calculated from a standard curve of malonaldehyde and express as mg malonaldehyde/kg sample.

Fatty acid analysis

Fatty acids of total lipid were transesterified to methyl esters, using a base-catalyzed

transesterification, followed by a BF_3-MeOH-catalyzed esterification, according to the official method of AOCS Ce 1b-89 (AOCS, 2009), to obtain fatty acid methyl ester (FAMEs). The FAMEs were dissolved in iso-octane and injected into a model GC 17A gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a Zebron ZB-wax fused silica wall-coated open tubular column (0.25 mm i.d. x 30 m, 0.25 µm in film thickness: Torrance, CA, USA) and flame-ionization detector. The column oven and injection port temperature were held initially at 170°C for 2 min, then programmed to 240°C at a rate of 5°C/min, from 240°C to 250°C at a rate of 1.6°C/min, and finally held at 250°C for 10 min. Nitrogen was used as a carrier gas with an inlet pressure of 2.0 kg/cm^2.

Objective color measurement

Color was measured using a Hunter Lab colorimeter (DP 9000, Hunter Associates Laboratory, Reston, VA, USA) with the angle 10° and a D65 illuminant standard observed. Color evaluation was made through the CIE L*, a*, b* system. CIE L*, a*, b* values were determined as indicators of lightness, redness/greenness, and yellowness/blueness, respectively.

Measurement of water activity (A_w)

The A_w was determined at 25°C using Novasina model AWC 200 water activity meter (Pfäffikon, Switzerland).

Statistical analysis

Microsoft Excel 5.0 (Microsoft Co., Washington, USA) was used for all statistical analyses. Data were analyzed using one-way ANOVA, and means were compared using Duncan's multiple range test. Differences were considered to be significant at $P < 0.05$.

Result and Discussion

Changes in moisture contents and Water activity (A_w)

Changes in moisture contents of salted dried snakehead fish during refrigerated storage are depicted in Figure 1. At the beginning, the moisture content of all samples ranged between 65.81 ± 0.20 and 66.80 ± 0.30% ($P < 0.05$). During storage time, the moisture content in all samples slightly increased. No significant differences ($P < 0.05$) in the moisture contents were observed among the four groups.

Changes in the A_w of salted dried snakehead fish during refrigerated storage are shown in Figure 2. From the result, the A_w of salted dried snakehead fish

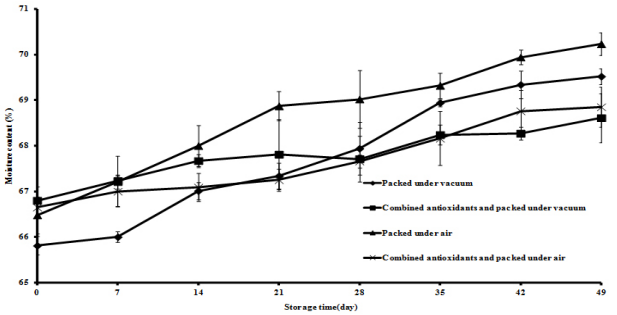

Figure 1. Changes in moisture contents of salted dried snakehead fish during storage at 4°C

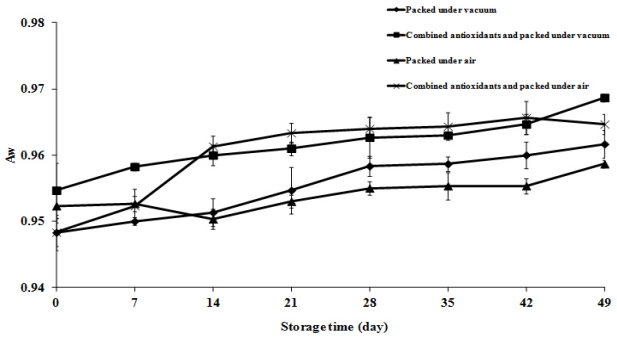

Figure 2. Changes in A_w contents of salted dried snakehead fish during storage at 4°C

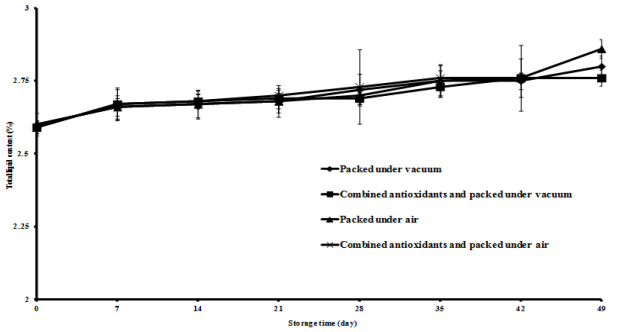

Figure 3. Changes in total lipid contents of salted dried snakehead fish during storage at 4°C

remained unchanged during storage time. The mean contents of A_w ranged 0.95 and 0.97.

