Effect of Different Cooking Methods on Histamine Levels in Selected Foods

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Background: Histamine in food is known to cause food poisoning and allergic reactions. We usually ingest histamine in cooked food, but there are few studies about the influence of cooking method on the histamine level. Objective: The purpose of this study was to determine the influence of cooking methods on the concentration of histamine in foods. Methods: The foods chosen were those kinds consumed frequently and cooked by grilling, boiling, and frying. The histamine level of the food was measured using enzyme-linked immunosorbent assay. Results: Grilled seafood had higher histamine levels than raw or boiled seafood. For meat, grilling increased the histamine level, whereas boiling decreased it. For eggs, there was not much difference in histamine level according to cooking method. Fried vegetables had higher histamine levels than raw vegetables. And fermented foods didn’t show much difference in histamine level after being boiled. Conclusion: The histamine level in food has changed according to the cooking method used to prepare it. Frying and grilling increased histamine level in foods, whereas boiling had little influence or even decreased it. The boiling method might be helpful to control the effect of histamine in histamine-sensitive or susceptible patients, compared with frying and grilling. (Ann Dermatol 29(6) 706~714, 2017)

Keywords- Biogenic amines, Food, Histamine

INTRODUCTION

Food-derived histamine is associated with non-allergic food intolerance and food poisoning, whereas endogenous histamine stored in mast cells is responsible for food allergy reactions. At high concentrations, they are risk factors for food intoxications, whereas moderate levels, may lead to food intolerance. Histamine intolerance and poisoning resulted from the disequilibrium of accumulated histamine and the capacity for histamine degradation by the enzyme diamine oxidase (DAO). Ingested histamine can be metabolized by DAO, which is mainly present in intestinal epithelial cells. If we ingest excessive histamine, un-degraded histamine could be absorbed into the body. Impaired histamine degradation based on reduced DAO activity, and the resulting excess of histamine, may cause numerous symptoms mimicking an allergic reaction. There are some concrete clinical examples of reduced DAO activity including the subgroup of patients those with atopic dermatitis, that are pregnant, and other chronic disease (e.g., liver cirrhosis, anorexia nervosa, inflammatory bowel disease: ulcerative colitis and Crohn’s disease), and those that use anticancer drugs. Even the abundance of histamine is not the only causative factor for food allergy, there are some evidences which address...
higher levels of histamine-containing food could induce more adverse reaction than lower levels of histamine-containing food in susceptible patients.\textsuperscript{5,7,8,13} Various approaches, such as modified atmosphere packaging, irradiation, high hydrostatic pressure, and food additives and preservatives have been applied to control the accumulation of histamine in food products.\textsuperscript{14} These methods of controlling histamine content rely mainly on growth inhibition of histamine-producing bacteria and histamine decarboxylase activities.\textsuperscript{15}

There are many reports regarding the histamine content in raw food.\textsuperscript{2,16-18} High histamine levels are found in food such as tuna, mackerel, anchovy, spinach, wine, cheese, sausage and fermented foods.\textsuperscript{2,18} However, there are few reports regarding the histamine content in cooked food and the influence of cooking methods. Because we eat cooked food more often than raw, we need to know how cooking methods influence histamine level. To determine the influence of cooking method on the concentration of histamine, we started with a list of foods rich in histamine; then compared the histamine level of these foods when raw, and after household processing (e.g., frying, grilling and boiling).

**MATERIALS AND METHODS**

**Foods**

Because there are numerous food items, the most commonly eaten foods were given priority. Twenty-seven foods often consumed by Koreans were selected for this study. We got help determining representative food items by consultation with those from the Department of Nutrition at Hallym University Kangnam Sacred Heart Hospital, Hallym University College of Medicine. These were categorized as 1) fishery products and processed marine products, 2) meat, processed meat, and eggs, 3) vegetables, 4) fermented pastes and dairy products (milk and cheese). The detailed foods list is presented in Table 1. Processed marine products included canned tuna (Light Tuna Can; Dongwon, Seoul, Korea) and processed meat included sausage (a mixture of 19% pork and 64% chicken, Bek-Sul Kentucky Frank; Cheiljedang, Seoul, Korea), and ham (pork only, Spam; Cheiljedang). Fermented foods were bought; soybean paste (Chamdoenjang; Pulmuone, Seoul, Korea), red pepper paste (Haechandeul Taeryangcho gold red pepper paste; Cheiljedang), salty fermented anchovies (Haechandeul Haseonjeong fermented anchovy; Cheiljedang), and Kimchi with cabbage (Pulgipeun taste Junlado Kimchi; Pulmuone), Kimchi with radish (Pulgipeun taste Junlado Kkakdugi; Pulmuone) and the cheese was hard, sliced cow cheese (de Vinch; Namyang, Seoul, Korea). These foods were all bought at local hypermarket on the day of experiment (E-mart Yeouido, Seoul, Korea).

