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Effect of zinc sulphate on gelling properties of phosphorylated protein isolate from yellow stripe trevally

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ABSTRACT

Impacts of zinc sulphate ($ZnSO_4$) (0–140 μmol/kg) on gel properties of yellow stripe trevally surimi added with sodium tripolyphosphate (STPP) (0.25% and 0.5%, w/w) and protein isolate phosphorylated with STPP at 0.25% and 0.5% (w/w) were studied. Gels from surimi added with 60 μmol $ZnSO_4$/kg in the absence and presence of 0.5% STPP had the increases in breaking force and deformation by 20.9% and 33.3%, and 11.6% and 18.6%, respectively, compared with the control surimi gel (without additives). Gel of protein isolate phosphorylated with 0.5% STPP containing 100 μmol $ZnSO_4$/kg had the increases in breaking force and deformation by 14.87% and 5.6%, respectively, compared with the gel from non-phosphorylated protein isolate at the same $ZnSO_4$ level, suggesting that the phosphorylated protein isolate was more crosslinked by Zn^{2+}. The addition of $ZnSO_4$ at the suitable level lowered the expressible moisture content, but increased whiteness of surimi or protein isolate gels ($P < 0.05$). Non-covalent bonds, more likely salt bridge and ionic interactions, played a major role in cross-linking of proteins in both surimi and protein isolate added with $ZnSO_4$, regardless of phosphates incorporated. Microstructure study revealed that a gel having highly interconnected and denser network with smaller voids was formed when protein isolate phosphorylated with 0.5% STPP was added with $ZnSO_4$ at a level of 100 μmol/kg. Thus, gel with improved properties could be obtained from protein isolate from yellow stripe trevally phosphorylated with STPP in conjunction with addition of $ZnSO_4$ at an appropriate level.
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1. Introduction

Due to the over-exploitation of lean fish, which have been used for surimi production, dark fleshed fish have been paid increasing attention as a potential alternative raw material for surimi production, due to its abundance and low price (Chaijan, Benjakul, Visessanguan, & Faustman, 2004). However, it is difficult to obtain high quality surimi from those species due to the high content of dark muscle, which contains a large amount of lipids and myoglobin. To overcome this problem, a pH-shift process, developed by Hultin and Kelleher (2000), has been used to both increase yield and improve gel property (Kristinsson & Hultin, 2003). The extraction mechanism of this process is to solubilise the muscle protein at low or high pH to separate soluble proteins from bone, skin, connective tissue, cellular membranes, and neutral storage lipids through the centrifugation (Nolsøe & Undeland, 2009). The solubilised proteins are recovered by isoelectric precipitation to give a highly functional and stable protein isolate (Kristinsson & Ingadottir, 2006). The major advantages of this process include economical feasibility, high recovery yield and improved functionalities. In some cases, the better gel properties were obtained, compared with surimi prepared by the conventional process (Kristinsson, Theodore, Demir, & Ingadottir, 2005; Undeland, Kelleher, & Hultin, 2002). Gels prepared from rockfish and Atlantic croaker from proteins solubilised at alkaline pH exhibited better gel quality than those prepared from the acid-aided process (Perez-Mateos, Amato, & Lanier, 2004).

Divalent cations, e.g. calcium, magnesium and zinc are known to affect the functionality of proteins during gelation (Mathew, Shamasundar, Kumar, & Prakash, 2009; Morales, Ramirez, Vivanco, & Vazquez, 2001). At pH values sufficiently far from the isoelectric point of proteins, divalent ions such as Ca^{2+}, Zn^{2+}, Mg^{2+} induce protein cross-linking via the salt bridges between negatively charged carboxyl groups (Hongsprabhas & Barbut, 1997). Recently, Arfat and Benjakul (2012) have reported that the addition of zinc salts, especially zinc sulphate, induced the aggregation of natural actomyosin, mainly by the induction of higher hydrophobic interaction, salt bridge and ionic interaction.

Phosphates have been reported to dissociate protein complexes and are widely accepted as potential additives in fish and seafood to improve the functional properties of those products (Chang & Regenstein, 1997). Phosphates were used to improve gelling property of surimi from bigeye snapper (Julavittayanukul, Benjakul, & Visessanguan, 2006). Therefore, the addition of sodium

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tripolyphosphate in combination with zinc salt might be an approach to strengthen gel network of surimi via introducing the negatively charged phosphate, which can be subsequently served for 'salt bridge' induced by Zn^{2+}.

During pH-shift process, the proteins undergo dissociation via repulsion, thereby becoming solubilised. Phosphate compounds could react with –OH or $–NH_2$ groups on the side chains of proteins, particularly at alkaline condition (Ferrel Sung, 1982). For the new process, the simultaneous incorporation of phosphates during solubilisation under alkaline condition could not only improve the effectiveness of phosphorylation, but also has the potential to produce protein isolate with dominant negative charged side chains that could serve as a better substrate for cross-linking mediated by Zn^{2+} via salt bridge mechanism. As a consequence, the superior gel properties of fish muscle proteins can be obtained. Additionally, the excessive phosphates could be removed by the precipitation of proteins at their isoelectric point. Nevertheless, the use of divalent cations on gelling properties of phosphorylated fish protein isolate has not been reported previously. Therefore, the objective of this study was to investigate the effect of $ZnSO_4$ at different levels on the gel properties of surimi added with STPP and protein isolates from yellow stripe trevally phosphorylated with STPP.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Sodium dodecyl sulphate (SDS), β-mercaptoethanol (β-ME), glycerol, sodium tripolyphosphate and glutaraldehyde were purchased from Sigma (St. Louis, MO, USA). Sodium hydroxide, hydrochloric acid, *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED), acrylamide, and bisacrylamide were procured from Fluka (Buchs, Switzerland). Zinc sulphate ($ZnSO_4$) was purchased from Ajax Finechem, Pty. Ltd. (Taren Point, NSW, Australia).

