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Discrimination of Astaxanthin Fed Laying Hens and Their Peroxidated Carcasses by Electronic Nose

Young-An Kwon[1], Chanyong Lee[2], Bong-Duk Lee[3], Seung-Hyun Choi[4] and Gilhwan An[4,†]

[1]Department of Food Industry and Culinary, Woosuk University, Jeonbuk 565-701, Korea
[2]Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, Korea
[3]Department of Microbiology, Daejeon University, Daejeon 300-716, Korea.
[4]Department of Food Science and Technology, Chungnam National University, Daejeon 305-764, Korea

ABSTRACT The applicability of electronic nose was tested to detect lipid peroxidation in chickens and to measure antioxidant effect of astaxanthin in chicken carcasses. Two sources of astaxanthin were fed to 62-wk-old spent laying hens to improve meat quality: natural astaxanthin (NA) from the red yeast, *Phaffia rhodozyma*, and synthetic astaxanthin (SA) from chemical synthesis. One hundred forty four ISA Brown laying hens were used in a 6-wk feeding trial. Three treatments consisted of the basal diet (control), SA (100 mg astaxanthin/kg basal diet) and NA (50 mg astaxanthin/kg basal diet). The astaxanthin levels of SA and NA were set to give a similar degree of skin pigmentation. After 6-wk feeding of astaxanthin, the skins from NA and SA were discriminated from the control by electronic nose. However, electronic nose failed to distinguish between SA and NA skins after 6-wk feeding. The astaxanthin level differences between skins of SA and NA were not remarkable during the 6-wk trial. The lipid peroxide formation in skin was significantly decreased by SA but not by NA. The antioxidation effect of SA was detected by electronic nose because SA skin was discriminated from others. NA was a better pigmentation agent than SA, but the reverse was true in antioxidation. Electronic nose is applicable for detecting astaxanthin in chicken, and meat off-flavor caused by lipid peroxidation during storage.

(Key words : antioxidant, astaxanthin, chicken, electronic nose)

INTRODUCTION

Synthetic astaxanthin (SA), produced by Hoffman La Roche (Switzerland) and BASF (Germany), has been used for pigmentation of aquacultured animals (An et al., 2004). Natural astaxanthin (NA) produced by the red yeast, *Phaffia rhodozyma* (*Xanthophyllomyces dendrorhous*), successfully pigmented egg-yolks, bodies, and skins of laying hens and broiler chickens (Kim et al., 1996; Akiba et al., 2001).

Activity for pigmentation and antioxidation by two different sources of astaxanthin in broiler chickens were quite different (An et al., 2004). The effect of pigmentation NA was twice as good as SA for pigmentation and absorption in chicken. However, SA was superior to NA in antioxidation because chicken carcass fed with SA produced strongly decreased peroxides compared to NA. The weight increase with SA was significantly delayed in male mice but not with NA (Kim et al., 2009). SA decreased blood glucose, cholesterol, and triglyceride in mice by 4～21%. However, the increases in HDL-cholesterol were profound (64 and 32% in male and female mice respectively) by NA but not SA. Astaxanthin from *X. dendrorhous* was mainly (3R,3’R)-astaxanthin (Andrewes and Starr, 1976). The configuration ratio of synthetic astaxanthin was (3R,3’R):meso:(3S,3’S)=1:2:1 (The catalog for Carophyll Pink, Hoffman La Roche, Switzerland). Therefore, the quality of meat after slaughter could be strongly affected by the source of astaxanthin. Unfortunately, there is no study about the chemical and biological activity of chirally different astaxanthin.

Since the effect differences by astaxanthin sources were significant, the discrimination is important for determination of chicken meat quality. Since the astaxanthin analysis from chicken carcass by chromatography requires long time and large cost, there must be an easy and fast method.

In Japan and Korea, large quantities of roasted meat of spent laying hens are consumed. Laying hens were successfully pigmented by 6-wk trial of carotenoid feeding (Na et al., 2004).

