Asian-Aust. J. Anim. Sci.
Vol. 21, No. 7 : 1067 - 1072
July 2008

www.ajas.info

Effects of Fucoxanthin Addition to Ground Chicken Breast Meat on Lipid and Colour Stability during Chilled Storage, before and after Cooking

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ABSTRACT : Effects of fucoxanthin (FX), a major carotenoid in algae, on lipid peroxidation and meat colour in ground chicken breast meat were investigated. FX and/or α-tocopherol (Toc) were added to ground samples at a content level of 200 mg/kg. The samples were subjected to a chilling storage test before and after cooking. In the storage test before cooking, neither FX nor Toc affected the thiobarbituric acid reactive substances (TBARS) on days 1 and 6, and FX decreased the *L** value and increased the *a** and *b** values. In the storage test after cooking, both FX and Toc decreased TBARS values on days 1 and 6. FX decreased the *L** value and increased the *a** and *b** values, similar to what occurred in the storage test before cooking. Based on these results, we concluded that FX is a potent ingredient for improvement of the appearance and shelf life of chicken meat and its products. (**Key Words :** Fucoxanthin, Chicken Breast Meat, Lipid Peroxidation, Meat Colour, α-Tocopherol)

INTRODUCTION

Lipid peroxidation causes an important quality deterioration in meat and meat products during storage. Lipid peroxide products are the cause of warmed-over flavor in muscle foods (Rhee, 1988), and some lipid peroxide products are harmful to human health (Esterbauer et al., 1991). Many researchers have attempted to prevent lipid peroxidation in meat during storage by using natural antioxidants. For example, α-tocopherol (Toc), ascorbate, carotenoids, and flavonoids were examined for improvement of the stability of lipids during meat preservation (Mitsumoto, 2000).

Fucoxanthin (FX), (3*S*,5*R*,6*S*,3'*S*,5'*R*,6'*R*)-3'-acetoxy-5,6-epoxy-3,5'-dihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-β,β-caroten-8-one (Figure 1), is a major carotenoid in Phaeophyceae (algae), and it is the carotenoid most biosynthesized on our planet (Haugan et al., 1992). FX has proton-donative antioxidative activity (Nomura et al., 1997) and is characterized as the major antioxidative pigment in edible seaweed (Yan et al., 1999). Based on these established facts, we believe FX is potentially a useful natural antioxidant for improvement of lipid stability in chicken meat.

The addition of FX affects meat colour directly because it is a red- or yellow-colored pigment that has visible absorbance at 350-600 nm (Sugawara et al., 2002). Coloring effects of FX also must be investigated when considering FX as an ingredient in meat products for maintaining lipid stability of chicken meat and meat products.

In the present study, we investigated the effects of FX addition on lipid stability and meat colour in ground chicken breast meat during chilled storage before and after cooking. In addition, we compared the antioxidative and coloring effects of FX with those of Toc, a typical lipid-soluble antioxidant used to maintain meat quality during storage.

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Received November 13, 2007; Accepted March 1, 2008

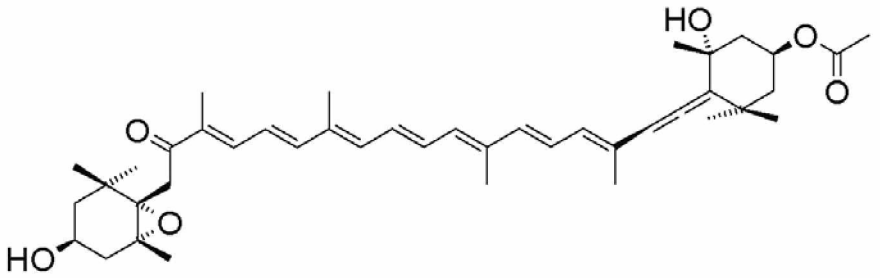

Figure 1. Structure of all-*trans*-fucoxanthin.

MATERIALS AND METHODS

Meat samples and chemicals

Vacuum-packed chicken breast meat samples from 50- to 53-day-old broilers (~2.0 kg of each package) were obtained from Nippon White Farm, Inc. (Aomori, Japan). Samples were transported and stored at 0°C for 4 days after slaughter. All chemicals were obtained from Wako Pure Chemicals (Osaka, Japan).

