Analysis of abietic acid and dehydroabietic acid residues in raw ducks and cooked ducks

Yongzhi Zhu,* Suzhen Zhang,*† Zhiming Geng,* Daoying Wang,* Fang Liu,* Muhan Zhang,* Huan Bian,* and Weimin Xu*

*Institute of Agricultural Products Processing, Jiangsu Academy of Agricultural Sciences, Nanjing, 210014, P.R. China; and †Key Laboratory of Meat Processing and Quality Control, Ministry of Education, Nanjing Agricultural University, Nanjing, 210095, P.R. China

ABSTRACT Rosin was once widely used for removal of duck feathers in China and is still being used secretly in some poultry processing enterprises. Abietic acid (AA) and dehydroabietic acid (DHAA) are the major compounds of rosin. In the present study, 90 duck samples were collected for investigation of AA and DHAA residues. Abietic acid and DHAA were simultaneously detected in 13 out 40 raw ducks, 8 out of 26 water-boiled salted ducks, and 7 out of 24 roasted ducks, respectively. In positive samples, averages of AA were significantly higher than those of DHAA in positive samples of the 3 types of ducks ($P < 0.05$). Averages of AA and DHAA in positive raw ducks were significantly higher than those in positive roasted ducks ($P < 0.05$). The results indicated that almost one-third of raw ducks were defeathered by means of rosin-containing defeathering agent, and cooking processes could reduce the AA and DHAA residues to some extent, but could not eliminate them completely.

Key words: abietic acid, dehydroabietic acid, defeathering, duck, high-performance liquid chromatography

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INTRODUCTION

Abietic acid (**AA**) and dehydroabietic acid (**DHAA**) are the major components of rosin, which is extensively used in manufacturing of solder, paper, printing ink, adhesive, and plasticizer. Abietic acid and DHAA (Figure 1) are well-known causes of pulmonary sensitization and skin allergy (Smith et al., 1997), and their oxidation products are regarded as the principal sensitizers (Karlberg et al., 1988). The AA and DHAA are reported to inhibit growth of the crustacean *Daphnia magna* (Kamaya et al., 2005). In cytotoxicology, AA has proved to cause lysis of human alveolar epithelial cells (Ayars et al., 1989), whereas DHAA has been reported to increase cell death in human epithelia and fibroblast cells (Söderberg et al., 1996). Abietic acid and DHAA can be detected in pulp and paper mill effluent (Liss et al., 1997), environmental samples (Smith et al., 1996; McMartin et al., 2002) and can also be found in many consumer products, such as medicaments (Lee et al., 1997), cosmetics, and industrial materials (Nilsson et al., 2008). Furthermore, both of them were reported to exist in food packaging materials and migrate into food (Mitani et al., 2007).

Rosin was once widely used as a defeathering agent in duck processing in China, the largest producer and consumer of duck in the world. The production and processing of duck can be traced back thousands of years, and the end products derived from duck (e.g., dry-cured, roasted, and water-boiled salted ducks) have great popularity in China as well as in Southeast Asia due to their delicate flavor and texture (Liu et al., 2006; Xu et al., 2008). In Nanjing, China, where these end products were originally developed, about 30 million ducks are consumed every year (Liu et al., 2006). Unlike chicken feathers, duck feathers, especially pin and down feathers, are difficult to remove by hand or machine. Therefore, removal of duck feathers is time-consuming and laborious. By using rosin, alone or in combination with other constitutes, duck feathers can be removed easily. However, such a defeathering agent is usually black and sticky, smells unpleasant, and looks like bitumen after repeated uses, which gave rise to concerns about the potential risk caused by such a defeathering agent in the last 20 yr in China. In 2009, the Food Safety Law of the People's Republic of China (2009) was made, which describes that the additives involved in food processing must be on the list of food additives given by the National Food Safety Standards. Rosin is

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[1]Corresponding author: zmgeng@163.com

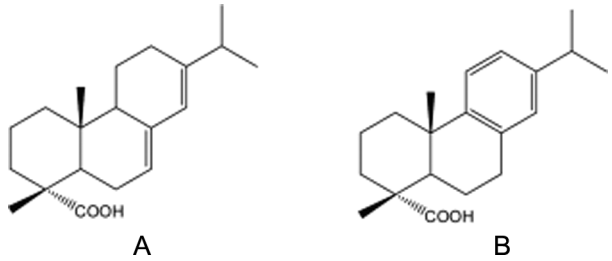

Figure 1. Structures of abietic acid (A) and dehydroabietic acid (B).

not on this list, which means rosin is no longer allowed to be used in food processing, including defeathering. However, it is still used secretly in some small poultry processing enterprises due to its outstanding defeathering performance, high maneuverability, and low cost. In our previous paper, we reported for the first time that rosin-defeathering could result in the accumulation of AA and DHAA residues in duck skins and both residues were detected in some commercial raw ducks from supermarkets in Nanjing (Zhu et al., 2014).

