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Effects of high-pressure processing on the quality of chopped raw octopus

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ABSTRACT

This study evaluated the effects of high-pressure treatment on microbial growth and production of off-flavor compounds in raw octopus during 16 days of refrigerated storage. Chopped raw octopus samples were treated at 150, 300, 450, and 600 MPa for 6 min using high-pressure laboratory food processing equipment. The number of psychrotrophic bacteria on day 16 was reduced by 0.1, 0.5, 1.3, and 2.8 CFU/g after pressure treatment at 150, 300, 450, and 600 MPa, respectively, as compared with control group. The amounts of trimethylamine (TMA) and dimethylamine (DMA) produced in the chopped raw octopus treated at 600 MPa was significantly reduced by 42.5% and 62.2%, respectively, as compared to the levels in the control. The production of biogenic amines (BAs) increased up to 1.82 mg/g in the control after 12 days of refrigerated storage, while the BA levels in the 450 MPa- and 600 MPa-treated octopus were 1.40 and 1.35 mg/g, respectively. Thus, high-pressure treatment effectively inhibited microbial growth and suppressed the formation of off-flavor compounds in raw octopus. High-pressure treatment is a promising alternative technology for extending the shelf life of raw octopus.

1. Introduction

High hydrostatic pressure processing (HPP) is an emerging nonthermal technology that can ensure the same level of food safety as heat pasteurization and produces fresher-tasting, minimally processed foods. This technology reportedly increases shelf life, while minimizing loss of quality. Additionally, it maintains the nutritional value and quality of food and therefore does not result in any undesirable changes associated with thermal processing (Bermúdez-Aguirre & Barbosa-Cánovas, 2011). The destructive effects of high pressure on microorganisms can be attributed primarily to inactivation of enzymes, damage to DNA, RNA, and ribosomes, and the destruction of membranes and cell walls. Membrane and cell wall destruction is caused by rapid changes in cell volume and protein denaturation (Lado & Yousef, 2002). The use of HPP has been approved by the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) and is a reliable technological alternative to conventional heat pasteurization in food processing procedures (Lebovka, Vorobiev, & Chemat, 2011). The advancements in HPP technology in the last decade have made its application in food processing for preserving quality and inactivating pathogens in many seafood items, such as oysters, mullet, salmon, cod, squid, and shrimp (Erkan, Üretener, & Alpas, 2010; Guo, Xu, Choi, Lee, & Ahn, 2010; Kaur, Kaushik, Rao, & Chauhan, 2013; Lakshmanan, Piggott, & Paterson, 2003; Ma & Su, 2011; Montiel, Alba, Bravo, Gaya, & Medina, 2012), economically feasible.

Octopus (*Octopus vulgaris*) belongs to the cephalopod family and is a popular seafood product in Asian countries; it is typically marketed in fresh, frozen, or dried-salted form. Spoilage caused by autolysis of the muscle is particularly high in the octopus because of the high level of proteolytic activity in the muscle, resulting from their highly active metabolism, and this favors rapid microbial growth (Hurtado, Montero, & Borderías, 2001). In addition, the deterioration of octopus products during refrigerated storage is characterized by the production of various off-flavor compounds, such as trimethylamine (TMA), dimethylamine (DMA), and biogenic amines (BAs). The formation of these products is mainly caused by microbial growth and enzymatic activities (Hu, Huang, Li, & Yang, 2012; Lin, Lee, & Chang, 1983). Spoilage bacteria belonging to *Pseudomonas* spp., *Aeromonas* spp., *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Flavobacterium*, and *Vibrionaceae* can reduce TMAO as a terminal electron acceptor. Endogenous enzyme (TMAO demethylase) is also

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involved in the degradation of TMAO into DMA and formaldehyde. Proteolytic activity in fish and squid muscle has been reported to be affected by high hydrostatic pressurization, and the degree of modification is largely dependent on the level and duration of pressurization (Hernández-Andrés, Gómez-Guillén, Montero, Pilar, & Pérez-Mateos, 2006; Lakshmanan, Patterson, & Piggott, 2005). A previous study has reported that HPP efficiently reduces proteolytic activity and microbial load in octopus muscles (Hurtado et al., 2001); however, the use of high pressure to prevent the formation of TMA, DMA, and BAs in raw octopus has not been studied. Therefore, this study aimed to examine the effects of different pressures during HPP of chopped raw octopus by measuring the production of TMA, DMA, and BAs and evaluating microbial growth during refrigerated storage.

2. Materials and methods

2.1. Sample preparation and high-pressure treatment

Freshly chopped octopus (*O. vulgaris*) was purchased from the local market. Samples (30 g each) of chopped octopus were packed in polyethylene bags under vacuum and treated in a 300-mL pressure vessel (Foodlab Plunger Press model S-FL-850-9 W; Stansted Fluid Power Ltd., Stansted, UK). The bags were placed in a cylindrical loading container at room temperature and pressurized at 150, 300, 450, or 600 MPa for 6 min. The pressure-transmitting fluid was deionized water. The come-up rate was approximately 300 MPa/min, and the deviation from the targeted pressure was ±10 MPa. The pressurized samples were stored at 4 °C. All analyses were performed in triplicate.