Fucoxanthin preparation

FX was prepared from the Japanese edible seaweed Wakame (*Undaria pinnatifida*), as described previously (Haugan et al., 1992). Briefly, acetone-methanol (70:30, v/v) extraction of dried Wakame samples was performed, and partition of hexane from aqueous methanol was done to remove chlorophylls and less polar carotenoids. The extract was applied to silica-gel column chromatography for FX purification, as described by Sugawara et al. (2001). For column chromatography, hexane-ethyl acetate (100:0, 80:20, 60:40, 50:50, and 40:60, v/v) was used for elution. The eluent of hexane-ethyl acetate from 50:50 to 40:60 was collected as the FX fraction.

We estimated the purity of FX by spectrophotometric assay and high-performance liquid chromatography (HPLC). The HPLC assay was performed as described by Haugan et al. (1992) on a Develosil CN-UG-5 column (250×4.6 mm). FX was monitored with an ultraviolet/visible detector (No. 119 UV/VIS detector, Gilson, Inc., Middleton, WI) set at 445 nm. The mobile phase was hexane-isopropyl acetate-acetone-methanol (76:17:7:0.1, v/v/v/v), and the flow rate was 2.0 ml/min. The purity of FX used in the present study was estimated as 94.2%. HPLC analysis showed that FX was composed of 95.9% all-*trans*-FX and 4.1% *cis*-FX.

Sample preparation and storage experiments

The chicken breast meat sample was minced twice and then divided into control, FX supplemented, Toc supplemented, and both FX and Toc supplemented groups. FX and Toc were dissolved in ethanol at the concentration of 20 mg/ml and added to the ground meat at 1.0 ml/100 g sample. Final concentrations of FX and Toc were 200 mg/kg meat, respectively. Ethanol without any antioxidants was added to the control group. The samples were mixed well and formed into circular patties 35 mm in diameter and 10 mm thick. The patties weighed approximately 10 g. These samples were subjected to storage tests before and after cooking. Each storage test was performed twice, with four patty samples prepared in each group for each trial.

The two trials were conducted with a new batch of ground meat, and different lots of chicken breast meat samples were used for tests before and after cooking.

In the chilling storage test before cooking, the patties were put into a weighing boat and over-wrapped by oxygen-permeable PVC film, and then stored in a refrigerator set at 4°C in the dark for 1 day or 6 days.

In the storage test after cooking, the patties were heat treated on a Toshiba ER-690SE (Toshiba Co., Tokyo) oven-grill range set at 180°C for 10 minutes. The cooked patties were cooled to room temperature and then subjected to the preservation test, similar to what occurred in experiment 1, for 1 day or 6 days.

After storage, the meat colour and thiobarbituric acid reactive substances (TBARS), an index for lipid peroxide, were determined as described below in the storage experiments before and after cooking.

Determination of meat colour and lipid peroxide

Meat color parameters such as L^*, a^*, and b^* values were analyzed by a UV-2400PC spectrophotometer (Shimadzu Co., Tokyo) equipped with an ISR-2200 integrating sphere (Shimadzu Co., Tokyo). Lipid peroxide was evaluated based on the TBARS values, which were determined by spectrophotometric assay as described previously (Witte et al., 1970) and later modified (Mitsumoto et al., 1993), and the values were expressed as nmol malondialdehyde equivalents per g samples.

Statistical analysis

L^*, a^*, b^*, and TBARS values were analyzed by the

Table 1. Effects of trial, fucoxanthin and α-tocopherol supplementation, and storage days on meat color and thiobarbituric-acid reactive substances (TBARS) in ground chicken breast meat during storage at 4°C before cooking

Effect	Item			
	L*	a*	b*	TBARS (nmol/g sample)
Trial	***	NS	NS	**
1st	54.0	10.7	23.5	4.72
2nd	51.2	11.3	20.2	2.02
Standard error	0.1	0.3	1.1	0.15
Fucoxanthin	***	***	**	NS
Control	56.2	4.0	13.6	3.39
Supplementation	49.0	18.1	30.0	3.35
Standard error	0.1	0.3	1.1	0.15
α-Tocopherol	*	NS	NS	NS
Control	52.3	11.2	21.1	3.52
Supplementation	52.9	10.8	22.5	3.22
Standard error	0.1	0.3	1.1	0.15
Storage days	***	***	***	***
Day 1	57.4	11.7	25.1	2.38
Day 6	47.8	10.3	18.6	4.36
Standard error	0.1	0.1	0.2	0.17
Fucoxanthin×α-tocopherol	NS	NS	NS	NS
Fucoxanthin×day	***	***	***	NS
α-Tocopherol×day	NS	NS	**	NS
Fucoxanthin×α-tocopherol×day	NS	*	*	NS