† To whom correspondence should be addressed : ghahn@cnu.kr

Since astaxanthin has been demonstrated to contribute to the flavor of salmonids (Josephson, 1987), accumulation of astaxanthin in spent laying hens might also increase favorable flavor. Astaxanthin also decreased the production of off-flavor by inhibiting lipid peroxidation in skin during storage of broiler chickens (Na et al., 2004). To promote the consumption of spent laying hens by improving meat quality, the effect of astaxanthin on pigmentation and antioxidant activity were tested with laying hens.

In a few studies the electronic nose was used for detecting volatile materials from poultry and its products. The impact of dietary sources of methionine for broiler was investigated by the electronic nose (Chavez et al., 2004). Also the electronic nose was used for detecting oxidation of lipid in poultry sausage (Olsen, 2005). In this study, the applicability of the electronic nose was determined to detect lipid peroxidation in chickens during storage. Also, the effect of natural and synthetic astaxanthin on preventing lipid peroxidation to improve meat quality was measured by the electronic nose.

MATERIALS AND METHODS

1. Birds and Management

A 6-wk feeding trial was conducted with 144 ISA Brown laying hens. At the starting point the chickens were 62 wk old. The birds were randomly allotted to wire cages (29×32×36 cm), 1 bird per cage. There were three treatments with 16 replicates each and 3 period points (2, 4 and 6 wk). A basal diet (Table 1) was used as the control. SA diet (100 mg astaxanthin/kg feed) was prepared by adding 1 g Carophyll Pink (10% astaxanthin, w/w; Roche, Switzerland) to 1 kg basal diet, and NA diet (50 mg astaxanthin/kg feed) by adding 25 g *Phaffia rhodozyma* to 1 kg basal diet. The yeast contained astaxanthin at 2 mg/g yeast. These astaxanthin levels were set to give the similar levels of pigmentation (An et al., 2004). Natural astaxanthin preparation with *Phaffia rhodozyma* was described in the previous report (An et al., 2006). Feed and water were provided *ad libitum.* Lighting schedule was 17 h light and 7 h dark. The room temperature was maintained at 6～12℃.

2. Analysis of Astaxanthin in Blood and Skin

The analysis methods measuring for astaxanthin contents in blood and skin were described previously (An et al., 2004). Briefly, blood was obtained from the wing vein and mixed with EDTA. Skin was roughly cut with scissors and homogenized (Polytron PT-MR2100, Kinematica Co., Switzerland). The homogenized samples were mixed with dimethyl sulfoxide, acetone, petroleum ether, and 20% aqueous NaCl solution and the upper petroleum ether layer was used for astaxanthin analysis using HPLC.

Table 1. Composition of chemical formulas of experimental diets for laying hens (Na et al., 2004)

Ingredient	Content (g/kg)
Maize	683.3~680.3[1)]
Soya bean meal (CP 440g CP/kg)	178.2
Maize gluten meal	36.0
Carotenoid	0~3[1)]
Limestone	84.0
Tricalcium phosphate	9.3
DL-methionine50	0.9
L-Lysine80	0.8
Vit. + Min. complex[2)]	5.0
Salts	2.5
	Calculated composition
Calculated ME (MJ/ kg)	11.7
CP (g/kg)	160.0
Ca (g/kg)	34.0
P (g/kg)	4.0
Methionine (g/kg)	7.6
Lysine (g/kg)	3.3

[1)]0～30 g/kg of carotenoid (purity: 100 g/kg): β-8-Apo-carotenoic acid ethyl ester (ACAEE), canthaxanthin, and β-carotene.
[2)]Contained followings per kg of diet : vitamin A, 1,600,000 IU; vitamin D_3, 300,000 IU; vitamin E, 800 IU; vitamin K_3, 132 mg; vitamin B_2, 1,000 mg; vitamin B_{12}, 1,200 mg; niacin, 2,000 mg; pantothenate Ca, 800 mg; folic acid, 60 mg; choline chloride, 35,000 mg; DL-methionine, 6,000 mg; iron, 4,000 mg; copper, 500 mg; manganese, 12,000 mg; zinc, 9,000 mg; cobalt, 100 mg; BHT, 6,000 mg; iodide, 250 mg.

