Food Bioscience 19 (2017) 57–64

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Image and chemical analyses of freezing-induced aggregates of fish natural actomyosin as affected by various phosphate compounds

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ARTICLE INFO

Chemical compounds used in this article:
Sodium tripolyphosphate (PubChem CID: 24455)
Tetrasodium pyrophosphate (PubChem CID: 24403)
Trisodium pyrophosphate (PubChem CID: 161081)
Disodium phosphate (PubChem CID: 24203)
Trimethylamine N-oxide (PubChem CID: 1145)
Formaldehyde (PubChem CID: 712)
Dimethylamine (PubChem CID: 674)
Ferrous chloride (PubChem CID: 24458)
Ascorbic acid (PubChem CID: 54670067)
L-Cysteine (PubChem CID: 5862)
Sugar (PubChem CID: 5988)
Sorbitol (PubChem CID: 5780), Triton X-100 (PubChem CID: 5590)

Keywords:
Fish natural actomyosin
Natural actomyosin
Pacific whiting
Merluccius productus
Freezing/thawing

ABSTRACT

The impact of various phosphate compounds [sodium tripolyphosphate (STPP), tetrasodium pyrophosphate (TSPP), STPP/TSPP, trisodium pyrophosphate (3SP), sodium hexametaphosphate (SHMP), and disodium phosphate anhydrous (DSPA)] on retarding freeze-induced protein denaturation and aggregation was evaluated. Using natural actomyosin (NAM) extracted from fresh Pacific whiting, the phosphate treatments were evaluated at various concentrations (0.1%, 0.3%, and 0.5%), with and without cryoprotectants (CP) (4% sorbitol and 5% sugar), after different freeze/thaw cycles (F/TC) (0, 3, and 9F/TC). Trimethylamine-*N*-oxide demethylase (TMAOase) activity, formaldehyde (FA) content, solubility, and turbidity were measured. The other NAM mixture containing the 0.5% STPP/TSPP, STPP, or TSPP along with CP showed low TMAOase activity, low FA production, high salt-soluble proteins solubility, and high turbidity. Among the phosphate treatments, STPP seemed to be the most effective compound in retarding both FA- and freeze-induced protein denaturation and aggregation. NAM without CP was more rapidly denatured and aggregated than NAM with CP as F/TC increased, resulting in a gradual increase in the degree of aggregation (DA). The DA, which was calculated based on graphical images, correlated with biochemical properties.

1. Introduction

Various inorganic phosphate compounds are commonly used as functional ingredients in the food industry because of their ability to provide pH adjustment, increase ionic strength, chelate metal ions, and dissociate myosin and actin (Hunt & Park, 2014; Okazaki & Kimura, 2014). In particular, pyrophosphate addition in surimi results in increased pH, improved water retention ability (WRA), and increased solubility of myofibrillar proteins (Park, 2000). A mixture (50:50) of STPP (sodium tripolyphosphate) and TSPP (tetrasodium pyrophosphate) (STPP/TSPP) is typically added at 0.3% into washed fish mince along with cryoprotectants (CP) (5% sugar and 4% sorbitol) in the production of commercial cold water fish surimi (Hunt & Park, 2014). The phosphate compounds have a different number (ortho, pyro, and poly) of phosphate groups (PO_4^{3-}) which are typically occupied by sodium or potassium ions at their anionic oxygen site. Orthophosphate mainly provides a high buffering capacity whereas poly- or pyrophosphates improve water retention (Singh, Korasapati, Juneja, & Thippareddi, 2010).

Freezing-induced protein denaturation followed by aggregation is primarily due to development and growth of intra- or intercellular ice crystals (Shenouda, 1980). Due to a different freezing rate between the exterior and interior of the cell, the formation of ice crystals and increased solute concentration occurs outside the cell. The unbalanced solute concentration possibly leads to moisture migration from the cell's interior to extracellular space due to osmosis, resulting in the growth of

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http://dx.doi.org/10.1016/j.fbio.2017.06.007
Received 28 September 2016; Received in revised form 11 June 2017; Accepted 15 June 2017
Available online 16 June 2017