The gradual increase of moisture and water activity of all samples could be due to moisture absorption by the food from the environment that gradually permeated through packaging materials and also from the respiration of the growing microorganisms (Sawaya *et al*., 1995).

Changes in total lipid contents

Changes in total lipid (TL) contents of salted dried snakehead fish during refrigerated storage are shown in Figure 3. The mean content of TL ranged between 2.59% and 2.86%.The amounts of the extracted lipids tended to increase slightly in all groups during the initial 7 days of storage time. This is probably due to the increased recovery of the extraction of lipids caused by changes in fish tissues. However, there were not significant different ($P < 0.05$) in total lipid

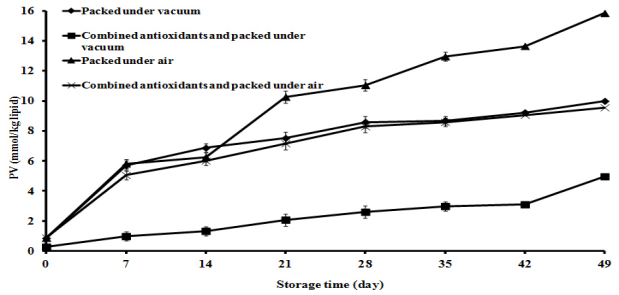

Figure 4. Changes in peroxide values of salted dried snakehead fish during storage at 4°C

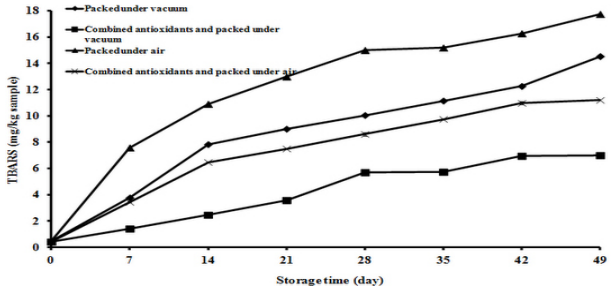

Figure 5. Changes in thiobarbituric acid reactive substances of salted dried snakehead fish during storage at 4°C

contents among the four groups from each other at any of the storage times. Similar results have been reported by Chedoloh *et al.* (2011), the most of the freshwater fish consumed in Thailand are *Channa* sp., *Clarias* sp., *Helostoma* sp., and *Puntius* sp. Fat contents of these freshwater species were between 1.08% for *Helostoma temmincki* to 2.77% for *Clarias batrachus.* For the most of fish had fat contents similar to those reported in earlier studies (Özogul *et al.*, 2007; Karapanagiotidis *et al.*, 2010)

Changes in PV

Changes in PV of salted dried snakehead fish during refrigerated storage are shown in Figure 4. The PV of all samples were significantly different ($p < 0.05$). The PV of the group without a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under air rapidly increased from 0.86 ± 0.03 to 5.81 ± 0.10 mmol/kg during the initial 7 days of storage and then gradually increased up to 15.85 ± 0.24 mmol/kg. In the group without a combined 100 ppm PG and 100 ppm ascorbate and packed under vacuum, the PV rapidly increased from 0.85 ± 0.01 to 5.71 ± 0.20 mmol/kg during the initial 7 days of storage and then gradually up to 9.99 ± 0.18 mmol/kg. In the sample group with combined antioxidants and packed under air, the PV rapidly increased form 0.88 ± 0.02 mmol/kg during the initial 7 days of storage and then gradually increased up to 9.56 ± 0.54 mmol/kg. For the sample with a combined antioxidant and packed under vacuum, the PV slowly increased from 0.80 ± 0.04 to 1.32 ± 0.56 mmol/kg during the initial day to 14 days of storage, followed by a gradual increase until 49 days of storage.