A HPLC system (SP930D, Younglin Instrument Co., Korea) with the Nucleosil column (100 Å, 4.6×250mm) (Nucleosil 100-5, MetaChem Technologies Inc., Torrance, USA), UV-visible detector at 476 nm, and mobile system (t-butyl methyl ether : hexane : isopropanol : methanol = 30 : 65 : 2.5 : 2.5) were used in this study. The column was used at room temperature and the flow rate was 0.5 mL/min.

3. Antioxidant Effect of Astaxanthin in Skin

The antioxidant activity of astaxanthin in skin was measured by measuring the inhibition of aldehyde formation during storage (Kosugi et al., 1989). Skin samples were incubated at 30℃ with continuous lighting for 15 days. Aldehyde contents were measured by using 2-thiobarbituric acid (TBA) method and malonaldehyde was used as a standard. The method with trichloroacetic acid was described previously (An et al., 2004).

4. Electronic Nose Analysis for Flavor Profiles

To determine the organoleptic characteristics, flavor patterns of chicken skin were analyzed using Electronic Nose (Fox 3000 Electronic Nose, Alpha M.O.S., France) equipped with a metal oxide sensor array (total 12 sensors) and an autosampler. One gram each of skin samples was placed separately in a 20 mL vial. The vials were then sealed with a silicon/PTFE septum and aluminum hole-cap. Six replications of each sample were analyzed three times by electronic nose. An air conditioning unit (air flow at 150 mL/h) was used to maintain the air (99.995% pure) at 20% constant relative humidity. Each sample vial was incubated in a heating chamber at 40℃ with agitation (500 rpm) for 10 min. Syringe temperature was set at 45℃, and headspace of the samples (2,000 μL) was injected into the sensors automatically. Acquisition and delay times of the sample were 120 s and 10 min, respectively. After acquisition, data processing software was used to collect raw data as the response of sensors. From the data obtained, principal component analysis (PCA) was conducted.

5. Statistical Analysis

Statistical analysis was performed with SPSS 14.0 (SPSS Science, Chicago, USA). When the F-value was significant ($p<0.05$), post-ANOVA test was conducted by using Tukey's test.

RESULTS AND DISCUSSION

1. Discrimination of Skins Fed with Astaxanthin by Electronic Nose

Our group previously reported that astaxanthin from *Phaffia rhodozyma* at 20～30 mg/kg feed was accumulated twice as much as that of synthetic astaxanthin (An et al., 2004). In this study, 62-wk-old laying hens were fed for 6 wk with NA or SA at 50 and 100 mg/kg feed, respectively, to get a rapid effect of pigmentation.

Astaxanthin accumulated in chicken skins were discriminated by electronic nose (Fig. 1). Compared to the control, the skins from 2-wk-fed chickens were discriminated but the electronic nose data showed that the distance between samples was not quite significant (Fig. 1). However, skins from 6-wk-fed chickens were successfully discriminated (Fig. 1). The 4-wk-fed samples were similar the 2-wk-fed ones (data not shown). However, skins from NA and SA were not discriminated by electronic nose. The astaxanthin contents in skins from NA and SA were not significantly different at 6th wk (Table 2). Even though SA was supplied twice as much as NA, the astaxanthin contents in skin from SA were lower than those from NA. The difference of astaxanthin contents in skins from NA and SA was largest after 4 wk feeding (1012 vs. 696 ng/g skin) but this difference disappeared after 6 wk (Table 2). This result indicated that accumulation of SA was slower than that of NA. To identify

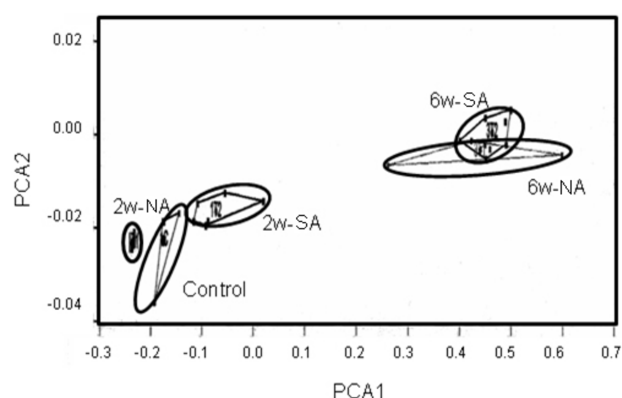

Fig. 1. Discrimination of pigmentation of chicken skin fed astaxanthin diet. Circles indicate grouping of 6.