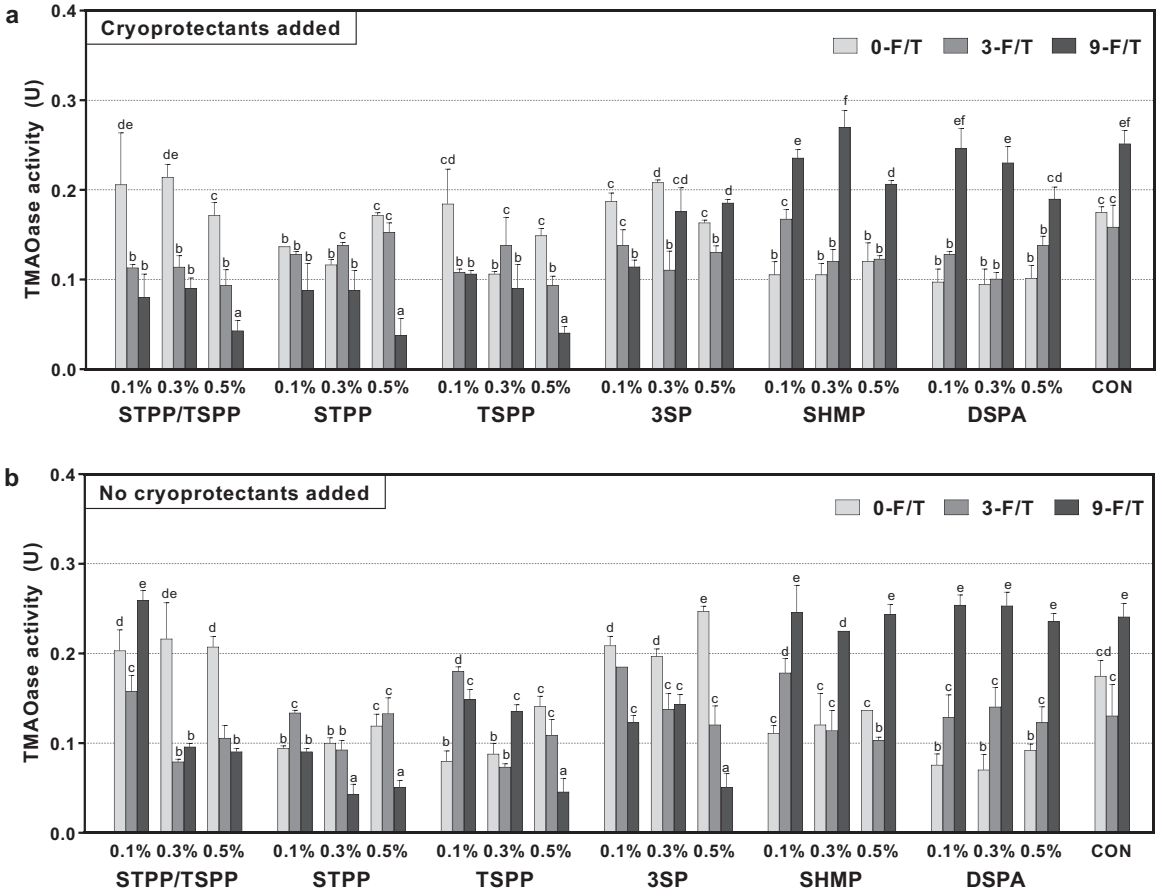

Fig. 1. TMAOase activity in NAM mixtures (20 mg/ml) with cryoprotectants (4% sorbitol and 5% sugar) (a) or without cryoprotectants (b) at different F/TC (0, 3, and 9). STPP/TSPP, STPP, TSPP, 3SP, SHMP, and DSPA were added in NAM mixtures at 0.1%, 0.3%, and 0.5% concentrations. CON: NAM mixture without phosphate compound. Different letters (a-f) on the bars indicate a significant difference at $p < 0.05$.

ice crystals (Hunt & Park, 2014). Therefore, the disruption of the cell membranes with the formation of larger ice crystals causes the release of mitochondrial and lysosomal enzymes such as trimethylamine-*N*-oxide demethylase (TMAOase) during frozen storage (Hamm, 1979). Endogenous TMAOase, which is an abundant lysosomal enzyme in gadoid cold-water fish species, may be released by the breakdown of a lysosomal membrane, resulting in denaturation of myofibrillar proteins by TMAOase-induced formaldehyde (FA) production during frozen storage (Lee & Park, 2016). Moreover, recrystallization and a rapid growth of ice crystals, occurring with multiple freeze/thaw cycles (F/TC) to mimic long-term frozen storage, leads to an increased protein denaturation (Srinivasan, Xiong, Blanchard, & Tidwell, 1997).

According to Leelapongwattana, Benjakul, Visessanguan, and Howell (2008), TMAOase from lizardfish, a tropical fish, was effectively inhibited (68–76%) by 0.2–0.4% TSPP. Pyrophosphate possibly functions to chelate the Fe ion, which is required for the maximum activity of TMAOase through a cycling mechanism between the ferrous (Fe^{2+}) and ferric (Fe^{3+}) states (Parkin & Hultin, 1986). However, the inhibition with various phosphate compounds, which differ in structure and function, on TMAOase activity in cold-water species has not been fully investigated. STPP, TSPP, trisodium pyrophosphate (3SP), sodium hexametaphosphate (SHMP), and disodium phosphate anhydrous (DSPA) are commonly used by the food industry for complexing metal ions and organic polyelectrolytes, facilitating chemical reactions with food constituents, increasing hydration and water binding, and for preserving foods (Ellinger, 1972). In this study, the objective was to measure the inhibition of various phosphate compounds on protein denaturation and aggregation physicochemically and photographically using a Pacific whiting natural actomyosin (NAM) system with or without CP (4% sorbitol and 5% sugar).

2. Materials and methods

2.1. Materials

Raw Pacific whiting (*Merluccius productus*) 48 h postharvest were randomly obtained at Da Yang Seafood Inc. (Astoria, OR, USA) in September 2015. The fish (200 ± 20 g) with ice on top and bottom of the ice cooler were transported to OSU Seafood Laboratory (Astoria, OR, USA) in 30 min. The immediately filleted and skinned fresh whiting (approximately 200 g) were subsequently ground (KitchenAid Food Chopper, KitchenAid®, Benton Harbor, MI, USA) in a cold room (2–4 °C) for 1 min. This mince was used to prepare the NAM. Six different sodium phosphates supplied by Innophos, Inc. (Cranbury, NJ, USA) including STPP/TSPP 50/50 mixture, STPP, TSPP, 3SP, SHMP, and DSPA. All non-phosphate chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Bradford reagent was purchased from Bio-Rad Lab (Hercules, CA, USA).

2.2. Preparation of NAM

NAM was prepared according to the method of Hemung, Li-Chan, and Yongsawatdigul (2008) with some modifications. Ground fish meat (50 g) was mixed with 250 ml of 50 mM NaCl buffer (20 mM tris-HCl, pH 7.0) and homogenized at 30,000 rpm for 2 min (Tissue Tearor Homogenizer, BioSpec Products Inc., Bartlesville, OK, USA). The homogenates were centrifuged at 5000 × *g* at 4 °C for 10 min (Beckman J6-MI centrifuge, Beckman Coulter, Fullerton, CA, USA). The pellet was