2.2. Microbiological analysis

The total psychrotrophic bacterial count in the samples was determined. Ten grams of each sample was obtained aseptically and homogenized with 90 mL of peptone saline solution (0.1%; Difco, Detroit, MI. USA) in a filter stomacher bag, using a stomacher (BigMixer, Interscience, France). To quantify psychrotrophic aerobic microorganisms, 1 mL of each dilution was pour-plated on Aerobic Count Plates (3 M Corporation, St. Paul, MN, USA). After incubation at 4 °C for 10 d, plates with 30–300 colonies were counted. Microbial data were transformed into logarithms of the number of colony-forming units (log CFU/g).

2.3. TMA determination

TMA was measured using the colorimetric method described by Guo, Xu, Choi, Lee, et al. (2010). Each sample (20 g) was mixed with 80 mL of 7.5% cold trichloroacetic acid (TCA) solution. The mixture was homogenized thoroughly using a high-speed mixer and then centrifuged at 3000 × *g* for 15 min. The extract was mixed with 20% formaldehyde (1 mL), anhydrous toluene (5 mL), and saturated potassium carbonate (3 mL). The mixture was screw-capped, mixed vigorously, and allowed to stand at 30 °C for 10 min. The toluene phase was mixed with 0.2 g of anhydrous sodium sulfate (Na_2SO_4). Water-free toluene was mixed with 5 mL of 0.02% picric acid. The absorbance was measured at 410 nm using a spectrophotometer (Labomed Inc., CA, USA). A standard curve for TMA was prepared using 0, 2.5, 5.0, 7.5, 10.0, and 12.5 mg/mL TMA in the same manner as that described for the sample.

2.4. DMA determination

The copper-dithiocarbamate method described by Guo, Lee, and Ahn (2010) was used to determine DMA levels in the samples. Each sample (25 g) was mixed with 50 mL of 75% cold trichloroacetic acid (TCA) solution. The mixture was homogenized thoroughly using a high-speed mixer for 3 min and then centrifuged at 3000 × *g* for 15 min. The supernatants (2 mL) were neutralized with 1 M NaOH and thoroughly mixed with 5 mL of 5% CS_2 in chloroform and 0.2 mL of an alkaline solution containing 40% NaOH and NH_4OH (1:1), followed by the addition of 1 mL of a copper-ammonia reagent and 1 mL of 30% acetic acid. The mixture was allowed to stand at 25 °C for 10 min. The chloroform layer was transferred into a screw-capped test tube and mixed with 0.2 g of anhydrous sodium sulfate. The absorbance was measured at 440 nm using a spectrophotometer. A standard curve for DMA was prepared using 0, 4, 8, 12, 16, and 20 mg/mL DMA in the same manner as that described for the sample.

2.5. Total BA determination

The amount of total BAs in squid samples was determined according to the enzyme-based colorimetric method (Yeh, Lin, & Hwang, 2004), with slight modifications. Each sample (20 g) was mixed thoroughly with 7.5% TCA (80 mL) and centrifuged at 3000 × *g* for 10 min. Ten milliliter supernatant was taken and diluted to 100 mL with distilled water. The precipitant was filtered. The pH of the supernatant was adjusted to 9 by adding 50% KOH and the supernatant was then centrifuged at 700 × *g* for 3 min. The collected supernatant (1 mL) was mixed vigorously with 0.45 mL of a color-developing reagent containing 1.5 M Tris buffer (pH 9.0), 400 mM 4-aminoantipyrine, 40 mM phenol, 300 mU/mL of diamine oxidase (0.45 mL), and 175 U/mL of horseradish peroxidase solution (0.05 mL). The mixture was incubated at 50 °C for 1 h, and the absorbance was measured at 505 nm using a UV/Vis spectrophotometer. A stock solution of histamine dihydrochloride was prepared in distilled water and diluted to 2, 5, 10, 20 ppm to obtain a standard curve. Distilled water was used as blank. The concentration of test solution was determined according to the standard curve. The total BAs were calculated as below and expressed in mg/g of squid.

$$\text{BAs (mg/g)} = X\text{(mg/g)} \times 100\text{ (mL)}/10\text{ (mL)} \times 80\text{ (mL)}/20\text{ g}$$

2.6. SDS-PAGE analysis

The change in protein profiles during refrigerated storage was examined by using SDS-PAGE. Fifty grams of chopped octopus was homogenized with 100 mL of distilled water. The homogenate was centrifuged at 3000 × *g* for 15 min, and the supernatant was mixed with an equal amount of denaturing Tris buffer (pH 6.7) containing 10% SDS, 0.002% 2-mercaptoethanol, and 0.002% bromophenol blue. The mixture was boiled for 10 min and cooled immediately in an ice bath. The protein concentration was estimated using the dye-binding method developed by Bradford (1976), with a dye reagent concentrate (500-0006, Bio-Rad, Hercules, CA, USA). The protein extracts were analyzed by 10% SDS-PAGE, and the protein bands on the gel were visualized by Coomassie Brilliant Blue R-250 staining.

2.7. Statistical analysis

All experimental results are expressed as the mean ± the standard deviation (SD) of triplicate determinations. The data were analyzed using analysis of variance (ANOVA) with statistical analysis software (SAS Inc., NC, USA). Significant differences between means were determined by Duncan's multiple range tests. Results were regarded as statistically significant when *p*-values were less than 0.05.