**Cooking methods**

We gave priority to the cooking methods most commonly used by Koreans, including boiling, grilling, and frying. When selecting representative cooking methods, we also got help and consultation from the same nutrition department. The foods were cooked by boiling, grilling, and frying. Because the histamine concentration is also dependent on the freshness of food, the foods were cooked immediately after purchase. Before cooking, all the raw foods were prepared by cutting into 1-g portions. The cooking temperature was monitored continuously using thermocouples.

Boiling was conducted at 90°C for 5 ∼ 10 minutes in a water bath (500 ml). Distilled water was also 90°C when it was boiled in aluminum foil cups. Grilling was performed at 150°C in a preheated pan for 1 ∼ 5 minutes without oil. Fishery products and processed marine products, eggs, meat and processed meat were grilled, or boiled until well done. For frying, fresh soy bean oil (10 ml) was used. Dried anchovy, eggs, onions, carrots, and laver seaweed were fried at 150°C for 1 ∼ 5 minutes in a preheated pan. Spinach was blanched for 30 seconds at 90°C in aluminum foil cups.

The laboratory room temperature was controlled to 25°C.

**Table 1. Food items in our study**

| Fishery and processed marine products | Dried anchovy, canned tuna, shrimp, squid, Spanish mackerel, saury, hairtail, mackerel |
|--------------------------------------|----------------------------------------------------------------------------------|
| Eggs, milk, meat, and processed meat | Eggs of chickens, milk*, pork, chicken, beef, sausage, ham                        |
| Vegetables                           | Spinach, carrots, onions, cabbage, radish, laver seaweed                         |
| Fermented foods                     | Soybean paste, red pepper paste, salty fermented anchovies, fermented cabbage Kimchi, fermented radish Kimchi, cheese* |

*Daily product (milk and cheese).
The laboratory workplace guidelines for humidity are 40%～60%. All our experiments were done under these conditions.

**Histamine measurements**

Before cooking, 30 gram samples of each fresh foods (dried anchovy, tuna in can, shrimp, Spanish mackerel, saury, hairtail, mackerel, pork, chicken, beef, sausage, ham, egg, onion, carrot, spinach, laver seaweed, cabbage, radish, soy bean paste, red pepper paste, salty fermented anchovies, Kimchi, fresh milk and cheese) were ground in a mixer (SFM-656CS; Shinil Industry, Seoul, Korea) for measurement of histamine level, respectively. After cooking, same method was done for each material to check histamine level. For each test, 1 g of food was homogenized with 5 ml 0.1 M HCl in a mixer for 5 min. The homogeneous samples were further centrifuged for 10 min at 3,000 × g. Then, 500 μl of samples were mixed with 500 μl of diluents (phosphate buffer saline) to reduce matrix interference. The histamine assay of each diluted food sample was performed with histamine enzyme-linked immunosorbent assay (ELISA) kit (Cat no. 5133-8; Diagnostic Automation, Inc., Calabasas, CA, USA). The instructions for this ELISA kit are based on detection of histamine only for food. Each histamine measure was expressed as ×10⁻³ ppm. During all experimental processes, three replicate measures per treatment food-combination were conducted.

**Statistical analysis**

All experimental values were expressed as means ± standard deviations of three replicates. The statistical significance of any differences between data was assessed by analysis of variance (ANOVA), followed by the Dunnett’s test or Tukey’s test. *p values < 0.05 were considered to be statistically significant. All statistical analyses were conducted using PASW Statistics ver. 18.0 (IBM Co., Armonk, NY, USA).

**RESULTS**

**Fishery products and processed marine products**

Foods in the fishery products and processed marine products group were cooked according to the representative cooking methods: frying, grilling or boiling. Then, we measured the histamine levels in cooked foods. Fig. 1 shows the histamine levels of the cooked fishery products and processed marine products.