Among the four groups, the combined 100 ppm PG and 100 ppm sodium ascorbate and packed under vacuum showed the lowest PV throughout storage time, followed by the combined 100 ppm PG and 100 ppm sodium ascorbate and packed under air group. These results suggest that the combined PG and sodium ascorbate and packed under vacuum was more effective in suppressing the oxidation of salted dried snakehead fish meat than that with no added antioxidant and packed under air. Aubourg *et al.* (2004) and Fagan and Gormley (2004) reported that usage of antioxidants and packing have a positive effect on delaying fat spoilage.

Changes in TBARS values

The effect of a combined use of PG and sodium ascorbate under packed vacuum inhibited TBARS formation in salted dried snakehead fish is shown in Figure 5. The TBARS values of salted dried snakehead fish were significantly ($p < 0.05$) affected by a combined of antioxidants and packing. Salted dried snakehead fish with a combined PG and sodium ascorbate under packed vacuum had a TBARS value of 0.40 ± 0.01 mg/kg sample and then increased gradually up to 6.98 ± 1.02 mg/kg sample in 49 days. The TBARS values of the group without a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under air increased rapidly from 0.47 ± 0.01 to 7.56 ± 0.01 mg/kg the initial 2 days of storage time and then increased gradually up to 17.73 ± 2.03 mg/kg. In the group without a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under vacuum, TBARS values increased gradually from 0.46 ± 0.03 to 14.52 ± 0.03 mg/kg. In the sample group with combined antioxidants and packed under air, TBARS values increased slightly from 0.40 ± 0.02 to 11.19 ± 0.03 mg/kg during 49 days of storage. These results showed that the sample treated with a combined use of PG and sodium ascorbate, packaged in vacuum and stored at 4°C showed the most effective retardation of lipid oxidation.

TBARS has been used to measure the concentration of relatively polar secondary reaction products, especially aldehydes (Nawar, 1996). The increased in TBARS indicated the formation of secondary lipid oxidation products (Kolakowska, 2002) which measures malonaldehyde content (Nishimoto *et al*., 1985). Malonaldehyde is formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (Fernandez *et al*., 1997). From the result, TBARS value of salted dried snakehead fish combined antioxidant and packed under vacuum showed the

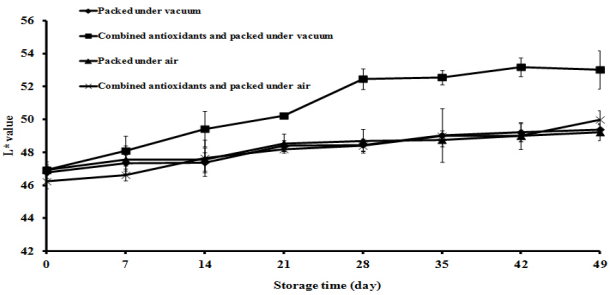

Figure 6. Changes in L^* values of salted dried snakehead fish during storage at 4°C

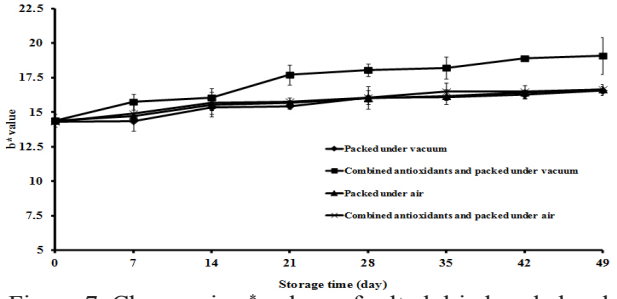

Figure 7. Changes in a^* values of salted dried snakehead fish during storage at 4°C

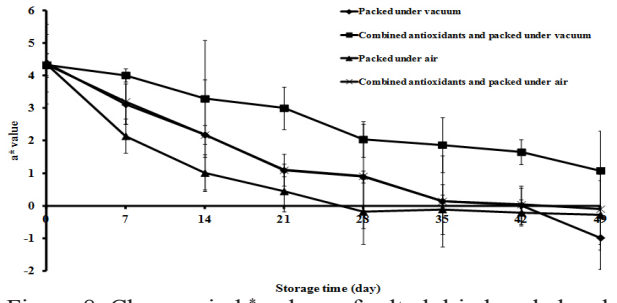

Figure 8. Changes in b^* values of salted dried snakehead fish during storage at 4°C