Abbreviations: NA, Natural astaxanthin (dried cells of the red yeast, *Phaffia rhodozyma*); SA, Synthetic astaxanthin (Carophyll Pink® purchased from Hofman La Roche); and w, feeding period in week.

Table 2. Concentration of astaxanthin in breast skin and blood

Week	Astaxanthin in skin (ng/g)		Astaxanthin in blood (ng/mL)	
	NA	SA	NA	SA
2	519±192[b]	530±228[b]	1,575±574[a]	2,661±733[**]
4	1,012±177[a]	696±68[b**]	1,560±590[a]	2,478±419[a**]
6	1,080±192[a]	959±261[a]	1,239±349[a]	3,000±564[a**]

[a,b]Within a column, mean values lacking common letters differ significantly ($p \leq 0.05$).

[**]Within a row, mean values (NA and SA) are significantly ($p \leq 0.01$) different.

Abbreviations: NA, Natural astaxanthin (dried cells of the red yeast, *Phaffia rhodozyma*); SA, Synthetic astaxanthin (Carophyll Pink® purchased from Hofman La Roche)

the limiting steps of astaxanthin accumulation in skins from SA diet, the astaxanthin levels in blood were measured. The astaxanthin level in blood from SA was about 2-fold higher than that from NA, paralleling to the levels of astaxanthin in feed (Table 2). This implied that the transport of blood astaxanthin to skin was slow in the case of SA. NA contained large amount of lipid because the yeast *Phaffia rhodozyma* is used after cell wall hydrolyzation and this yeast contains 17% of lipid in total composition (10). The major tissue accumulating astaxanthin is skin containing large amount of lipid. Because of hydrophobic character of astaxanthin it can be transported with lipid and accumulated in lipid part of the tissue (unpublished data). Therefore, lipid NA might be effectively transported to skin from blood, compared to SA.

2. Discrimination between NA and SA Based on Antioxidant Activity in Skin by Electronicnose

We showed that astaxanthin could be used to improve the quality of broiler meat (An et al., 2004). Astaxanthin delayed the lipid peroxidation in skin during storage, preventing the production of off-flavor. Therefore, the antioxidant activity of astaxanthin in skin was measured by accelerating the lipid peroxidation in skin. Skin samples were homogenized and incubated at 30℃ under illumination. Production of lipid peroxides during storage, measured by TBA values, was notably decreased by SA but not by NA (Fig. 2). The NA from *P. rhodozyma* was mainly (3R,3'R)-astaxanthin (Andrewes and Starr, 1976). The

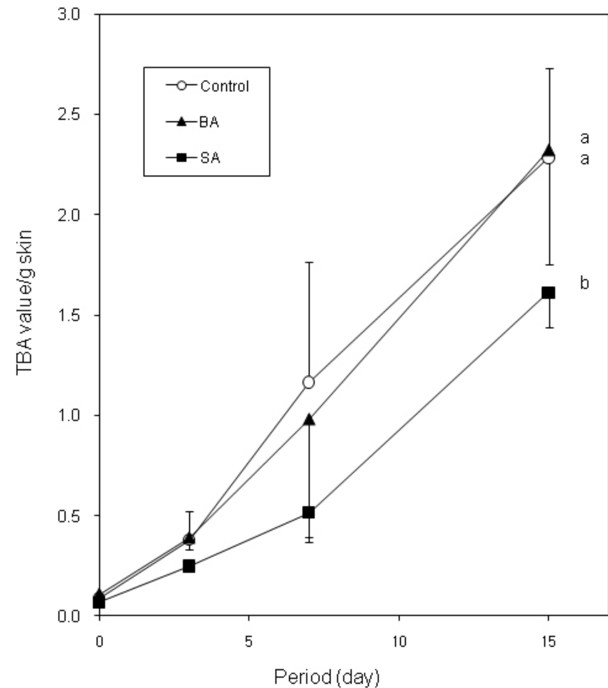

Fig. 2. Effects of astaxanthin diet on the prevention of skin lipid oxidation.