Fried dried anchovy (13,347 ± 10,738.81×10⁻³ ppm) showed the highest histamine level in this group, followed by grilled dried anchovy (2,669 ± 1,538.20×10⁻³ ppm). In the case of dried anchovy, the histamine level increased after frying (13,347 ± 10,738.81×10⁻³ ppm), grilling (2,669 ± 1,538.20×10⁻³ ppm), and boiling (105 ± 41.94×10⁻³ ppm). When fried, the histamine level of dried anchovy showed a >200-fold increase than when uncooked, and when grilled, it showed about a 45-fold increase. When boiled,
the histamine level increased only 2-fold. In the case of tuna, the histamine level was increased about 5-fold by grilling (717 ± 161.52 × 10⁻³ ppm), but decreased slightly after boiling (78 ± 63.55 × 10⁻³ ppm). For shrimp, saury, hairtail, and mackerel, the histamine level slightly increased or was not changed, by boiling. However, grilling caused it to increase remarkably (8 – 56 fold). The histamine level of Spanish mackerel was increased about 2-fold by grilling, while there was no distinctive change in histamine level from boiling. The method of cooking caused no distinctive changes in the histamine level of squid. Taken together, grilling in most of the fishery products and processed marine products group increased the histamine level more than boiling did. Also, regarding frying, only dried anchovy in the category of fishery/processed marine products was fried in our study.

**Meat, processed meat and egg**

Foods in the meat, processed meat and egg group were cooked by frying, grilling, or boiling; then we measured the histamine levels in the cooked foods (Fig. 2). Grilled pork showed the highest histamine level in this group (1,146 ± 1,016.90 × 10⁻³ ppm). For pork and chicken, the histamine level was increased about 1.5-fold by grilling, but was decreased 10% – 20% by boiling. The histamine level of beef changed less than other cooked meats, grilling caused a 1.8-fold increase, while there was no distinctive change due to boiling. In the case of sausage, the histamine level was slightly increased (1.03-fold) by grilling (502 ± 77.38 × 10⁻³ ppm), but decreased 60% after boiling (193 ± 20.42 × 10⁻³ ppm). The histamine level of ham was increased about 1.4-fold by grilling (283 ± 142.90 × 10⁻³ ppm), but decreased 60% after boiling (79 ± 19.52 × 10⁻³ ppm). These results showed that grilling increased the histamine level of most meat and processed meat, while boiling decreased their histamine levels.

For eggs, there was not much difference in histamine level in relation to cooking by boiling (12 ± 1.73 × 10⁻³ ppm) or frying (11 ± 1 × 10⁻³ ppm).

**Vegetables**

Foods in the vegetable group were cooked by frying or blanching. Fig. 3 showed the histamine levels of cooked vegetables. For onions and spinach, no distinct difference in histamine level resulted from these cooking methods. In the case of carrots, the histamine level was increased 2.5-fold by frying (31 ± 6 × 10⁻³ ppm). The histamine level of laver seaweed was increased about 4-fold by frying (168 ± 39.69 × 10⁻³ ppm). Frying increased the histamine level in carrots and laver seaweed.

**Fermented pastes and dairy products**

Fermented pastes, including soybean paste (1,164 ± 79 × 10⁻³ ppm) and red pepper paste (1,448 ± 98 × 10⁻³ ppm), showed high histamine, but not much difference in histamine level was caused by boiling. Salty fermented anchovies showed a much higher histamine level (1,157 ± 313 × 10⁻³ ppm) than did dried anchovies (59 ± 11 × 10⁻³ ppm). Additionally, we compared the histamine levels in fermented and fresh vegetables. Kimchi, including both fermented cabbage (378 ± 22 × 10⁻³ ppm) and radish (500 ± 74 × 10⁻³ ppm), showed 30 – 50-fold increase in histamine levels compared to fresh cabbage (11 ± 1 × 10⁻³ ppm) and fresh radish (10 ± 2 × 10⁻³ ppm). The histamine level of dairy cheese (418 ± 85 × 10⁻³ ppm) was significantly 20-fold greater than that of fresh milk (12 ± 7 × 10⁻³ ppm) (Fig. 4).
DISCUSSION

Biogenic amines are organic, basic nitrogenous compounds of low molecular weight, usually formed by de-carboxylation of free amino acids. In addition to their well-known occurrence and important role as endogenous regulators of several human physiological processes, biogenic amines occur in many different foods and beverages. Their concentrations vary extensively, not only between different food varieties, but also within the varieties themselves. It has been known for some time that uptake of biogenic amines from foods can have profound effects on human health and well-being. The most frequent food-borne intoxications and intolerance caused by biogenic amines, involve histamine. Whereas high histamine consumption causes life threatening intoxication, lower amounts can lead to headache, nausea, hot flushes, skin rashes, sweating, respiratory distress, and cardiac and intestinal problems to histamine-sensitive people.