lowest TBARS value ($P < 0.05$; Fig.5). This indicated that the combined PG and sodium ascorbate and vacuum packed had effective in controlling lipid oxidation in salted dried snakehead fish. Greene, (1969); Kelleher *et al.* (1992): Xiong *et al*. (1993) and Wan *et al.* (1993) reported propyl gallate is a well-known antioxidant that acts as an effective free radical scavenger to inhibit lipid oxidation. Thus, distribution of propyl gallate and its known potency as a strong free radical scavenger probably explain its effectiveness in inhibiting lipid oxidation in the present study. On the other hand, ascorbate is known to be a dual function agent, capable of both inhibiting and promoting lipid and myoglobin oxidation depending on its concentration and environment (Yin *et al*., 1993). Our results suggested that lipid oxidation occurring was most likely via a free radical mechanism. The difference in activity between the two antioxidants act also be due to a possible variation in distribution of these antioxidants in aqueous, nonaqueous, and water/lipid interphase of salted dried snakehead fish.

Oxidative rancidity may become a problem if higher than normal levels of oxygen are used (Finne, 1982). Oxygen causes oxidative rancidity in fatty fish, stimulates growth of aerobic bacteria and inhibits growth of strictly anaerobic bacteria (Church, 1998). Vacuum packaging has previously been shown to be effective in retarding oxidation in cooked meat (Cannon *et al*., 1995; Ho *et al*.,1995). Less oxidation in vacuum packs can be attributed to the elimination of oxygen. Shozen *et al.* (1997) reported the most effective method in controlling cholesterol oxidation in processed Anchovy was vacuum-packing.

Changes in color values

Color is often the first sensory quality by which foods are judged, and it may also provide an indication of chemical changes suffered by them. The relatively short shelf-life of meat is the single greatest concern to retail meat markets. Changes in L^*, a^*, and b^* measured by the CIELAB method values during refrigerated storage are shown in Figure 6-Figure 8. The L^* values (lightness) changed slightly increase during 49 days of storage time (Figure 6). The sample with a combined of PG and sodium ascorbate and under vacuum pack had the highest L^* value ($P < 0.05$) compared to those of other samples. With regard to b^* (yellowness) values, salted dried snakehead fish with a combined of PG and sodium ascorbate and under vacuum pack was significantly ($P < 0.05$) also had higher b^* values than those of other samples (Figure 7).

The color values of redness (a^*) are shown in Figure 8. There was significant ($P < 0.05$) colorless (decrease in redness) over storage time. A combined PG and sodium ascorbate and packed under vacuum was more effective in maintaining color than other treatments. The decrease in a^* values has frequently been associated with the formation of metmyoglobin and thus with meat discoloration (Jeremiah, 2001). Very low oxygen (residual) concentration in vacuum pack could be avoided by using O_2 scavengers to maintain the color stability (Rousette and Rennere, 1990). Hunter a^* values of salted dried snakehead fish showed non-significant differences ($P < 0.05$) among treatments during the initial day of storage (Figure 8). After storage time, a^* values for sample with a combined PG and sodium ascorbate and packed under vacuum had the most bright red color, with the highest a^* values, whereas the treatment with a combined PG and sodium ascorbate and packed under air and treatment without a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under vacuum and air resulted in a more purplish red color. The inhibitory effect by a combination of PG and sodium ascorbate was stronger than the sample

Table 1. Changes in fatty acid compositions of total lipid in salted dried snakehead fish (the group with a combined PG and sodium ascorbate and packed under vacuum) during storage at 4°C

