Effects of storage temperatures, vacuum packaging, and high hydrostatic pressure treatment on the formation of biogenic amines in Gwamegi

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Received: 3 September 2015 / Accepted: 24 September 2015 / Published online: 15 January 2016

Abstract This study investigated the effects of storage temperatures, vacuum packaging, and high hydrostatic pressure (HHP) on the formation of biogenic amines (BAs) in Gwamegi. The Gwamegi samples were subjected to 400 MPa pressure for 10 min at 20 °C or vacuum packaging, and then stored at −16, 5, or 25 °C for 28 days. The amounts of six BAs (histamine, tyramine, cadaverine, putrescine, spermine, and spermidine) and the total plate counts were checked weekly. Compared with the control at 5 °C, the total plate counts were 2–3 log CFU lower for the HHP-treated samples and 1–3 log lower for the vacuum-packaged samples. Tyramine, spermine, and spermidine were found in Gwamegi stored at −16 °C and tyramine, cadaverine, spermine, and spermidine at 5 °C, whereas all six BAs were found at 25 °C. Histamine was relatively low in amount in all the treated samples. The BAs reached a high level during storage at 25 °C, with the total concentration reaching 1099 ppm in 28 days. After HHP treatment or vacuum packaging, the concentration of cadaverine and spermidine decreased, whereas that of tyramine and spermine increased. Tyramine was the dominant BA in all the samples and during storage at 5 °C in the control (0–56.67 ppm), HHP-treated (0–105 ppm), and vacuum-packaged (0–90 ppm) samples. Both HHP treatment and vacuum packaging effectively reduced the formation of some types of BAs and improved the microbial quality in Gwamegi. However, simultaneously, they also promoted the formation of others types of BAs such as tyramine and spermine.

Keywords Biogenic amines · Gwamegi · High hydrostatic pressure · Temperature · Vacuum packaging

Introduction

Biogenic amines (BAs) are found naturally in living organisms and in high amounts in some foods and fermented foods, such as cheese, sausage, fermented vegetable, wine, and fish. BAs are nitrogenous, basic organic compounds of low molecular weight produced by the decarboxylation of amino acids, or by the transamination and amination of aldehydes and ketones as a result of plant, animal, and microbial metabolic activities (ten Brink et al. 1990; Silla-Santos 1996). The formation of BAs in foods depends on factors such as the presence of microorganisms with amino acid decarboxylase, the availability of precursors (amino acids), and optimum conditions for microbial growth and decarboxylating activity (ten Brink et al. 1990). Histamine, tyramine, putrescine, cadaverine, spermine, spermidine, 2-phenylethylamine, and tryptamine are the most common BAs present in foods (Silla-Santos 1996). The main interests in BAs are due to their potential toxicity to human health and their role as indicators of food quality. The presence of BAs could represent poor hygiene in the food processing or handling, and contamination by microorganisms. Normally, small amounts of BAs can be detoxified by the human intestinal amine oxidases (Lehane and Olley 2000; Ancín-Azpilicueta et al. 2008). However, the risk to health has been suggested to become serious when the activity of amine oxidases is disturbed or inhibited as a result of the ingestion of a high BA amount (Soufleros et al. 2007; Spano...
et al. 2010). The most prevalent BAs involved in food poisoning are histamine, tyramine, cadaverine, and putrescine. Numerous studies have identified histamine as the cause of scombroid poisoning (Hwang et al. 1995; López-Sabater et al. 1996; Becker et al. 2001; Koutsoumanis et al. 2010), which frequently occurs just between several minutes and 3 h after the consumption of fish or fish products with more than 1000 mg kg$^{-1}$ histamine (Luten et al. 1992). The signs of this poisoning include faintness, dizziness, itching, an inability to swallow and a mouth-burning sensation (Anonymous 1975). The risk is considered more severe for sensitive people who have used the antidepressant drug which is known to inhibit amino oxidase activity (Maríñez-Font et al. 1995). Ladero et al. (2011) reported the maximum total BA concentration of 750–900 ppm as being the intake limit, whereas 1000 ppm was proposed by Silla Santos (1996). However, establishment of the toxic dose of BAs still faces some difficulties as a result of the different characteristics of the individual. Therefore, it is imperative to quantify the BAs in foods and to discover effective strategies to minimize or reduce them.

