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Production and assessment of Pacific hake (*Merluccius productus*) hydrolysates as cryoprotectants for frozen fish mince

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ABSTRACT

The aim of this study was to investigate application of fish protein hydrolysates (FPHs) as cryoprotectants for cod fish mince subjected to freeze-thaw abuse. Response surface methodology revealed little difference in cryoprotectant ability between FPHs produced from Pacific hake muscle within the range of conditions studied, namely Flavourzyme® enzyme/substrate ratio (E/S 1–4%), time (1–6 h) and pH (5–7). When added at 4% or higher concentrations, FPH minimized expressible moisture and cook loss, while maximizing salt extractable protein from freeze-thaw abused fish mince, providing similar or better cryoprotection compared to an 8% sucrose-sorbitol blend, and a stabilizing effect of FPH on myosin was observed by differential scanning calorimetry. Sensory evaluation showed that addition of 8% FPH in fish ball products increased the perception of fishiness, saltiness, bitterness and firmness while decreasing moistness. FPH could be a viable alternative to the sugar-based cryoprotectants currently used for frozen fish products.

1. Introduction

In a world where the demand for seafood is increasing, and not limited to coastal populations, preservation of these foods is of the utmost importance. Frozen storage has long been used as a means of preservation to slow down the microbial and enzymatic degradation of fish. However, long-term storage of frozen fish has drawbacks, including the loss of protein solubility and water holding capacity over time (Nikoo, Benjakul, & Rahmanifarah, 2016; Park, 2013), leading to an overall decline in fish quality. To minimize the degradation of frozen fish, low molecular weight sugars, such as sugar and sorbitol, are commonly used in the industry as cryoprotectants. Addition of a sucrose-sorbitol blend to fish before freezing has been shown to limit protein denaturation and aggregation, particularly of the myosin globular heads, by minimizing the formation of disulfide bonds while helping to retain surface hydrophobicity and gel strength (Sultanbawa & Li-Chan, 2001; Korzeniowska, Cheung, & Li-Chan, 2013). Unfortunately, there are drawbacks to using sugar-based cryoprotectants, including undesirable sweetness (Sych, Lacroix, Adambounou, & Castaigne, 1990), as well as health concerns of diabetics or others wishing to avoid products with added sugar (Jenkelunas, 2013).

A possible alternative to the sugar-based cryoprotectants is hydrolyzed protein, particularly from under-utilized species or processing by-products. For example, gelatin and other protein hydrolysates from marine sources have been reported to exert cryoprotective effects during frozen storage or freeze-thaw abuse of seafood products and their constituent proteins, including natural actomyosin proteins (e.g., Korzeniowska et al., 2013), surimi (e.g., Khan et al., 2003; Kittiphattanabawon, Benjakul, Visessaguan, & Shahidi, 2012; Limpisophon et al., 2015), and fish mince (e.g., Cheung, Liceaga, & Li-Chan, 2009; Mueller & Liceaga, 2016; Nikoo, Benjakul, & Xu, 2015). The effectiveness of cryoprotection is commonly assessed after a period of frozen storage or several cycles of freeze/thaw treatment by "denaturation indices", such as Ca^{2+}-ATPase activity, surface hydrophobicity, sulfhydryl & disulfide bond content, water holding capacity, expressible moisture, salt soluble protein content, gel strength, gel texture and unfrozen water content (Nikoo et al., 2016). Differential scanning calorimetry and low-field proton nuclear magnetic resonance are also techniques that have been used in recent years to study the cryoprotective effects of hydrolysates on seafood systems (Nikoo et al., 2016).

It is therefore evident that there is now a growing body of knowledge that supports the production of protein hydrolysates from marine sources for potential use as cryoprotectants in seafood products. Nevertheless, a number of questions still remain concerning the practicality of using these protein hydrolysates as a

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food cryoprotectant: what are the optimal processing conditions for creating hydrolysates with cryoprotective abilities? What level of hydrolysate is needed for sufficient cryoprotection in fish products, and how does the incorporation of hydrolysates into these products affect the taste? The need to address these questions forms the basis for the current study (Jenkelunas, 2013).

The specific objectives of this research were (1) to apply response surface methodology to identify the best production conditions for generating cryoprotective fish protein hydrolysates from Pacific hake, and (2) to characterize the properties of the fish protein hydrolysate produced under the optimized conditions, by measuring various indices of denaturation, as well as by evaluating taste attributes of minced fish balls containing fish protein hydrolysate before and after freeze-thaw treatment. Control (no added ingredient) and sucrose-sorbitol blend were used as reference materials to assess the cryoprotective effectiveness of fish protein hydrolysates.