Skin samples were obtained from the chickens 6-wk-fed with astaxanthin. Symbols: ○, control; ▲, natural astaxanthin; ■, synthetic astaxanthin.

Bars indicate S.D.

Abbreviations: NA, Natural astaxanthin (dried cells of the red yeast, *Phaffia rhodozyma*); SA, Synthetic astaxanthin (Carophyll Pink® purchased from Hofman La Roche).

configuration ratio of SA was (3R,3'R):(3R, 3S'S):(3S,3'S)=1:2:1 (The catalog for Carophyll Pink, Hoffman La Roche, Switzerland). Probably, meso- or (3S,3'S)-astaxanthin has a strong antioxidant activity, compared to (3R,3'R)-astaxanthin. However, the effect of isomeric structure on antioxidant activity was not reported so far. At the TBA points higher than 0.4, meat samples usually emit unacceptable off-flavor (unpublished data). After 3 days of incubation at 30℃, TBA values of the skins of the control and NA were about 0.4 but TBA values of SA was about 0.2, suggesting less off-flavor by human nose. Therefore, SA could be used for lengthening the shelf life of chicken meat.

The volatile materials produced in skins were also discriminated by electronic nose. By PCA of the electronic nose, skins containing astaxanthin were discriminated with the control skin (Fig. 3, 0 day). After incubation for 7 days, the skin pigmented

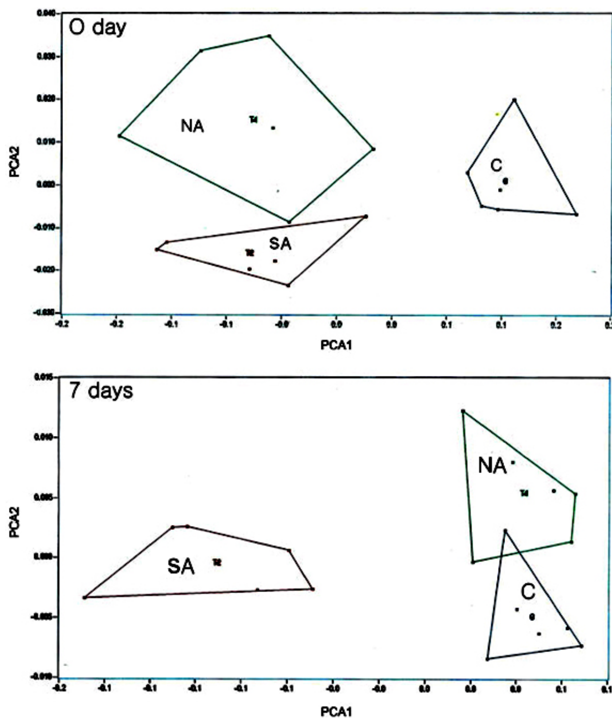

Fig. 3. Discrimination of skin oxidation by electronic nose. Skin samples were obtained from the chickens 6-wk-fed with astaxanthin.
Abbreviations: NA, Natural astaxanthin (dried cells of the red yeast, *Phaffia rhodozyma*); SA, Synthetic astaxanthin (Carophyll Pink® purchased from Hofman La Roche); and C, control. Panels: 0 day, control; and 7 days, incubation of skin in an incubator for 7 days at 30℃.

by NA moved to the direction of the control skin (Fig. 3, 7 days). Consequently, electronic nose could discriminate SA skins from NA and the control skins based on their antioxidant activities.

In conclusion, electronic nose can discriminate chickens fed with astaxanthin. It also can discriminate astaxanthin sources (NA vs. SA) based on peroxide detection. Since the developed method with electronic nose is easy and fast, it can be applied for the quality control in chicken meat industry.

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