BA production can be controlled by several methods. Nieto-Arribas et al. (2009) gave some evidence of the inhibition of BA accumulation in food from the application of techniques such as the use of high-quality raw material, good manufacturing practice, temperature control, and the use of amine-negative microorganisms as starter cultures.

High hydrostatic pressure is a non-thermal technique which is based on pressure, and is used to preserve food by inactivating or eliminating pathogenic or spoilage microorganisms. The application of HHP on raw material or final products of fermented foods is supposed to reduce the bacterial population and BA accumulation (Naila et al. 2010). On the other hand, there is also some controversy over the effect of pressure processing on BA production. Based on the study of Novella-Rodríguez et al. (2002), 50 MPa of pressure for 72 h at 14 °C did not induce any reduction of BAs in goat cheese ripened for 28 days; on the contrary, the BA amount increased significantly.

Storage temperature is the most important factor contributing to BA accumulation in foods, as certain enzymes of the decarboxylase family in microorganisms require specific temperatures for their action. Several researchers have clarified that a high content of BAs can be formed under high temperature (Wei et al. 1990; Du et al. 2002; Kim et al. 2002; Rodtong et al. 2005; Ruiz-Capillas et al. 2007).

Modified atmosphere packaging is a significant preservation method which supplies a changing gaseous mixture of the environment surrounding the food product. This technique could prevent the formation of BAs, as it has been found to possess the ability to inhibit the growth of microorganisms or enzymes with decarboxylation activity. Vacuum packaging is one type of modified atmosphere packaging and has been used on several food products. Compared with air packaging, vacuum packaging is better at extending the shelf life of the food (González-Montalvo et al. 2007).

Gwamegi is a Korean half-dried processed seafood product that is produced during the winter by naturally drying fish such as herring or saury. Generally, the drying process takes about 15 days or until the moisture content reaches 30 % under the sea wind. As Gwamegi is made by a traditional method, some concerns related to poor processing which could produce biogenic amines have been considered. Therefore, in order to improve the quality of Gwamegi, the effects of temperature, HHP treatment, and vacuum packaging on BA formation in Gwamegi were investigated in this study.

Materials and methods

Materials

Gwamegi (semi-dried saury fillet) was purchased from a store located in Korea. They were kept refrigerated during the transportation and stored in a refrigerator until analysis.

Standard BAs (histamine dihydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, and tyramine hydrochloride) and reagents (hydrochloric acid 0.1 M, sodium hydroxide, sodium bicarbonate, sodium chloride, dansyl chloride, acetone, ammonium hydroxide 25 %, and acetonitrile) were purchased from Sigma Chemical Co. (USA).

Sample preparation and storage

The Gwamegi samples were divided in three groups: HHP treatment, vacuum packaging, and control (without HHP treatment or vacuum packing). The samples were pressurized at 400 MPa for 10 min at 20 °C in a 5 L HHP 600 MPa pilot unit (Bao Tou Kefa High Pressure Technology Co., Ltd., China). For vacuum packaging, the sample was packed into vacuum seal bags (FoodSaver®) by using a vacuum sealer (FoodSaver, Vac 1075). All the samples were stored for 28 days at three different temperatures: −16, 5, and 25 °C.

Microbiological analysis

A 5 g sample was added to 45 ml of a sterilized dilute NaCl solution (0.85 %, w/v) in a sterile plastic bag. After homogenizing with a stomacher (Bagmixer, 400 vW; Interscience, France), appropriate decimal dilutions were poured-plated (1 mL) on the plate count agar medium (Difco, USA) and cultured at 35 ± 1 °C for 48 h in an incubator. All counted colonies were converted to logarithm units per gram.
Biogenic amine analysis

The content of the BAs was analysed by high-performance liquid chromatography (HPLC). All the procedures were based on the method developed by Ben-Gigirey et al. (1998) with minor modifications. The BAs were extracted by adding 20 mL of hydrochloric acid (0.1 M) to 5 g of sample, and then homogenizing the mixture for 30 min in an ultrasonic centrifuge, followed by centrifugation at 10,000 rpm at 4 °C for 20 min. The residue was extracted again with the same conditions. The supernatants were collected at both extractions and the volume was adjusted to 50 mL with hydrochloric acid (0.1 M).

