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Effects of ozone on the removal of geosmin and the physicochemical properties of fish meat from bighead carp (*Hypophthalmichthys nobilis*)

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ABSTRACT

Two manners of ozone treatment (washing with ozonized water and ozone-flotation) were selected to remove the geosmin in the fish muscle from bighead carp. A total of 42.09%–54.28% and 42.78%–69.19% geosmin in fish muscle could be eliminated by ozone water and ozone-flotation washing for 5–20 min, respectively. Treatment with ozone of an appropriate condition significantly increased the salt-extractable protein, Ca^{2+}-ATPase activity, total sulfhydryl and active sulfhydryl contents, carbonyl content of the myofibrillar protein, and gel strength without improving PV and TBA values too much. No apparent changes in the protein distribution and no new patterns appeared in SDS-PAGE after the ozone water treatment. The results indicate that ozone water treatment is a prospectively mild oxidation protocol than ozone flotation to enhance the physicochemical properties of fish protein from bighead carp as well as to eliminate muddy flavours.

Industrial relevance: Ozonation has been used for years to disinfect water for drinking purposes in Europe. A number of other commercial uses have been found for ozone including disinfection of bottled water, swimming pools, prevention of fouling of cooling towers, and wastewater treatment. Ozone use may have many advantages in the food industry. There are suggested applications of ozone in the food industry such as food surface hygiene, sanitation of food plant equipment, reuse of waste water, and lowering of the biological oxygen demand (BOD) and chemical oxygen demand (COD) of food plant waste.

Ozone is a strong oxidant that also has decolorizing and deodorizing effects, and thus offers many advantages in the food industry, and has been traditionally used in freshwater aquaculture systems in the treatment of fish, disinfection of eggs, sterilization of water to improve the water quality, and decomposition of odorous compounds in natural water in addition to its applications for improving the sensory quality and shelf life of fish. Since 1920, scientists have attempted to exploit the wide-range disinfection characteristics of ozone to slow the decomposition for improving the safety of fishing products. A number of commercial uses have been found for ozone, including the disinfection and preservation of aquatic products. In the United States, ozone received Generally Recognized as Safe (GRAS) classification in 1997, and in 2001, the FDA officially approved ozone for use in the food industry and for direct contact with food products, including fish, meat, and poultry. Ozone has continued to gain momentum in the food processing industry as the safest, most cost-effective, and a chemical-free way of dealing with food safety management.

1. Introduction

Ozone is a strong oxidant that also has decolorizing and deodorizing effects (Bailey, 1982; Chang et al., 1995; Chang et al., 1996), and thus offers many advantages in the food industry, and has been traditionally used in freshwater aquaculture systems in the treatment of fish, disinfection of eggs, sterilization of water to improve the water quality (Rice, 1997), and decomposition of odorous compounds (GSM and 2-MIB) in natural water in addition to its applications for improving the sensory quality and shelf life of fish (Rice et al., 1982; Morioka et al., 1993; Kötters et al., 1997; Kim, Yousef, & Dave, 1999; Campos et al., 2006). Since 1920, scientists have attempted to exploit the wide-range disinfection characteristics of ozone to slow decomposition for improving the safety of fish products. A number of commercial uses have been found for ozone, including the disinfection and preservation of aquatic products (Chen et al., 1992; Silva et al., 1998). In the United States, ozone received Generally Recognized as Safe (GRAS) classification in 1997, and in 2001, the FDA officially approved ozone for use in the food industry and for direct contact with food products, including fish, meat, and poultry (Lin & Chang, 1995; Mielcke & Ried, 2004; Vaz-Velho et al., 2006). Ozone has continued to gain momentum in the food processing industry as the safest, most cost-effective, and a chemical-free way of dealing with food safety management (Vaz-Velho et al., 2006).

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Ozone participates in direct or indirect oxidation by ozonolysis and catalysis. A basic scientific explanation for the action of ozone on proteins during ozone reactions has been established. Ozone causes the oxidation of the polypeptide backbone of proteins, peptide bond cleavage, protein cross-linking, and a range of amino acid side-chain modifications (Kelly & Mudway, 2003). Ozone-treated protein molecules undergo changes in their visual folding and binding ability (Cataldo, 2003), which influences their functionality. However, several studies have shown that reactive oxygen species (ROS)-mediated oxidative stress on proteins is a major cause of chemical deterioration in foods, mainly in meat and meat products (Choe & Min, 2006). Numerous ROS are able to trigger oxidative damage to proteins, and oxidizing lipids are known to play a role in this oxidative damage (Stadtman & Levine, 2003). In such a manner, many oxidative modifications of proteins may occur, such as an increase in the number of carbonyl groups, the oxidative degradation of essential amino acids (e.g., histidine, tryptophan, methionine, and cysteine), loss of sulfhydryl groups, and formation of intra and/or intermolecular cross-linkages (Stadtman & Levine, 2003). It is generally accepted that oxidative degradation involves the loss of essential amino acids, changes in texture, alteration in protein functionality, and impaired digestibility (Lund et al., 2011). The impact of protein oxidation on meat quality is still the subject of multiple studies but little is known about the changes in the functional physicochemical properties of proteins in food systems after oxidation by ozone.

