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Effect of high pressure processing on the quality of squid (*Todarodes pacificus*) during refrigerated storage

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ABSTRACT

The influence of high pressure processing (HPP) on the inhibition of trimethylamine-*N*-oxide demethylase (TMAOase) activity and off-odour production in squid treated at 300 MPa for 20 min was investigated during 12 days of refrigerated storage. TMAOase activity of raw squid (21.5 nkat/g) was significantly decreased to approximately 5 nkat/g after 20 min of HPP. The production of dimethylamine (DMA) in HPP-treated squid for 20 min was significantly decreased to 0.31 μmol/g after 12 days of storage. The decrease in DMA was correlated with the decrease in TMAOase activity. At 300 MPa, the number of total aerobic bacteria in squid was reduced by 1.26 log units after 20 min of HPP. The HPP-treated samples effectively reduced the amount of trimethylamine (TMA). Therefore, the HPP could be used as a promising alternative technology to retard the quality deterioration of squid by inhibiting TMAOase activity and microbial growth.

1. Introduction

Squid (*Todarodes pacificus*), the class of Cephalopod, is one of the most popular seafood products in oriental countries, including China, Japan and Korea. Raw and semi-dried squid products are commonly consumed. However, the squid products are likely to produce various off-odour components during refrigerated and frozen storage. The quality deterioration of squid products is mainly caused by trimethylamine-*N*-oxide (TMAO) reduction and microbial contamination (Chiou, Chang, Lo, Lan, & Shiau, 2000; Fu et al., 2006). TMAO that plays an important role as an osmoprotectant is abundant in fish (Barrett & Kwan, 1985; Fu et al., 2006; Santos, Iobbi-Nivol, Couillault, Giordano, & Mejean, 1998). TMAO is converted to dimethylamine (DMA), formaldehyde (FA), and trimethylamine (TMA), which are responsible for characteristic fishy odours during storage (Benjakul, Visessanguan, & Tanaka, 2004; Kimura & Kimura, 2001; Santos et al., 1998). Two main possible pathways for the degradation of TMAO have been proposed. One is that the enzymatic reaction produces equimolar DMA and FA from TMAO, which is catalysed by trimethylamine-*N*-oxide demethylase (TMAOase) (Fu et al., 2008; Leelapongwattana, Benjakul, Visessanguan, & Howell, 2008b; Nitisewojo & Hultin, 1986; Stanley & Hultin, 1984). The other suggested pathway is a microbial spoilage reaction, resulting in redox-potential decrease, pH increase, and electrical conductance increase, in which TMAO degrades to trimethylamine (TMA) (Ayensa & Gomez-Guillen, 1999; Fu et al., 2008; Krzymien & Elias, 1990; Lundstrom & Racicot, 1983; Nitisewojo & Hultin, 1986; Stanley & Hultin, 1984).

With increasing concern about the quality and safety, the control of off-odours and microbial spoilage during storage has been a major endeavour of the seafood industry, which has led to continuous developments in preservation techniques. Low-temperature storage is mainly used to extend shelf-life and retard microbial growth (Leelapongwattana, Benjakul, Visessanguan, & Howell, 2008a; Wang, Liceaga-Gesualdo, & Li-Chan, 2003). However, the enzymatic and microbial reduction of TMAO could occur during prolonged low-temperature storage of seafood products, leading to quality deterioration (Krzymien & Elias, 1990; Lundstrom, Correia, & Wilhelm, 1982; Matser, Stegeman, Kals, & Bartels, 2000; Nielsen & Jorgensen, 2004). Chemical preservatives such as sodium citrate, pyrophosphate, hydrogen peroxide, sodium alginate, sucrose, and sorbitol have also been employed as chelating agents and cryoprotectants in seafood products to decrease the TMAOase activity (Leelapongwattana et al., 2008b; Parkin & Hultin, 1982). Recently, high pressure processing (HPP) has attracted great attention because it can effectively inhibit pathogenic and spoilage bacteria with less adverse effects on the nutritional quality and organoleptic properties (Amanatidou et al., 2000; Diez et al., 2008). HPP can control the fish enzymes, including cathepsin C, collagenase, chymotrypsin, and trypsin-like enzymes, for preserving fresh-like texture (Ashie & Simpson, 1996;

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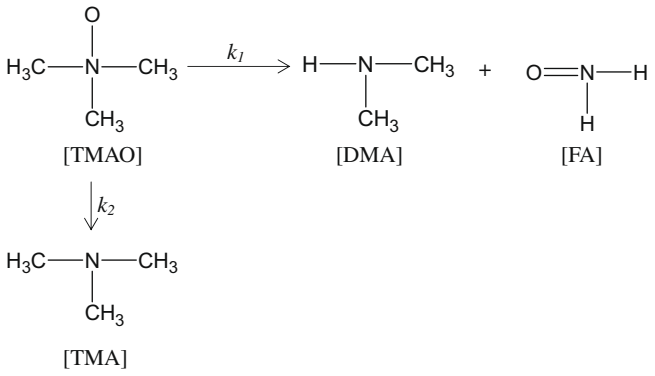

Fig. 1. Postulated reactions for DMA, FA, and TMA formation. k_1 and k_2 represent the rate constants catalysed by TMAOase and microbial growth, respectively.