For the assay, 1 mL of each standard solution or extracted sample was mixed with 300 µL of saturated sodium bicarbonate and 200 µL of sodium hydroxide (2 M). Then, 2 mL of dansyl chloride solution (10,000 ppm) prepared in acetone was added to the mixture, which was then incubated for 45 min at 40 °C. Residual dansyl chloride was removed by adding 100 µL of ammonium hydroxide (25 %) to the mixture and incubating at room temperature for 30 min, following which the mixture was adjusted to 5 mL with acetonitrile. Finally, the mixture was centrifuged at 2500×g for 5 min, and the supernatant was filtered through 0.2-µm-pore-sized filters.

An HPLC unit (Waters 600S Controller; Waters, USA) equipped with a Waters 996 photodiode array detector and Empower Pro software was employed. A C18 column (4.6 mm × 250 mm, 5 µm particle size) was used, with water (solvent A) and acetonitrile (solvent B) as the mobile phases running at the flow rate of 1 mL/min. The sample injection volume was 20 µL and the BAs was monitored at 254 nm.

Statistical analysis

Results are expressed as the mean with standard deviation (SD). The data were analysed from the triplicate tests by two-way analysis of variance. All statistical analyses were interpreted by using SPSS (19.0). Significant difference was considered at values of p < 0.05.

Results

Total plate counts of Gwamegi stored at three different temperatures for 28 days

The total plate counts from the Gwamegi samples during storage for 28 days at three different temperatures are shown in Fig. 1. According to the data, the overall total plate counts increased as the temperature and storage duration were increased. Moreover, the total plate counts showed significant differences (p < 0.05) among storage temperature conditions and storage duration. The microbial numbers were low in the −16 °C samples, whereas they were very high in samples stored at 25 °C. The initial total plate count in Gwamegi at day 0 was 1.77 ± 0.26 log CFU/g. The total plate counts in the 5 and 25 °C samples started to increase dramatically at day 7 of storage, where the increase was almost threefold at 5 °C (5.26 ± 0.24 log CFU/g) and almost fivefold at 25 °C (8.38 ± 0.08 log CFU/g) relative to the initial count of day 0. After day 7, there was no notably change of microbial amount in the Gwamegi samples with further storage up to 28 days at 5 and 25 °C. At day 28, the total plate counts fluctuated, reaching 6.65 ± 0.07 log CFU/g for the 5 °C storage samples, and decreasing slightly to 7.65 ± 0.03 log CFU/g for the 25 °C samples. However, the total plate counts in the sample stored at −16 °C remained relatively constant at 1.83 ± 0.03 log CFU/g throughout the 28 days.

Figure 2 gives information about the effect of HHP treatment and vacuum packaging on the total plate count from Gwamegi stored at 5 °C. The total plate counts changed during storage depending on the processing method used. Initially, there was no total plate count in the HHP-treated sample. The total plate counts from the HHP-treated and vacuum-packaged samples were lower than that from the control (p < 0.05) during the entire storage period, reaching an average value of 2.65 ± 0.16 and 4.02 ± 0.02 log CFU/g, respectively, at day 7. Thereafter, the total plate count from the HHP-treated sample remained stable until day 21, after which it increased and leveled at 3.95 ± 0.07 log CFU/g after 28 days of storage.
On the other hand, the total plate count from the vacuum-packaged samples continued to increase at day 14, giving a value of $4.47 \pm 0.19 \log \text{ CFU/g}$, and then decreased for the rest of the storage duration, reaching $3.05 \pm 0.06 \log \text{ CFU/g}$ at day 28. Thus, HHP treatment and vacuum packaging significantly reduced the total plate count in Gwamegi by 2–3 log and 1–2 log CFU/g, respectively, during storage at 5 °C. This result indicates the good quality of the products, as the microbial counts were less than the accepted values for fish and fish products recommended by the Food and Drug Administration (5.69 log or 105 CFU/g) (FDA 1998).