Most foods are usually eaten after cooking in various ways and we eat cooked food more often than raw. However, data on the effect of cooking on the histamine content of foods are still incomplete. In the present study, we evaluated the effect of cooking practices on the concentration of histamine in foods by comparing the histamine levels between raw and cooked foods. The histamine level of most fishery products and processed marine products, except for squid, was increased by grilling, and it seems that most of them increased greatly. Regarding frying, only dried anchovy in the category of fishery/processed marine products was fried in our study. Therefore, further study will be needed to determine the effect of frying on the lev-
el of histamine in other kinds of food in the category of fishery/processed marine products. However, in this group, boiling had little influence on histamine level, although it did increase very slightly. The histamine level of meat and processed meat was also increased by grilling, but not as much as fishery products and processed marine products. Meanwhile, boiling of most meats decreased the histamine level. For eggs, no significant changes in histamine level were observed in relation to frying and boiling. Fried vegetables had higher histamine levels than raw vegetables. As expected, the fermented food showed generally high histamine level. However, fermented pastes showed no changes in histamine level by boiling.

Pre-requisites for the formation of histamine are availability of free amino acid, such as histidine, presence of decarboxylase active microorganisms, and favorable conditions for decarboxylation of amino acids. Scombroid species of fish have naturally high levels of histidine in their muscle tissue, which can be used by microorganisms capable of producing the enzyme histidine decarboxylase, to convert histidine to histamine during growth. Evidently, food rich in free histidine, such as some fish species (anchovies, scombroid fish, and herring) are potentially more likely to contain high histamine levels. In our study, fishery group foods in their raw forms showed high histamine levels. High free histidine level and availability in the food is thought to result in high histamine level in food.

In our study, as expected, fermented foods, including Kimchi, soybean paste, and pepper paste, showed high histamine levels. Kimchi, soybean paste, and red pepper paste are traditional foods that Koreans enjoy eating, and there are some studies on histamine levels of these fermented foods. Kimchi is made by fermenting vegetables, such as salted cabbage or radish, with a number of other ingredients, including such as red pepper powder, garlic, and ginger. It is fermented by lactic acid bacteria at low temperatures, ensuring proper ripening and preservation. Soybean paste is generally made by additional fermentation of the solid material that separates from a mixture of Meju (fermented soybean lumps) and Ganjiang fermented soy source. The latter is prepared by soaking Meju in solar salt solution (approximately 16%–18% [w/v] salts) for approximately 1–2 months. Red pepper paste is produced by fermenting powdered red pepper combined with powdered Meju (fermented soybean powder), salt, malt-digested rice syrup, and rice flour for about six months. The fermentation process extends the storage period while increasing the bioavailability of bioactive ingredients such as free amino acids, peptides, alcohols, organic acids, capsaicin, and flavonoids. In fermented foods the contaminating microflora is mainly responsible for the generation of increasing histamine levels. Besides that, salt, sugar, red pepper or food additives can affect the level of histamine.

Cooking causes inactivation of histamine-producing spoilage bacteria. However, histamine is heat resistant, so it can remain intact in cooked products. Therefore, if histamine is produced in the product before cooking, it can cause illness if it presents in the product at toxic concentrations. The degradation change of histamine by a heating process, such as frying, has rarely been reported. In our study, heating processes, such as grilling and frying, increased the histamine levels in foods. The possible reason for these changes may have been that the moisture lost by evaporation during grilling or frying could cause the histamine concentration to increase. This also showed how the histamine level of boiling in some foods decreased. Previous study found that the food absorbed water while boiling, so the histamine concentration was decreased by dilution. The cellular components of foods might be softened and broken by boiling and consequently released into the boiling water. However, further studies will be needed to determine the losses from foods due to cooking (e.g., moisture loss) to confirm the precise mechanism of effect of cooking method on the histamine level in foods.

Also, another possible reason for the differences is that the histamine formation is affected by histidine decarboxylase activity. Histidine decarboxylase is the enzyme that converts the histidine in food into histamine. The formation of histamine in food requires the presence of histidine decarboxylase-positive microorganisms, in conjunction with conditions allowing the growth and enzyme activity of these bacteria. It is known that there are several factors (e.g., pH, temperature, and NaCl concentration) that affect histidine decarboxylase activity. This enzyme activity increased with increasing temperature to 30°C–40°C and decreased above 50°C. Although histidine decarboxylase activity decreased at high temperature, histamine production would continue until the enzyme became inactive. Histamine, once formed in food, is heat stable even if heating inactivates both the enzyme and the source microorganisms. Consequently, during heat treatment the histamine in food would accumulate continuously until the enzyme was inactive. This may explain why the histamine level was increased by heat treatment of most seafood and meat in our study. Boiling is also one of the heat treatments that elevated the histamine level, but the effect could be reduced due to dilution, as mentioned. However, further studies monitoring the levels of histamine-producing bacteria and histidine-decarboxylase activity in raw and cooked foods are necessary to confirm the precise mechanisms of histamine formation.
activity will also be needed to confirm this mechanism for the change of the histamine levels in cooked foods.