The impact of deterioration of the marine environment has led to a decline in the proportion of seafood, making the rational use of resources of freshwater fish products and the development of effective processing technologies to keep pace with the development of freshwater aquaculture of utmost importance. The rapid growth, high yield, and low price of freshwater fish makes it attractive for processing into surimi-based products, which is one of the major processing approaches for preserving the nutritional value, and is convenient and popular among consumers. Thus, the processing of low-value freshwater fish into frozen surimi is expected to gain importance as a means of deep processing of freshwater fish, with good prospects for the development of aquatic products. However, because of the environment in which freshwater fish is grown, it is characterized by certain types of unpleasant odours from semi-volatile compounds that confer a discernible muddy and musty odour from geosmin (GSM) and 2-methylisoborneol (2-MIB) produced by planktonic and benthic algae (particularly cyanobacteria), fungi, bacteria, and actinomycetes (Persson, 1980; Mallevaille & Suffet, 1987), which limits its consumption.

Up to now, there have been few studies about eliminating the muddy flavour of freshwater fish through ozone treatment; moreover, the effects of ozone-induced oxidation on the physicochemical properties of the fish protein were not investigated. This work may provide a scientific basis for application of ozone for the elimination of off-flavours as well as the enhancement of the physicochemical properties of fish.

2. Materials and methods

2.1. Materials and chemicals

To obtain fishes enriched with off-flavours, fresh bighead carp weighing about 2 kg were purchased from a local supermarket. All the chemicals used were of analytical grade and were purchased from Sigma (St. Louis, MO, USA) or Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of different concentrations of ozone water

Ozone gas (velocity of flow = 0.3 m^3/h) generated by an ozone generator (Model, WFG-5, Weizhen Environmental Protection Equipment Factory, Shanghai, China) was added to distilled water (20 L) by controlling the access time of the ozone; thus, different concentrations of ozone water were prepared. A 100 mL aliquot of ozone water was added to 20 mL of 2% potassium iodide containing 3 mL of 0.5 M sulfuric acid to keep the solution acidic. After reaction in the dark for 5 min, the solution was titrated with 0.01 mol/L sodium thiosulfate standard solution (1% starch solution as indicator) until the blue colour disappeared. Various concentrations of ozone water were 3.3 mg/L, 5.1 mg/L, and 7.6 mg/L when access time of the ozone was 5 min, 10 min, and 15 min, respectively.

2.3. Preparation of fish meat treated with ozone

Bighead carp back muscles weighing 500 g were picked and minced followed by rinsing with a 5-fold (*w*/*v*) quantity of a certain concentration of ozone water for 20 min. The ozone water was then removed and the sample was rinsed again by replacing the ozone water with the same volume of distilled water. The water was decanted through a filtration cloth. Various samples of fish meat were prepared by changing the concentration of ozone water. Another 500 g of bighead carp back muscles were picked and minced followed by rinsing with a 5-fold (*w*/*v*) distilled water, ozone gas (velocity of flow = 0.3 m^3/h) was added into the water through a stainless steel diffuser (2 μm pore size), different ozone gas-flotation-treated fish meat were obtained by controlling the access time of the ozone for 5 min, 10 min, and 15 min, respectively. The rinse was continued until the total time was up to 20 min. After that, the fish meat was rinsed with the same volume of distilled water and then was decanted through a filtration cloth. All of the aforementioned operations were performed at a temperature below 10 °C.

2.4. Determination of geosmin from fish meat

The distillate of fish meat by microwave-assisted distillation was added 4-fold (*w*/*v*) NaCl, and then extracted by solid phase microextraction with PDMS/DVB (65 μm). The extraction was conducted at 60 °C and kept for 30 min. After that, the sample was quickly put into gas chromatography vaporization chamber for thermal analysis and GC/MS analysis. HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used for GC/MS analysis, the experimental parameters were as follows: initial temperature of the column temperature was from 40 °C to 250 °C at a speed of 8 °C/min and kept for 10 min, He gas flow at the rate 0.8 mL/min, ion source temperature was 230 °C, and transfer line temperature was 280 °C. Selected ion chromatograms (EIC) were collected for quantitative analysis of geosmin.

2.5. Preparation of myofibrillar protein

A certain quantity of bighead carp fish meat was combined with 5 times the volume of Tris-maleate buffer (50 mM KCl/20 mM Tris-maleate, pH 7) and then homogenized. The homogenate was centrifuged at 9000 rpm using an Anke GL-20G-II centrifuge (Anting Scientific Instrument Factory, Shanghai, China) at 4 °C for 10 min. The supernatant was removed; the precipitate was washed twice, combined with Tris-maleate buffer (0.6 M KCl/20 mM Tris-maleate, pH 7.0), and then homogenized at 10,000 rpm for 1.5 min. The homogenate was extracted and kept at 4 °C for 30 min to allow sufficient dissolution of the proteins and then centrifuged at 9000 rpm at 4 °C for 20 min. The supernatant was then precipitated by pouring the mixture into 10 times the volume of distilled water. The obtained precipitate was a myofibrillar protein, which was then dissolved in the same volume of 0.6 M KCl for use.

2.6. Preparation of oil of fish meat

A certain quantity of bighead carp fish meat combined with 5 times the volume of methanol was sufficiently homogenized, after that, 5 times the volume of chloroform was added for filtration. The filtered