2. Materials and methods

2.1. Materials

Whole Pacific hake (*Merluccius productus*) caught during the spring-summer season (May-August) was provided by Steveston Seafood Direct Ltd. (Richmond, BC, Canada). Immediately after reaching land, the fish were transported on ice to the University of British Columbia food science pilot plant where they were filleted and stored in a −25 °C freezer. Fresh Pacific cod (grey cod, *Gadus macrocephalus*) fillets were purchased from Albion Fisheries Ltd (Vancouver, B.C.). The cod was transported on ice, to the University of British Columbia, no more than 24 h after reaching land. Flavourzyme® 1000 L (1000 Leucine Aminopeptidase Units LAPU/g) was acquired from Novozymes North America Incorporated through Brenntag Canada (Langley, BC). Sucrose and D-sorbitol were purchased from Fisher Scientific (Ontario, Canada). Bovine serum albumin was purchased from Sigma-Aldrich (Ontario, Canada) and bicinchoninic acid protein reagents A and B were purchased from Thermo Fisher Scientific Inc. (Illinois, United States). HPLC peptide standard mixture (Product No. H 2016) was purchased from Sigma-Aldrich (Ontario, Canada), Egg albumen was acquired from Inovatech (now Neova Technologies Inc.) in Abbotsford, BC, Canada. Ingredients for the sensory evaluation (bread crumbs, corn oil, garlic, onion, salmon, salt, skim milk powder, sugar, sunflower oil and wheat flour) were bought at a local supermarket (Canada Safeway, Vancouver, BC, Canada).

2.2. Optimization of conditions for fish protein hydrolysate production

2.2.1. Experimental design

Optimization of the conditions to produce fish protein hydrolysates from Pacific hake (hereinafter abbreviated as "FPH") for cryoprotection was conducted using response surface methodology (RSM) and a three-factor central composite rotatable design (CCRD). A rotatable design is one where the prediction variance has the same value at any two locations that are the same distance from the design center (Myers & Montgomery, 2002). Flavourzyme® was used as the enzyme preparation for hydrolysis based on its ability to produce hydrolysates with high degree of hydrolysis and low bitterness (Cheung & Li-Chan, 2010). Furthermore, the combination of endoproteinase and exopeptidase activity in this enzyme preparation (Merz et al., 2015) was expected to be beneficial since amino acids, as well as peptides, have been reported to exhibit cryoprotectant properties (Noguchi & Matsumoto, 1971, 1975).

Using the CCRD, twenty FPH were produced from Pacific hake fillets under experimental conditions varying in the levels of pH, hydrolysis time and% enzyme/substrate (%E/S) (Supplementary material Table S1). The temperature of hydrolysis was held at 50 °C, the optimum temperature for Flavourzyme (Novozymes, 2001). The range of pH in the CCRD was selected to fall between 5 and 7, which is the range for optimal enzyme activity (Novozymes, 2001). The range of time for hydrolysis, between 1 and 6 h, was selected based on literature indicating that hydrolysates obtained using times over 6 h would exhibit less desirable taste and colour properties. Imm and Lee (1999) found that a 6-h hydrolysis of red hake with Flavourzyme (2% E/S) yielded a flavour stock with highest consumer acceptability, while a study by Dong et al. (2008) linked darkening of silver carp hydrolysates with increasing hydrolysis times. The recommended concentration of Flavourzyme is 1–2% E/S for debittering or flavour production applications, but higher ratios of enzyme are used for more extensive hydrolysis (Imm & Lee, 1999; Novozymes, 2001); therefore, a range of 1–4% E/S was explored in this study.

2.2.2. Production of fish protein hydrolysates

FPH was produced using Pacific hake fillets as described by Cheung et al. (2009). Five hundred grams of Pacific hake fillets were chopped into 2.5-cm pieces and placed in a 4-L beaker with 1 L of double distilled water. For each of the twenty batches of FPH to be produced under the conditions given by the CCRD, the pH of the fish mixture was adjusted using 1 N HCl and 1 N NaOH to a level between 5 and 7 (Table S1), and the temperature was quickly raised to 50 °C by submersion in a water bath. The predetermined concentration of Flavourzyme (Table S1) was then added to the fish (w/w protein), assuming ~18.31% protein in Pacific hake muscle (National Oceanic and Atmospheric Administration Fisheries, 2016). The hydrolysis progressed at 50 °C for the allotted time between 1 and 6 h (Table S1) while being stirred at 400 rpm with an overhead stirrer, before being terminated by boiling for 15 min in a steam kettle. The resulting hydrolysate was centrifuged at 12,000*g* for 15 min at room temperature; the supernatant was collected and then freeze dried to yield FPH, which was stored in sealed 50 ml Falcon™ tubes at −25 °C.

Based on the results from response surface methodology regarding effects of the production variables on cryoprotective effectiveness of the FPH, additional batches of FPH were produced for analysis of hydrolysate composition and taste attributes, using the following conditions: no pH adjustment, and hydrolysis for 1 h using Flavourzyme at 1% E/S ratio. The original pH of the samples was ~6.8.

2.3. Preparation of fish mince for assessment of cryoprotection by FPH

Fish mince was prepared from Pacific cod fillets using a BEEM-GIGANT Grinder, Model TYP EF5-10 (BEEM California Corp.; California, USA), with a 4-mm screen. FPH was added to 250 g portions of fish mince at 8% (w/w) concentration for comparing each of the 20 different batches of FPH from the CCRD and for the sensory evaluation of taste attributes, and also at four different concentrations between 2 and 8% to assess the effect of dose on the resulting denaturation indices. Portions of fish mince with no added ingredients (control), or with 8% sucrose-sorbitol in a 1:1 ratio (hereinafter referred to as "suso") were prepared at the same time for comparison.

Each sample was mixed thoroughly using a Kitchen-Aid bowl mixer on a setting of 4, for 1 min, then divided into two separate polyethylene pouches and heat-sealed. One pouch of each sample was analyzed that day, while the other pouch was analyzed after 6 freeze thaw cycles, with each freeze thaw cycle involving 18 h at −25 °C followed by 6 h at 4 °C (Cheung et al., 2009).