Fatty acid	Storage time (day)							
	0	7	14	21	28	35	42	49
C14:0	3.06	3.01	3.01	2.99	2.95	2.98	2.97	2.89
C15:0 I	0.41	0.41	0.42	0.41	0.40	0.40	0.41	0.41
C15:0	2.56	2.54	2.55	2.54	2.56	2.55	2.56	2.55
C16:0 I	1.07	1.07	1.08	1.07	1.08	1.07	1.08	1.07
C16:0	22.54	22.50	22.58	22.57	22.60	22.58	22.60	22.56
C17:0 I	1.21	1.23	1.22	1.21	1.22	1.22	1.23	1.21
C17:0	2.08	2.08	2.08	2.04	2.04	2.05	2.06	2.04
C18:0	6.54	6.55	6.61	6.56	6.52	6.52	6.53	6.54
C20:0	0.27	0.25	0.26	0.26	0.26	0.25	0.25	0.23
C24:0	0.96	0.97	0.99	0.97	0.96	0.97	0.65	0.96
Saturated	40.7	40.61	40.8	40.62	40.59	40.59	40.34	40.46
C16:1 n7	4.28	4.29	4.30	4.28	4.29	4.30	4.27	4.29
C16: 1 n5	0.7	0.70	0.72	0.71	0.72	0.72	0.71	0.76
C18:1 n9	9.73	9.75	9.69	9.70	9.80	9.75	9.73	9.73
C18:1 n7	5.3	5.43	5.39	5.38	5.32	5.33	5.36	5.31
C18:1 n5	0.43	0.48	0.48	0.43	0.42	0.41	0.43	0.44
C20:1 n11	8.68	8.60	8.60	8.68	8.66	8.69	8.68	8.66
C20:1 n9	1.18	1.17	1.17	1.18	1.16	1.15	1.14	1.19
C22:1 n11	1.07	1.06	1.09	1.07	1.08	1.07	1.07	1.08
Monoenoic	31.37	31.48	31.44	31.43	31.45	31.42	31.39	31.46
C16:2 n4	1.52	1.54	1.53	1.52	1.54	1.54	1.52	1.5
C16:2 n3	0.58	0.56	0.59	0.55	0.56	0.58	0.57	0.58
C17:2 n8	1.35	1.34	1.35	1.34	1.35	1.35	1.35	1.37
C17:2	0.28	0.25	0.28	0.26	0.38	0.36	0.26	0.26
C18:2 n6	7.55	7.54	7.58	7.56	7.54	7.56	7.56	7.57
C18:2 n4	3.87	3.87	3.88	3.87	3.88	3.89	3.88	3.87
C18:3 n4	0.49	0.48	0.50	0.49	0.49	0.48	0.48	0.48
C18:3 n3	0.9	0.86	0.86	0.85	0.87	0.89	0.90	0.9
C18:4 n1	1.18	1.18	1.19	1.19	1.18	1.20	1.18	1.24
C20:2	0.22	0.22	0.24	0.22	0.24	0.24	0.23	0.25
C20:4 n6	2.59	2.59	2.59	2.50	2.50	2.48	2.45	2.46
C20:5 n3	0.45	0.40	0.42	0.41	0.39	0.04	0.39	0.38
C21:5 n3	1.43	1.43	1.48	1.43	1.40	1.40	1.38	1.37
C22:5 n3	2.17	2.10	2.11	2.12	2.10	2.09	2.07	1.84
C22:6 n3	4	3.75	3.70	3.60	3.09	3.08	3.01	2.9
Polyenoic	28.58	28.11	28.30	27.91	27.51	27.178	27.23	26.97

Table 2. Changes in fatty acid compositions of total lipid in salted dried snakehead fish (the group without a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under air) during storage at 4°C