For eggs, no significant changes in histamine level were observed in relation to cooking methods. The change in the water content of eggs was reported to be minimal after boiling and frying, which may account for the small change in the histamine level after cooking. In addition, because the histamine level was relatively low for eggs ($10 \pm 2 \times 10^{-3}$ ppm), there is a possibility of detection failure because the changes after different cooking methods could also be too trivial to be measured\(^45\).

When interpreting the results for frying, the influence of soybean oil should be considered. However, because soybean oil has a relatively low histamine level ($14 \times 10^{-3}$ ppm), it is thought to have little effect on the level of histamine in fried foods.

Numerous factors during manufacture and distribution should be considered to affect on histamine contents in food. Previous study demonstrated that large variations in the amine content were found in retail Belgian sausages, and that these were related to the method of manufacture\(^46\). Furthermore, wide variations were observed in the amine content of different batches of the same commercial brand of fermented Spanish products\(^47\). Previous studies reported that fish could be contaminated with histamine-producing microorganisms during postharvest handling of the fish\(^24\). Fresh scombrotoxin-forming fish contain negligible amounts of histamine ($< 1$ ppm), but high levels of histamine occur when harvested fish are held at temperatures above $15^\circ C$ for several hours, permitting spoilage microorganisms to grow\(^44,48,49\). Several studies clearly show that immediate storage on ice drastically decreases the rate of histamine formation\(^50,52\). Also, high levels of histamine in commercially produced canned-fish products have occurred and are primarily due to temperature abuse before canning\(^55\). Therefore, the histamine contents in foodstuff can be affected by numerous extrinsic factors during manufacture and distribution. Confirming the variability of histamine level regarding manufacture and distribution will require examining the histamine content in more food items, in similar foods from more manufacturers, and in similar foods distributed in other ways.

The cooking time is another characteristic of a cooking method. The effect of cooking time on the histamine level should be considered. However, in our study, we created some variation in cooking time in the same cooking method. Fishery products and processed marine products, eggs, meat, and processed meat were grilled, or boiled until well done. It was considered meaningful to measure histamine levels of foods in actual ready-to-eat condition. Because the time needed to cook each food to reach that condition is different, we could not make the cooking time uniform.

In our study, we determined the histamine levels in raw and cooked foods. Because the levels detected in raw and cooked food in this study were significantly lower that the toxic level set by Food and Drug Administration (50 ppm), these levels or the increase caused by cooking could not induce food poisoning or intolerance. However, the susceptibility to histamine varies according to the enteral environment and DAO activity of each individual\(^5\). Sensitive persons, with insufficient DAO activity, could suffer from numerous undesirable reactions after intake of foods containing low amounts of histamine. We speculated that using the fresh foods with good pre-cooking condition could affect the low level of histamines in most foods of our study.

There are several limitations of this work and this article. The priority of the food items and cooking methods used in this work was based on their frequency of utilization by Koreans. Therefore, the outcome of this article would not definitely reflect the dietary characteristics of other (foreign) countries. Also, foodstuffs can be affected by numerous extrinsic factors during manufacture and distribution; so variability might exist among the same products. Also, only a few food items in each food category were used in this article; so it might be hard to generalize the results. It is necessary to confirm the difference in histamine levels related to more cooking methods and in more foods from more manufacturers.

To the best of our knowledge, there are no previous studies about direct comparison between cooking methods; so it is meaningful to make lists of the histamine levels of various cooked foods. We also tried to find some tendencies between the cooking methods regarding the level of histamine. In our study, it seems that grilled and fermented food showed increased histamine levels. However, due to the wide variation of basal histamine levels, there was no statistical significance in the differences between any of the food items. Also, among the categories of food items, the tendencies were a bit different; so it was hard to perform direct comparison between the cooking methods.

To conclude, this study showed that the histamine level in foods can change according to the cooking method used to prepare it. In our study, frying and grilling seems to increase histamine level in foods, whereas boiling had little influence or even decreased it. Considering our results, boiling, compared with frying, grilling and fermenting was found to be a more effective method for reducing the histamine content. This study could be beneficial for se-
lection of cooking practice in histamine-sensitive people with food intolerance, by providing data for reducing the histamine level in their diets.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

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