TBARS values are indicated as malondialdehyde equivalents.
Values are expressed as means and standard errors. NS: not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

General Linear Model (GLM) procedure, SAS version 9.12 (SAS Institute, Cary, NC). Trial, FX supplementation, and Toc supplementation were designated as the main plot, and storage time, two- or three-factor interactions of FX×Toc, FX×storage day, Toc×storage day, and FX×Toc×storage day were designated as the sub-plot. The PDIFF option of the GLM procedure was used to determine the difference among least square means of FX×Toc×storage day.

RESULTS

Study on chilling storage before cooking

Table 1 presents the effects of trial number, FX and Toc supplementation, and storage days on meat color and TBARS values during chilling storage before cooking. FX decreased the *L** value and increased the *a** and *b** values

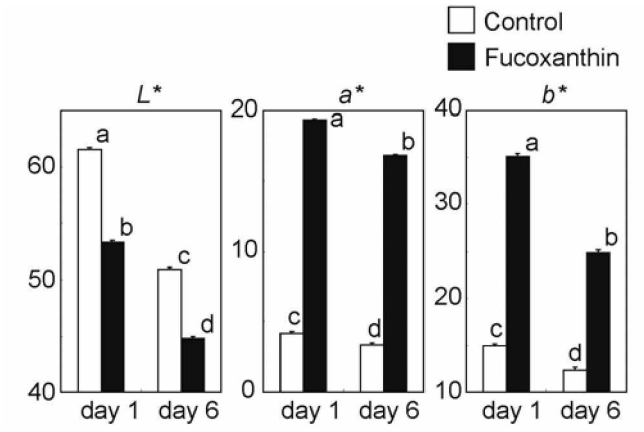

Figure 2. Effects of fucoxanthin addition and storage days on *L**, *a**, and *b** values in ground chicken breast meat during chilling storage at 4°C before cooking. Values are expressed as least square means±standard errors. Values with different superscripts differ significantly ($p<0.05$) in each panel.

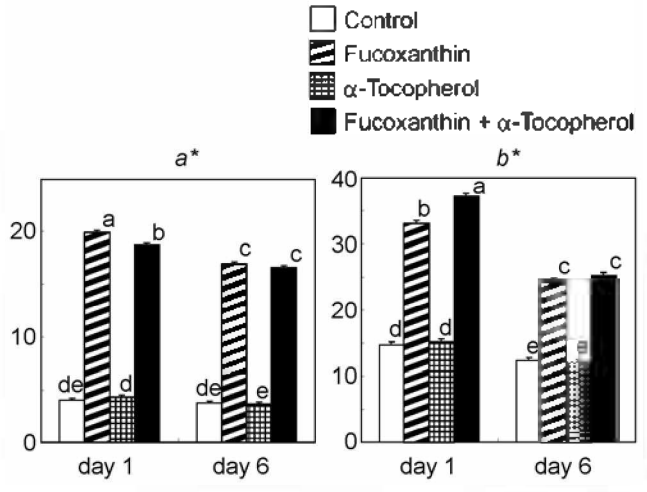

Figure 3. Effects of fucoxanthin addition, α-tocopherol addition, and storage days on *a**, and *b** values in ground chicken breast meat during chilling storage at 4°C before cooking. Values are expressed as least square means±standard errors. Values with different superscripts differ significantly ($p<0.05$) in each panel.

Table 2. Effects of trial, fucoxanthin and α-tocopherol supplementation, and storage days on meat color and thiobarbituric-acid reactive substances (TBARS) in ground chicken breast meat during storage at 4°C after cooking

Effect	Item			
	L*	a*	b*	TBARS (nmol/g sample)
Trial	*	NS	***	NS
1st	71.8	6.89	21.9	60.1
2nd	78.1	3.32	25.8	32.1
Standard error	0.8	1.48	0.1	11.8
Fucoxanthin	**	*	***	NS
Control	78.5	0.73	16.9	58.1
Supplementation	71.5	9.48	30.8	34.0
Standard error	0.8	1.48	0.1	11.8
α-Tocopherol	NS	NS	**	NS
Control	75.2	4.53	23.2	64.6
Supplementation	74.8	5.68	24.5	27.6
Standard error	0.8	1.48	0.1	11.8
Storage days	**	***	***	***
Day 1	74.4	5.45	25.0	22.5
Day 6	75.6	4.76	22.7	69.7
Standard error	0.3	0.11	0.3	2.4
Fucoxanthin×α-tocopherol	NS	NS	***	NS
Fucoxanthin×day	NS	**	***	***
α-Tocopherol×day	NS	***	NS	**
Fucoxanthin×α-tocopherol×day	NS	NS	NS	NS