Biogenic amines in Gwamegi subjected to different treatments and stored at three different temperatures for 28 days

The storage temperatures effectively influenced the BA concentration ($p < 0.05$), as the higher temperature, the higher the amount of BAs. The change of BA concentration in Gwamegi during storage at $-16\, ^\circ\text{C}$ is shown in Fig. 3. Only tyramine, spermine, and spermidine were present in Gwamegi stored at $-16\, ^\circ\text{C}$, where their content between day 0 and day 28 was remarkably low (tyramine ranged from 0 to 35 ppm, spermine from $17.5 \pm 2.88$ to $32.5 \pm 2.74$ ppm, and spermidine from 0 to $23.33 \pm 5.77$ ppm). At 5 °C, considered as the commercial temperature for storing Gwamegi, there was a slight but statistically insignificant increase in amount of these three BAs relative to that at $-16\, ^\circ\text{C}$, and cadaverine was also present. As shown in Fig. 4, during the storage at 5 °C between day 0 and day 28, tyramine ranged from 0 to $56.67 \pm 5.16$ ppm, cadaverine from 0 to 20 ppm, spermine from $17.5 \pm 2.88$ to $33.33 \pm 5.16$ ppm, and spermidine from 0 to $23.33 \pm 5.16$ ppm. All of the six BAs were found at 25 °C storage and all in large amounts. According to the data in Fig. 5, 25 °C was the worst temperature at which to store the samples. At 28 days, the amounts of tyramine (360 ppm), cadaverine (334 ppm), and putrescine (300 ppm) had surpassed the toxic dose. Several other studies had similar findings.

Commercially, the Gwamegi products are stored at refrigerated temperature, and thus 5 °C was chosen for study in terms of comparing the effects of treatments on BA formation. The comparison shown in the results describe only the concentrations at days 0 and 28. Gwamegi samples were found to be safe and low in BA content when stored at $-16$ and 5 °C. Furthermore, there was no histamine present in the Gwamegi at these temperatures, adding another aspect of safety.

After the HHP treatment, the concentration of some BAs increased and simultaneously some decreased significantly ($p < 0.05$) throughout the storage period. The

![Fig. 2](image-url) Changes of total plate count in Gwamegi with different treatments during storage at 5 °C for 28 days

![Fig. 3](image-url) Changes of biogenic amines concentration in Gwamegi during storage at $-16\, ^\circ\text{C}$ for 28 days

![Fig. 4](image-url) Changes of biogenic amines concentration in Gwamegi during storage at 5 °C for 28 days
concentration of tyramine increased significantly from 0 to 56.67 ± 5.16 ppm in the control and leveled up, whereas it ranged from 0 to 105 ± 7.07 ppm in HHP-treated samples during storage (Fig. 6). Following the same trend, spermine was also found to be higher in the samples treated with HHP (17.5 ± 2.88 to 33.33 ± 5.16 ppm) than in the control (17.5 ± 2.88 to 63.33 ± 5.16 ppm) (Fig. 7). The concentrations of cadaverine (Fig. 8) and spermidine (Fig. 9) were dropped from 0 to 20 ppm and 0–23.33 ± 5.16 ppm, respectively, in the control samples down to 0–0 and 0–10 ppm in the HHP-treated samples.

The vacuum of vacuum packaging applied to Gwamegi was identified to have the same effect on BAs as HHP treatment. The two main amines in the vacuum-packaged samples were tyramine, which ranged from 0 to 90 ± 10 ppm (Fig. 6), and spermine, which ranged from 17.5 ± 2.88 to 61.67 ± 4.08 ppm (Fig. 7). Spermidine at
0–10 ppm (Fig. 9) and cadaverine at 0–10 ppm (Fig. 8) were comparatively low in concentration. Based on these results, only spermidine and cadaverine were decreased by vacuum packaging, whereas tyramine and spermine were not affected but instead increased ($p < 0.05$).

**Discussion**

In general, histamine occurs in various amounts in many foods, especially the fish products, and is involved in scombroid poisoning. The absence of histamine in Gwamegi samples stored at −16 and 5 °C was not by chance, and there are several lines of evidence to prove this. Traditionally, Gwamegi is produced naturally during the winter time by drying with the sea wind, and this processing can be one of the factors preventing histamine formation. Histamine-forming bacteria are capable of growing and of producing histamine over a wide temperature range. Growth is more rapid, however, at high-abuse temperatures (e.g., 21.1 °C) than at moderate-abuse temperatures (e.g., 7.2 °C) and is dramatically rapid at temperatures around 32.2 °C (FDA 2011). Histamine is more commonly the result of high-temperature spoilage than of long-term, relatively low-temperature spoilage. In addition, many researchers have illustrated that most cases of high histamine production were caused by high-abuse temperatures (particularly around 25 °C) applied to the fish processing or storage (Kim et al. 2001; Staruszkiwicz et al. 2004; Visciano et al. 2007; Tao et al. 2009). In a study of the influence of storage time and freezing temperature on histamine formation in anchovies, the freezing process was capable of slowing down or preventing histamine formation (Rossano et al. 2006). According to FDA (2011) recommendations, fish that are caught or harvested should be chilled as rapidly as possible to 40 °F or 4.4 °C in order to prevent or inhibit histamine formation. Additionally, in the European Regulation (2004), it is compulsory to maintain a temperature approaching that of ice freezing for fresh fishery products, cooked, and chilled products from crustaceans and mollusks and thawed unprocessed fishery products.