2.2. Preparation of fish mince

Fresh yellow stripe trevally (*Selaroides leptolepis*) with an average weight of 80–90 g/fish were caught from Songkhla coast along the Gulf of Thailand during December, 2012 and January, 2013. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand within 1 h. The fish were immediately washed, gutted, cleaned and filleted. Fillets were subjected to mincing using a mincer with the hole diameter of 5 mm. Mince obtained was placed in polyethylene bag and imbedded in ice until use.

2.3. Preparation of surimi and fish protein isolate

To prepare surimi by the conventional washing process, fish mince was washed with cold water (4 °C) using a water/mince ratio of 3:1 (w/w). The mixture was stirred gently for 10 min in a cold room (4 °C) and the washed mince was filtered with a layer of nylon screen. Washing was performed three times. Finally, the washed mince was centrifuged at 700×*g* for 15 min using a basket centrifuge (Model CE 21 K, Grandiumpiant, Belluno, Italy). The resulting pellet was referred to as 'surimi'.

To prepare the protein isolate, the alkaline solubilisation process was used following the method of Undeland et al. (2002) with a slight modification. The mince was prewashed with 3 cycles of cold water using a water/mince ratio of 3:1 (w/w). The mince (250 g) was homogenised for 1 min with 2.25 l of cold distilled water (4 °C) using an IKA homogeniser (Selangor, Malaysia) at a speed of 11,000 rpm. The homogenate was adjusted to the pH of 11 using 2 N NaOH. Thereafter, the solubilised proteins were added with 0.25% or 0.5% (w/w) STPP and stirred continuously for 1 h at 4 °C. For the control protein isolate, no STPP was incorporated. The mixtures were then centrifuged at 10,000×*g* for 20 min using a refrigerated centrifuge (Avanti-JE Centrifuge, Beckman 163 Coulter Inc., Fullerton, CA, USA) at 4 °C to remove the insoluble materials. The soluble proteins were then precipitated by adjusting the pH to 5.5 using 2 N HCl. Precipitated proteins were collected by centrifuging at 10,000×*g* for 20 min at 4 °C. The pH of obtained pellet was adjusted to 7.0 using 2 N NaOH. The recovered proteins were referred to as 'phosphorylated protein isolate'.

One set of surimi, protein isolate, and phosphorylated protein isolate were subjected to analyses. Another set of those samples was added with cryoprotectants including 4% sucrose and 4% sorbitol, mixed well and frozen using an airblast freezer (Patkol Co., Ltd, Bangkok, Thailand). The frozen samples were kept at −18 °C until used for gel preparation. The storage time was not more than 1 month.

2.4. Study on chemical compositions and Ca^{2+}-ATPase activity of surimi, protein isolate and phosphorylated protein isolate

Surimi, protein isolate and phosphorylated protein isolate without cryoprotectants were determined for chemical compositions and Ca^{2+}-ATPase activity.

2.4.1. Moisture and ash contents

The samples were analysed for moisture and ash contents according to the method of AOAC (2000) with the analytical No. of 950.46 and 920.153, respectively.

2.4.2. Determination of lipid and phospholipid contents

Lipid content was determined by the Soxhlet apparatus according to the method of AOAC (2000) with the analytical No. of 920.39B. Phospholipid content was measured based on the direct spectrophotometeric measurement of complex formation between phospholipids and ammonium ferrothiocyanate as described by Stewart (1980). Lipids were firstly extracted by the Bligh and Dyer method (Bligh & Dyer, 1959). Thereafter, lipids (20 μl) were dissolved in chloroform to obtain a final volume of 2 ml. One millilitre of thiocyanate reagent (a mixture of 0.10 M ferric chloride hexahydrate and 0.40 M ammonium thiocyanate) was added. After thorough mixing for 1 min, the lower layer was removed and the absorbance at 488 nm was measured. A standard curve was prepared using phosphatidylcholine (0–50 ppm). The phospholipid content was expressed as mg/100 g dry sample.

2.4.3. Determination of phosphate content

The phosphate content was determined according to the method of Suzuki and Suyama (1985) with a slight modification. To the samples (0.6–0.8 g), 20 ml of 4 M NaOH were added and mixed vigorously. The samples were heated in a boiling water bath (90–95 °C) for 30 min and then cooled at room temperature for 1 h. The mixture was mixed with 20 ml of 4 M HCl for neutralisation. The supernatant (0.2 ml) was mixed with 2 ml of phosphate reagent (4.2% ammonium molybdate solution: 0.045% malachite green, 1:3 v/v). The mixture was then incubated at room temperature for 30 min. The absorbance was measured at 620 nm. The phosphate content was calculated from a phosphate standard curve. Disodium hydrogen phosphate solutions with concentrations of 0–15 μg/ml were used as standard. Phosphate content was expressed as mg/100 g sample.

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