Cheret, Hernandez-Andres, Delbarre-Ladrat, de Lamballerie, & Verrez-Bagnis, 2006). However, relatively few studies have focused on the effect of HPP on the reduction of unpleasant fish off-odours produced in the postulated reactions (Fig. 1). Therefore, the objective of this study was to investigate the potential of using HPP to reduce the formation of DMA and TMA in squid as measured by TMAOase activity and microbial growth during refrigerated storage. To our knowledge, the present study is the first application of high pressure processing to retard the off-odour development in squid.

2. Materials and methods

2.1. Chemicals

TMAO, DMA, TMA, and FA were purchased from Sigma–Aldrich Chemicals Inc. (St. Louis, MO, USA). Solvents for the extraction of TMAOase, DMA, and TMA were obtained from Fisher Scientific Inc. (Fair Lawn, NJ, USA). Ascorbic acid and L-cysteine were acquired from Fluka Chemical Co. (Milwaukee, WI, USA) and Tris was purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA).

2.2. Sample preparation

Squids (*T. pacificus*) were purchased from the local fisheries market (National Federation of Fisheries Cooperatives, Chuncheon, Gangwon, Korea) within 48 h of capture (February 2009) and transported in ice to the laboratory. The average weight of squids was 0.7 kg (approx. 35 cm length). The squid samples were eviscerated, and each squid sample (25 g of mantle) was vacuum-packaged under a vacuum setting of 20 kPa and sealing time of 0.1 s (Innova, Nozzle Vacuum Sealer, Gasungpak Co., Ltd., Gwanju, Gyeonggi, Korea). Sample pouches (5 × 3 cm, 0.0762 mm thickness) were made from sterile polyethylene filter bags (01-002-57; Fisher Scientific).

2.3. High pressure treatment

The packaged samples were loaded into a high pressure processor (QFP-6, Flow Autoclave Systems, Columbus, OH, USA) and subjected to 300 MPa at 20 °C for 0, 5, 10, and 20 min. The high pressure-treated samples were stored at 4 °C for 0, 4, 8, and 12 days. The pressurisation rate was approximately 5.4 MPa/s, and the depressurisation occurred in less than 10 s. The process hold time did not include the pressure come-up or the de-pressurisation times. The samples without high pressure treatment (0.1 MPa) were used as the control.

2.4. pH measurement

Each sample (25 g) was thoroughly mixed with 50 ml of distilled water for 3 min in a high speed mixer (SFM-555SP; Shinil Industrial Co., Ltd., Seoul, Korea). pH values were measured for all treatments on day 0, 4, 8, and 12 by using a pH metre (Mettler–Toledo International Inc., Toledo, OH, USA).

2.5. TMAOase extraction

The TMAOase extract was according to the methods of Benjakul et al. (2004) with slight modifications. The treated samples (25 g each) were mixed with 50 ml 20 mM Tris–acetate buffer (TA; pH 7.0) containing 0.1 M NaCl and 0.1% Triton X-100. The mixtures were thoroughly blended for 3 min using a Kitchen Aid mixer and centrifuged at 38,500*g* for 30 min at 4 °C (Supra 22 K Plus, High Speed Refrigerated Centrifuge, Hanll Science Industrial Co., Ltd). The supernatants were collected and used as TMAOase crude extract.

2.6. TMAOase activity assay

The TMAOase activity was measured using TMAO as a substrate in the presence of cofactors (cysteine, ascorbate, and $FeCl_2$). The enzymatic reaction of the crude extract (0.5 ml) was initiated by the addition of 24 mM TA (2.5 ml) containing 24 mM TMAO, 2.4 mM cysteine, 2.4 mM ascorbate, and 0.24 mM $FeCl_2$ (pH 7.0). The mixtures were incubated in a water bath (Fisher Scientific) at 25 °C for 20 min. The reactions were terminated by adding 30% trichloroacetic acid (TCA). The reaction mixtures were immediately cooled in an ice-bath to avoid further reactions and centrifuged at 8000*g* for 15 min. The collected supernatants were used for DMA determination. The blank without enzyme extract was run to estimate the nonenzymatical production of DMA, which was subtracted from the total production of DMA. TMAOase activity was expressed as 1 nmol of DMA production under the incubation with an enzyme for 1 s (nkat). The 'katal' is defined as the catalytic amount that produces 1 mol of DMA per second.

2.7. DMA and TMA determination

Samples (25 g) were homogenised with 50 ml of 7.5% cold TCA solution using a high speed mixer (SFM-555SP; Shinil Industrial Co., Ltd.) for 3 min. The homogenates were centrifuged at 3000*g* for 15 min, and the supernatants were neutralised with 1 M NaOH and used for the analyses of DMA and TMA.

2.7.1. DMA analysis

The copper-dithiocarbamate method of Dyer and Mounsey (1945) with slight modifications was used to determine DMA in the samples. The neutralised supernatant extract (2 ml) was thoroughly mixed for 2 min with 5 ml of 5% CS_2 in chloroform and 0.2 ml of alkaline solution containing 40% NaOH and NH_4OH (1:1), followed by the addition of 1 ml of 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 sulphate. The absorbance was measured at 440 nm using a Spectro UV–Vis Dual Beam Scanning Spectrophotometer. A standard curve for DMA was prepared at 0, 4, 8, 12, 16, and 20 mg/ml in the same manner as described above.

2.7.2. TMA analysis

TMA was measured using the colourimetric method described by Conway and Byrne (1936) with minor modifications. The sample extract (2 ml) was mixed with 1 ml of 20% FA (pH 7.0) to reduce