Pinho et al. (2001) observed that tyramine and putrescine increased dramatically during the storage at room temperature (25 °C). At 25 °C, histamine was found in mackerel (4610 ppm), albacore (3430 ppm), and mahi-mahi (3340 ppm) within a 24 h storage period (Kim et al. 2002). The tyramine content in a meat-fat mixture produced by *Carnobacterium divergens* was higher at 25 °C than at 15 °C (Masson et al. 1999). Krizek et al. (2002) reported that the BA content was increased in carp meat (a freshwater fish) when the temperature was increased (3, 15, and 25 °C) after 3 days of storage. Moreover, BAs produced by mesophilic bacteria are considered to form significantly between 20 and 37 °C (EFSA 2011).

Among the six types of BAs tested, only spermine was initially present in Gwamegi at day 0, with the value of 17.5 ± 2.88 mg kg⁻¹. In fact, spermine is found in a wide variety of organisms and tissues and is involved in numerous physiological processes of living organisms (Bardóczi 1995). For example, several studies reported the existence of spermine in raw fish, of which saury and herring are also included (Kim et al. 2009; Park et al. 2010). The presence of spermine in fish or fish products may be caused by incorrect handling or storage conditions which could optimize the growth or contamination of microorganisms.

Unlike tyramine and spermine, the cadaverine and spermidine amounts were reduced by the HHP treatment. These findings were in accord with the results by several studies. Paarup et al. (2002) reported that high pressure treatment was not effective in retarding BA formation in vacuum-packaged squid mantles, and the levels of tyramine were higher than that of controls after applying 400 MPa for 15 min and storage at 4 °C. Latorre-Moratalla et al. (2007) reported that 200 MPa treatment for 10 min at 17 °C produced no effect on the inhibition of tyramine aggregation in meat batter. Moreover, HHP treatment was demonstrated to optimize the formation of tyramine and spermine in sausages during 4 weeks of storage compared with controls (Simon-Sarkadi et al. 2012). The increase of tyramine may be related to the increase of microorganisms which could produce tyramine. Tyramine was also previously noted to be increased in vacuum-packaged chilled pork during storage (Li et al. 2014). The growth of tyramine in vacuum-packaged samples has been suspected to be influenced by microorganisms. Buňková et al. (2010) reported that a higher production of tyramine was always found in anaerobic environments, as they proved that the tyramine producer *Enterococcus durans* CCDM 53 produced the highest tyramine amount during cultivation under anaerobic condition.

In contrast, Ntzimani et al. (2008) found that vacuum packaging contributed to the reduction of tyramine, spermine, and other BAs in smoked turkey breast fillets stored at 4 °C compared with air storage. The effectiveness of vacuum packaging was also demonstrated in the study on the effect of packaging on the BA content in sardines during storage at 4 °C (Özogul and Özogul 2006). In addition to vacuum packaging, other modified atmosphere packaging methods are available and can be more effective than vacuum packaging to control BA formation. Carbon dioxide or nitrogen gas has been widely used to pack food products to increase their shelf life and assure quality.

The application of HHP treatment and vacuum packaging improved the microbial quality of the Gwamegi and...
produced negative effects on BA formation during storage. Comparing with the control samples, both HHP treatment and vacuum packaging inhibited the production of cadaverine and spermidine, while optimizing tyramine and spermine formation. However, it is necessary to conduct further research on BA-producing microbial strains to obtain more evidence of the effect of HHP treatment and vacuum packaging on BA formation in Gwamegi. Moreover, the use of modified atmosphere packaging with carbon dioxide, nitrogen, or oxygen as alternatives should be another measure to investigate in terms of controlling BAs in Gwamegi.

Acknowledgments This research was supported by the Chung-Ang University Research Scholarship Grants.

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