TBARS values are indicated as malondialdehyde equivalents.
Values are expressed as least square means and standard errors. NS: not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

significantly ($p<0.05$). Toc raised the L^* value significantly ($p<0.05$). During 6 days of storage, L^*, a^*, and b^* values were significantly ($p<0.001$) decreased and the TBARS value was significantly ($p<0.001$) increased. FX and Toc, however, did not affect lipid peroxidation ($p>0.05$).

Figure 2 indicates the interactive effects of FX addition and storage days on meat colour values. Figure 3 presents the interactive effects of FX and Toc addition and storage days on a^* and b^* values. The a^* and b^* values were different between the FX and FX+Toc groups at day 1 ($p<0.05$), although Toc alone did not affect the a^* and b^* values ($p>0.05$). By day 6, the addition of Toc did not have any effect on either the a^* value or the b^* value.

Study on chilling storage after cooking

Table 2 shows the effects of trial, FX and Toc supplementation, and storage days on meat colour and TBARS values during chilling storage after cooking. As in the storage after cooking test, FX decreased the L^* value and increased the a^* and b^* values significantly ($p<0.05$). Toc increased the b^* value significantly ($p<0.05$) but did not affect the L^* and a^* values ($p<0.05$). During 6 days of storage, the L^* value increased and the a^* and b^* values decreased significantly ($p<0.05$). The TBARS value increased during 6 days of preservation ($p<0.05$). Although TBARS values were not affected by FX or Toc alone ($p>0.05$), significant interactions between FX or Toc and storage time were observed ($p<0.01$).

As shown in Figure 4, FX addition raised the a^* and b^* values, and the a^* and b^* values derived from FX had decreased by day 6. FX decreased TBARS values at both days 1 and 6. Toc also decreased TBARS values at both days 1 and 6, as shown in Figure 5.

DISCUSSION

Various antioxidants have been investigated for their potential to maintain lipid quality in chicken meat during pre- and post-cookery storage. For example, postmortem supplementation of carnosine (O'Neil et al., 1999), grape seed extracts and green tea (Rababah et al., 2004), cocoa polyphenol (Hassan and Fan, 2005), spice mix and curry leaf (Biswas et al., 2006), and tea catechins (Mitsumoto et al., 2005) inhibited lipid peroxidation in chicken meat during storage. FX is a known antioxidative carotenoid and also a red or yellow pigment (Sugawara et al., 2002). We examined the effects of FX supplementation on lipid peroxidation and meat colour in ground chicken breast meat.

FX decreased the L* value in meat patties before cooking, and it increased the a^* and b^* values in chilling storage tests both before and after cooking. The a* and b* values derived from FX, however, had decreased by day 6 in the storage tests before and after cooking, as shown in Figure 2 and 4. Because FX is an antioxidant that is susceptible to oxidative attack, the decrease of a^* and b^* values in FX admixed meat patties during chilling storage may have been caused by oxidative degradation of FX.

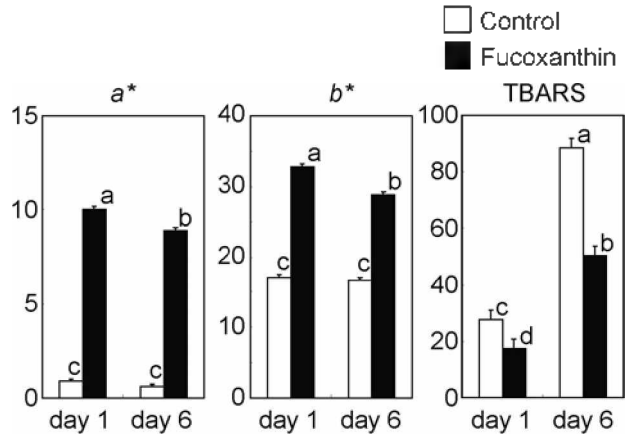

Figure 4. Effects of fucoxanthin addition and storage days on *a**, *b**, and thiobarbituric acid reactive substances (TBARS) values in ground chicken breast meat during chilling storage at 4°C after cooking. Values are expressed as least square means±standard errors. Values with different superscripts differ significantly ($p<0.05$) in each panel.