The aim of the present paper was to further investigate the residues of AA and DHAA in raw ducks from farm product markets and cooked ducks (i.e., roasted and water-boiled salted ducks) from cooked food shops in Nanjing, and evaluate the differences of the residues between raw and cooked ducks.

MATERIALS AND METHODS

Forty raw duck samples, stored in a polyethylene bag at −20°C with an average weight of approximately 2.0 kg, were purchased from farm product markets in Nanjing. These raw ducks were from poultry processing enterprises in Jiangsu, Shandong, Anhui, and Henan provinces, the major provinces of duck producing and processing in China. Fifty cooked duck samples were collected from cooked food shops in Nanjing, including 24 roasted ducks and 26 water-boiled salted ducks, both of which are major end products of duck in Nanjing. For determination of AA and DHAA, skin (including subcutaneous fat) of duck was collected from the breast and the thigh, followed by mincing and mixing. A portion of each sample (100 g) was kept in a polyethylene bag at −20°C until HPLC analysis. Results were expressed as means of 4 replicates of each duck sample.

HPLC Analysis of AA and DHAA

The analysis of AA and DHAA was conducted on an e2695 HPLC system (Waters, Milford, MA), coupled with photodiode array detector (**PAD**) and fluorescence detector (**FLD**) in series, according to our earlier paper (Zhu et al., 2014) with some modifications. In brief, 5 g of sample was weighed into a 50-mL centrifuge tube, followed by the addition of 20 mL of acetonitrile. The tube was capped and shaken in an ultrasonic shaker for 15 min, followed by centrifugation at 1,644 × *g*, 25°C, for 15 min. Four milliliters of supernatant was transferred into another tube and mixed with 4 mL of water, followed by centrifugation at 1,644 × *g*, 25°C, for 5 min. The supernatant was purified by using a C18 SPE cartridge (Sep-Pak C18, 500 mg/3 mL, Milford, MA). The eluant was dried under a gentle stream of nitrogen at 40°C, and the residue was dissolved in an adequate amount of mobile phase (depending on the levels of analytes), followed by filtration through a 0.22-μm syringe filter before HPLC analysis.

The analytes were separated on an Xbridge C18 column (4.6 × 250 mm, 5 μm, Waters, Milford, MA) by methanol/2 m*M* phosphoric acid (86/14, vol/vol), and AA was detected at 240 nm by PAD, whereas DHAA was detected by FLD with excitation wavelength at 225 nm and emission wavelength at 287 nm.

Statistical Analysis

Statistical analysis was carried out by SPSS software, version 17.0 for Windows (Chicago, IL). The ranges, means, medians, and Q_L~Q_U (the range from the lower quartile to the upper quartile) of AA and DHAA were assessed by descriptive statistics. The differences of AA and DHAA among raw, roasted, and water-boiled salted ducks were evaluated by tests for several independent samples, and the levels of AA and DHAA were compared by one-way ANOVA.

RESULTS AND DISCUSSION

Concentrations of AA and DHAA in Raw, Salted, and Roasted Ducks

The AA and DHAA residues in raw and cooked ducks were determined simultaneously by HPLC-PAD-FLD, and the results are summarized in Table 1. The AA and DHAA were detected in 13 out of 40 raw ducks, indicating that approximately one-third of raw ducks were defeathered by means of rosin. Compared with our previous report (Zhu et al., 2014), the positive rate of raw ducks seemed to be much higher. In the present study, samples were from farm product markets, whereas the samples were from supermarkets in the earlier study. In China, supermarkets grew in the last decade, and there are still a lot of farm product markets. Relatively speaking, supermarkets carry out rigid evaluations of commodities and suppliers, whereas there are almost no such evaluations in farm product markets. Such differences might be the major contributor to the difference in prevalence rates. The AA and DHAA were detected in cooked ducks with the positive rates of 30.8 and 29.2% in water-boiled salted and roasted ducks, respectively. These positive prevalence rates, which were almost identical to those in raw duck, indicated that neither roasting nor water-boiling seemed to eliminate the residues in raw ducks.