Fatty acid	Storage time (day)							
	0	7	14	21	28	35	42	49
C14:0	3.18	3.20	3.56	3.98	4.01	4.25	4.22	4.25
C15:0 I	0.38	0.36	0.38	0.35	0.36	0.38	0.38	0.39
C15:0	2.79	2.80	2.79	2.80	2.81	2.81	2.80	2.82
C16:0 I	0.99	0.99	1.00	0.98	1.01	1.04	1.04	1.05
C16:0	22.9	23.58	23.68	23.98	24.59	25.59	26.89	26.96
C17:0 I	1.18	1.18	1.15	1.20	1.19	1.19	1.23	1.26
C17:0	2.26	2.28	2.24	2.26	2.24	2.24	2.24	2.26
C18:0	6.21	6.96	6.99	7.00	7.12	7.58	7.85	7.98
C20:0	0.23	0.25	0.24	0.26	0.27	0.27	0.26	0.27
C24:0	0.94	0.94	0.95	0.95	0.98	0.98	0.98	0.99
Saturated	41.06	42.54	42.98	43.76	44.58	46.33	47.89	48.23
C16:1 n7	4.2	3.81	3.74	3.45	3.05	3.04	2.97	2.98
C16: 1 n5	0.86	0.84	0.82	0.89	0.91	1.03	1.03	1.09
C18:1 n9	9.01	9.00	8.97	8.89	8.77	8.68	8.28	8.19
C18:1 n7	5.65	5.84	5.37	5.45	5.75	5.60	5.60	5.66
C18:1 n5	0.47	0.58	0.69	0.78	0.84	0.88	1.02	1.11
C20:1 n11	8.37	8.35	8.30	8.35	8.33	8.32	8.41	8.37
C20:1 n9	1.21	1.24	1.22	1.25	1.26	1.30	1.50	1.54
C22:1 n11	1.16	1.17	1.17	1.15	1.18	1.19	1.19	1.17
Monoenoic	30.93	30.83	30.28	30.21	30.09	30.04	30	30.11
C16:2 n4	1.51	1.50	1.53	1.47	1.40	1.42	1.42	1.39
C16:2 n3	0.4	0.40	0.41	0.39	0.39	0.38	0.34	0.34
C17:2 n8	1.76	1.77	1.74	1.76	1.78	1.75	1.78	1.8
C17:2	0.34	0.35	0.37	0.60	0.34	0.30	0.31	0.3
C18:2 n6	7.26	7.20	7.29	7.20	7.19	7.18	7.15	7.17
C18:2 n4	3.71	3.70	3.69	3.68	3.70	3.69	3.66	3.62
C18:3 n4	0.33	0.33	0.34	0.31	0.32	0.33	0.30	0.3
C18:3 n3	0.95	0.90	0.94	0.92	0.90	0.89	0.90	0.89
C18:4 n1	1.41	1.42	1.38	1.39	1.39	1.36	1.38	1.37
C20:2	0.26	0.29	0.29	0.30	0.30	0.31	0.32	0.33
C20:4 n6	2.49	2.39	2.38	2.39	2.39	2.34	2.30	2.25
C20:5 n3	0.69	0.50	0.51	0.50	0.48	0.47	0.43	0.3
C21:5 n3	1.1	1.00	1.01	0.98	0.97	0.80	0.83	0.85
C22:5 n3	2.1	1.78	1.50	1.42	1.33	1.30	1.20	0.99
C22:6 n3	4.34	4.24	4.04	3.89	3.25	3.01	2.68	2.07
Polyenoic	28.65	27.77	27.43	27.2	26.13	25.53	24.99	23.97

Table 3. Changes in fatty acid compositions of total lipid in salted dried snakehead fish (the group with a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under air) during storage at 4°C

Fatty acid	Storage time (day)							
	0	7	14	21	28	35	42	49
C14:0	3.51	3.52	3.54	3.56	3.59	3.60	3.61	3.63
C15:0 I	0.91	0.97	0.98	1.00	1.14	1.80	1.20	1.24
C15:0	2.46	2.44	2.49	2.45	2.45	2.40	2.45	2.47
C16:0 I	1.01	0.90	0.91	0.90	0.91	0.89	0.90	0.88
C16:0	22.45	22.46	22.48	22.48	22.50	22.80	22.99	23.61
C17:0 I	1.11	1.10	1.11	1.13	1.10	1.09	1.14	1.12
C17:0	1.98	1.98	1.95	1.96	1.99	1.96	1.96	1.72
C18:0	6.13	6.17	6.60	6.61	6.70	6.89	7.12	7.33
C20:0	0.24	0.28	0.29	0.30	0.30	0.31	0.34	0.33
C24:0	0.83	0.83	0.86	0.83	0.85	0.85	0.86	0.89
Saturated	40.63	40.65	41.21	41.22	41.53	42.59	42.57	43.22
C16:1 n7	4.66	4.45	4.46	4.63	4.45	4.44	4.47	4.01
C16: 1 n5	0.86	0.93	0.93	0.95	0.98	0.98	0.97	0.97
C18:1 n9	9.08	9.58	9.92	9.95	9.97	9.99	9.97	10.04
C18:1 n7	5.67	5.53	5.21	5.64	5.60	5.66	5.68	5.7
C18:1 n5	0.39	0.75	0.40	0.54	0.54	0.56	0.48	0.66
C20:1 n11	8.23	8.23	8.17	8.36	8.36	8.39	8.76	8.79
C20:1 n9	0.79	0.78	0.79	0.79	0.80	0.75	0.77	0.78
C22:1 n11	1.43	1.43	1.45	1.48	1.42	1.44	1.42	1.48
Monoenoic	31.11	31.67	31.33	32.33	32.12	32.21	32.52	32.43
C16:2 n4	1.52	1.49	1.50	1.48	1.44	1.50	1.54	1.51
C16:2 n3	1.15	1.18	1.17	1.18	1.15	1.16	1.17	1.13
C17:2 n8	1.45	1.45	1.45	1.45	1.46	1.45	1.46	1.48
C17:2	0.29	0.28	0.29	0.29	0.28	0.27	0.27	0.28
C18:2 n6	7.96	7.96	7.90	7.91	7.91	7.93	7.88	7.9
C18:2 n4	3.54	3.54	3.55	3.56	3.54	3.52	3.52	3.51
C18:3 n4	0.61	0.60	0.63	0.64	0.65	0.65	0.67	0.69
C18:3 n3	0.35	0.34	0.37	0.35	0.36	0.37	0.37	0.38
C18:4 n1	1.52	1.52	1.56	1.55	1.53	1.54	1.53	1.52
C20:2	0.31	0.36	0.33	0.34	0.35	0.35	0.34	0.34
C20:4 n6	2.39	2.29	2.28	2.70	2.70	2.26	2.22	2.19
C20:5 n3	0.49	0.42	0.42	0.40	0.39	0.38	0.35	0.36
C21:5 n3	1.01	1.00	1.02	1.00	0.99	0.98	0.99	0.97
C22:5 n3	2.23	2.22	2.24	2.23	2.20	2.18	2.10	2
C22:6 n3	3.93	3.90	3.59	3.25	3.14	3.12	2.98	2.69
Polyenoic	28.75	28.55	28.3	28.33	28.09	27.66	27.39	26.95