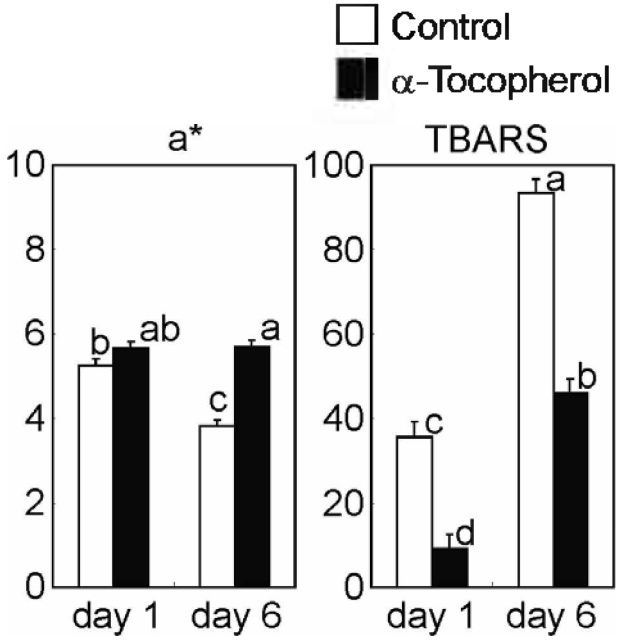

Figure 5. Effects of α-tocopherol addition and storage days on *a** and thiobarbituric acid reactive substances (TBARS) values in ground chicken breast meat during chilling storage at 4°C after cooking. Values are expressed as least square means±standard errors. Values with different superscripts differ significantly ($p<0.05$) in each panel.

The effect of the interaction between FX and Toc on meat colour was different in chilling storage before and after cooking. In storage examination before cooking, the interaction between FX and Toc was observed in the *a** and *b** values only on day 1 (Figure 3). On the other hand, the interaction of FX and Toc affected the *b** value during chilling storage after cooking. Additional investigation is needed to determine the interactive effects of FX and other antioxidative substances on meat color.

For lipid peroxidation, FX was effective only during chilling storage after cooking. In the storage test after cooking, FX addition decreased TBARS values on day 1 and 6, as shown in Figure 4. Toc addition also decreased TBARS values (Figure 5). FX prevented TBARS formation at the ratio of 63.1% and 58.5% on day 1 and 6, respectively. Toc prevented TBARS formation at the ratio of 25.6% and 49.3% on day 1 and 6, respectively. Based on these results, we conclude that FX is a potent and useful antioxidant for maintaining lipid stability of chicken meat, but the antioxidative activity of FX is lower than that of Toc. In addition, a synergistic effect of FX and Toc was not observed in the chilling storage test either before and after cooking.

No antioxidative effect of either FX or Toc was observed during chilling storage before cooking for each measurement period (Table 1), although postmortem Toc supplementation inhibits lipid peroxidation in raw beef and pork during storage (Mitsumoto, 2000; Chae et al., 2006). The results of Toc supplementation in storage before cooking may be caused by the initial antioxidative status of chicken meat samples. Additional investigation about antioxidative properties of FX and Toc in raw chicken meat is needed.

In conclusion, FX supplementation increased redness and yellowness in ground chicken breast meat, and it inhibited lipid peroxidation in chilling storage after cooking. The antioxidative activity of FX during chilling storage after cooking was lower than that of Toc. We concluded that FX is one of the potent ingredients, found in large quantities in nature, of chicken meat for improvement of meat colour and lipid stability, especially in cooked chicken meat. On the other hand, we examined the effects of FX at the concentration of only 200 mg/kg meat. Additional investigation is needed that tests a number of concentrations of additives to evaluate the antioxidative and colouring properties of FX. In addition, further studies are necessary to clarify the antioxidant and colouring mechanisms of FX in muscle systems.

ACKNOWLEDGMENTS

The present study was financed by the Japan Fisheries Agency of the Ministry of Agriculture, Forestry and Fisheries of Japan.

The authors are grateful for the help of Yumiko Endoh of the National Institute of Livestock and Grassland Science, Japan.

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