Table 1. Concentrations of abietic acid (AA) and dehydroabietic acid (DHAA) in raw and cooked ducks

Sample	Range (μg/g)	Mean (μg/g)	Median (μg/g)	Q_L-Q_U[1] (μg/g)	Positive rate (%)
Raw duck					
AA	0.60–9.64	3.08	2.30	1.40–4.31	32.5 (13/40)
DHAA	0.58–3.75	1.55	1.38	0.93–1.79	32.5 (13/40)
Water-boiled salted duck					
AA	0.65–3.83	1.76	1.33	1.00–2.53	30.8 (8/26)
DHAA	0.31–2.39	1.01	0.79	0.47–1.52	30.8 (8/26)
Roasted duck					
AA	0.65–1.96	1.23	1.16	0.73–1.54	29.2 (7/24)
DHAA	0.16–1.17	0.64	0.60	0.42–0.91	29.2 (7/24)
Total[2]					
AA	0.60–9.64	2.24	1.55	1.16–2.85	31.1 (28/90)
DHAA	0.16–3.75	1.17	0.95	0.61–0.95	31.1 (28/90)

[1]Q_L, the lower quartile (25 %); Q_U, the upper quartile (75 %).

[2]Total, sum of samples, including raw, water-boiled salted, and roasted ducks.

The AA and DHAA occurred simultaneously in all the positive samples, which was in agreement with our previous study (Zhu et al., 2014). Both residues demonstrated huge variations, especially for raw ducks, in which AA and DHAA varied from 0.60 to 9.64 and 0.58 to 3.75 μg/g, respectively. In common practices, rosin is usually mixed with other ingredients to improve its defeathering performance. The differences in defeathering agent recipes, together with the differences in processing conditions (e.g., defeathering temperature and time) among poultry processing enterprises might lead to the remarkable differences of AA and DHAA residues in ducks.

Differences and Correlations Between Concentrations of AA and DHAA in Positive Ducks

The major components of rosin, AA and DHAA, are organic weak acids with hydrophobic characteristics. Because rosin is a natural product, its composition varies with source. In general, rosin contains 30 to 50% of AA and 3 to 20% of DHAA (Wang et al., 2007; Botham et al., 2008; Nilsson et al., 2008). Therefore, in most cases, AA content is usually much higher than DHAA in the variety of matrix (Lee et al., 1997; Mitani et al., 2007; Zhu et al., 2014). In the present study, averages of AA were 1.99, 1.74, and 1.92 times higher than those of DHAA in positive raw, water-boiled salted, and roasted ducks, respectively, and all the averages of AA were significantly different from those of DHAA in the 3 types of positive ducks ($P < 0.05$; Table 2). Correlation analysis indicated that AA and DHAA were significantly correlated with each other in positive raw ducks ($P < 0.05$) and in total of 28 positive ducks ($P < 0.01$), whereas there was no such relation in positive water-boiled salted ducks and positive roasted ducks due to the small size of positive samples. Obviously, the differences and relationship between AA and DHAA contents in positive raw and cooked ducks resulted from rosin's physicochemical characteristics.

Resin acid of the abietic type is not stable at high temperature (Barton, 1949; Zeiss, 1948), and AA is more likely to be oxidized than DHAA, and the oxidation products are regarded as the principal allergens (Nilsson et al., 2008). Furthermore, a toxicology study indicated AA is more toxic than DHAA toward *Daphnia magna* as well as rainbow trout (Peng and Roberts, 2000). Cooked ducks are usually processed at 85 to 180°C (Liu et al., 2006). Therefore, the significant higher level of AA in positive ducks, especially in positive raw ducks, should be of greater concern.

Comparisons of AA and DHAA Residues Among Positive Raw, Water-Boiled Salted, and Roasted Ducks

To evaluate the differences of AA and DHAA residues among the 3 types of positive ducks, comparisons of AA and DHAA levels in positive raw, water-boiled salted, and roasted ducks were conducted by 1-way ANOVA (Figure 2). Average of AA in positive

Table 2. Differences and correlations between concentrations of abietic acid (AA) and dehydroabietic acid (DHAA)

Sample	AA/DHAA[1]	Difference (t)	Correlation (r)
Raw duck	1.99	2.779*	0.631*
Water-boiled salted duck	1.74	2.802*	0.603
Roasted duck	1.92	3.628*	0.461
Total[2]	1.91	3.874**	0.689**

[1]AA/DHAA, ratio of average of AA to average of DHAA.

[2]Total, sum of positive ducks, including positive raw, water-boiled salted, and roasted ducks.

*, **Significant at $P < 0.05$ and $P < 0.01$, respectively.

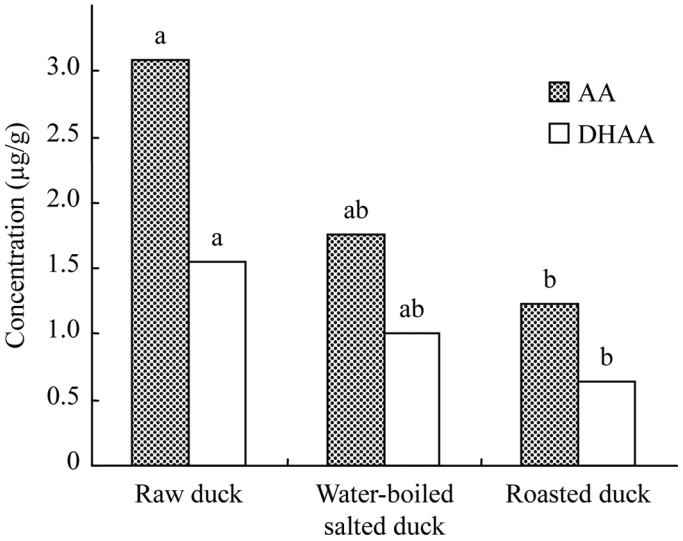

Figure 2. The average concentrations of abietic acid (AA) and dehydroabietic acid (DHAA) in different ducks. Different letters (a,b) indicate significance at $P < 0.05$.