Table 4. Changes in fatty acid compositions of total lipid in salted dried snakehead fish (the group without a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under vacuum) during storage at 4°C

Fatty acid	Storage time (day)							
	0	7	14	21	28	35	42	49
C14:0	3.15	3.38	3.79	3.79	3.86	3.94	3.97	4.01
C15:0 I	0.45	0.46	0.44	0.47	0.48	0.45	0.45	0.45
C15:0	2.74	2.75	2.76	2.78	2.79	2.80	2.81	2.81
C16:0 I	1.08	1.08	1.07	1.09	1.10	1.10	1.11	1.12
C16:0	21.55	22.91	22.99	23.01	23.57	23.85	24.52	24.96
C17:0 I	1.24	1.25	1.25	1.28	1.29	1.28	1.29	1.3
C17:0	2.15	2.18	2.16	2.18	2.14	2.14	2.14	2.15
C18:0	6.5	6.58	6.74	6.78	6.91	6.99	7.02	7.04
C20:0	0.26	0.25	0.26	0.28	0.28	0.27	0.26	0.26
C24:0	0.94	0.95	0.98	0.96	0.95	0.98	0.98	0.97
Saturated	40.06	41.79	42.44	42.62	43.37	43.8	44.55	45.07
C16:1 n7	4.78	4.28	4.20	4.14	4.00	3.98	3.83	3.87
C16: 1 n5	0.94	0.97	0.99	0.97	0.98	1.00	1.04	1.1
C18:1 n9	8.99	8.80	8.78	8.23	8.04	8.00	7.98	7.91
C18:1 n7	4.95	4.95	4.99	4.96	4.98	4.95	4.92	4.96
C18:1 n5	0.36	0.38	0.40	0.59	0.63	0.69	0.70	0.72
C20:1 n11	8.15	8.19	8.20	8.21	8.27	8.28	8.30	8.35
C20:1 n9	1.56	1.59	1.62	1.63	1.65	1.78	1.80	1.81
C22:1 n11	1.31	1.30	1.29	1.29	1.31	1.32	1.32	1.33
Monoenoic	31.04	30.46	30.47	30.02	29.86	30	29.89	30.05
C16:2 n4	1.57	1.53	1.45	1.40	1.39	1.30	1.28	1.29
C16:2 n3	0.32	0.30	0.39	0.38	0.28	0.24	0.22	0.2
C17:2 n8	2.12	2.15	2.15	2.13	1.14	1.15	1.15	1.16
C17:2	0.86	0.85	0.85	0.84	0.83	0.82	0.83	0.82
C18:2 n6	7.91	7.90	7.89	7.84	7.88	7.85	7.86	7.87
C18:2 n4	3.47	3.45	3.44	3.46	3.45	3.43	3.45	3.44
C18:3 n4	0.49	0.49	0.46	0.47	0.47	0.46	0.46	0.47
C18:3 n3	0.93	0.93	0.90	0.91	0.92	0.91	0.92	0.93
C18:4 n1	1.17	1.15	1.14	1.11	1.12	1.11	1.12	1.11
C20:2	0.21	0.21	0.23	0.25	0.25	0.26	0.28	0.26
C20:4 n6	2.4	2.38	2.38	2.36	2.35	2.30	2.30	2.28
C20:5 n3	0.69	0.59	0.60	0.60	0.54	0.50	0.53	0.49
C21:5 n3	1.39	1.31	1.34	1.34	1.30	1.29	1.29	1.28
C22:5 n3	2.2	2.01	2.00	1.99	1.97	1.80	1.80	1.78
C22:6 n3	3.95	3.68	3.10	2.93	2.87	2.45	2.35	2.01
Polyenoic	29.68	28.93	28.32	28.01	26.76	25.87	25.84	25.39

without a combined antioxidants.

Lipid peroxidation and oxidation of oxymyoglobin to metmyoglobin are two closely linked phenomena in muscle system. Hultin (1980) indicated the free radicals derived from lipid hydroperoxides were involed in the oxidation of meat pigment and that in vitro lipid peroxidation may precede pigment oxidation. Although many factors can influence meat color stability, metmyoglobin formation by free radicals is predominant (Renerre and Labas, 1987). Ascorbic acid may be able to function as a metmyoglobin reductant until lipid peroxidation products or free radicals become numerous thereby either destroying the ascorbic acid activity or overpowering ascorbic acid (Greene *et al.*, 1971). Protection of meat tissue against lipid oxidation by a combined PG and ascorbic acid, as in the present study, was also shown by several researchers in various muscle types (Liu and Xiong, 1996; Lee *et al.*, 1999).

Change in fatty acid compositions

Changes in fatty acid compositions of the TL of salted dried snakehead fish during refrigerated storage are shown in Table 1-4. For the group with a combined PG and sodium ascorbate and packed under vacuum (Table 1), the predominant fatty acid were C14:0, C18:0, C16:1n-7, C18:1n-9, C20:1n-11, C18:2n-6, C18:2n-4 and C22:6n-3 which together accounted for 70.25% of total fatty acids. Total fatty acids were composed of 40.7% of saturated, 31.37% monoenoic and 28.58% of polyenoic acids. During 49 days of storage, C14:0 and C16:0 decreased, C18:0, C16:1n-7 and C18:1n-9 remained unchanged, while C20:5n-3 and C22:6n-3 decreased. Total saturated fatty acids and monoenoic acids in the combined of PG and sodium ascorbate increased while total polyenoic acids decreased at a slower rate than those in the other groups. For the group without a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under air (Table 2), C14:0, C16:0 and C18:0 increased and C16:1n-7, C18:1n-9, C20:5n-3 and C22:6n-3 decreased. The saturated fatty acids and monoenoic acids increased and total polyenoic acids decreased. For the group with a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under air (Table 3) and the group without a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under vacuum (Table 4), C14:0, C16:0, C18:0 and C18:1n-9 increased and C16:1n-7, C20:5n-3 and C22:6n-3 decreased. Total saturated fatty acids and monoenoic acids increased and total polyenoic acids decreased at a slower rate than those in the group without a combined 100 ppm PG and 100 ppm sodium ascorbate and packed under air.

Fresh snakehead fish, with a fat content of 3.25% (Rahman, 1995), usually has high content C14:0 C16:0, C18:0, C16:1n-7, C18:1n-9, C18:2n-6, C18:4n-3 and C22:6n-3 and the level of PUFA are higher than those of saturated fatty acids. In the present study, total polyenoic acids in total lipid of the combined PG and sodium ascorbate and packed under vacuum group decreased at the slower rate than the those of other groups, suggesting that in the combined PG and sodium ascorbate and packed under vacuum group, lipid oxidation decreased slowly as is also shown in the PV and TBARS changes. These different behaviours between the four experimental groups in total polyenoic acid changes must be due to differences in oxidative stabilities. The declined of PUFA in the group without the combined antioxidants are consistent with higher susceptibility to autoxidation. Rapid oxidation of PUFA, within several days of refrigerated storage of cooked fish meat has been reported (Jittrepotch *et al.*, 2006). The PUFA may be involved in the heat-induced degradation of lipids which results lipid oxidation. Therefore, the high susceptibility of salted dried snakehead fish to lipid oxidation could be controlled by a combined use of PG and sodium ascorbate and packed under vacuum.

Conclusions

In the present study, it could be summarized that a combination of PG and sodium ascorbate were effective in delaying lipid oxidation and showed a greater antioxidant activity in terms of the lower PV and TBARS values than did not with antioxidants. The most effective method in controlling of lipid oxidation was vacuum-packing.

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