raw ducks was 1.75 and 2.50 times higher than those in positive water-boiled salted ducks and in positive roasted ducks, respectively, whereas average DHAA in positive raw ducks was 1.53 and 2.42 times higher than those in positive water-boiled salted ducks and positive roasted ducks, respectively. There were significant differences of AA and DHAA levels between positive raw ducks and positive roasted ducks ($P < 0.05$). However, the averages of AA and DHAA in positive water-boiled salted ducks were found to be nonsignificantly different from those in positive raw ducks, and positive roasted ducks as well.

This result indicated that different cooking processes might have different effects on reducing AA and DHAA residues in raw ducks. Roasted ducks are processed at high temperature (more than 175°C) for a long period of time (about 45 min), whereas water-boiled salted ducks are processed at a much lower temperature (85–90°C; Liu et al., 2006). During the making of roasted ducks, roasting at high temperature for a long time causes oil to be exuded from the duck bodies continuously, which contains a lot of AA and DHAA due to their hydrophobic characteristics. In addition, AA and DHAA may undergo isomerisation, oxidation, or other reactions at high temperature to produce other products that cannot be measured by the method dedicated to analysis of AA and DHAA (Peng and Roberts, 2000). These factors contribute to the significant decreases of AA and DHAA in roasted ducks compared with those in raw ducks. Water-boiled salted ducks are manufactured at mild conditions, and AA and DHAA residues may be decreased because of the fat dissolving in hot water when cooking the salted ducks in water at about 90°C. The AA and DHAA levels in water-boiled salted ducks were lower to some extent than those in raw ducks, but the differences were not significant. To evaluate the effects of cooking modes on the reduction of AA and DHAA residues in raw duck, corresponding tests are being carried out in our laboratory, in which water-boiled salted and roasted ducks were cooked according to the classic protocols using rosin-defeathered ducks as raw ducks, followed by analysis of AA and DHAA. The tentative result indicated AA in raw ducks were decreased by 37 and 62% after making water-boiled salted and roasted ducks, respectively (done in our laboratory, unpublished data). This result supported the finding in the present paper, indicating that roasting seems to be a more efficient mode to reduce resin acid residues in raw ducks compared with water-boiled salting.

It is worth noting that rosin is not a disposable defeathering agent in the processing of raw ducks. In common practices, rosin is heated to the melting point followed by cooling repeatedly, with periodic additions of fresh rosin for keeping the amount as well as the defeathering performance. During the continuous circles of heating and cooling, rosin becomes darker and darker because of oxidation or other reactions, and corresponding products are produced. The physicochemical properties of these products and their residues in raw duck remain largely unknown. In addition, although AA and DHAA in raw ducks can be reduced to some degree after cooking, there are still comparable amounts of AA and DHAA residing in cooked ducks, which will be ingested by humans when the end products are consumed. Besides AA and DHAA, their oxidized products, the major skin and pulmonary sensitizers, may be produced and coexist with AA and DHAA during cooking of ducks, especially roasting ducks at high temperature for a long period of time.

Rosin has been prohibited to be employed in defeathering of ducks since 2009 in China; however, almost one-third of ducks were still defeathered by means of rosin or rosin-containing defeathering agent, which suggests that more rigid measures should be taken to monitor the abuse of rosin in processing of ducks and prevent the rosin-defeathered ducks from entering circulation. In addition, more effort should be made to develop a corresponding substitute for rosin, which should have excellent performance with low price and be accepted extensively by duck processors.

Conclusion

Abietic acid and DHAA were simultaneously detected in 13 out 40 raw ducks, 8 out of 26 water-boiled salted ducks, and 7 out of 24 roasted ducks, respectively. In all positive samples of the 3 types of ducks, averages of AA were significantly higher than those of DHAA ($P < 0.05$). Averages of AA and DHAA in positive raw ducks were significantly higher than those in positive roasted ducks ($P < 0.05$). The results indicated that almost one-third of raw ducks in circulation were still being defeathered by means of a rosin-containing defeathering agent, and cooking processes could reduce the AA and DHAA residues to some extent, but could not eliminate them completely.

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