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Response surface optimization of bromelain-assisted gelatin extraction from surimi processing wastes

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ABSTRACT

Response surface methodology (RSM) with a 4-factor, 5-level central composite design (CCD) was used to optimize the extraction conditions for fish gelatin from surimi processing wastes assisted with enzyme, bromelain. The fish waste used was from only one fish species, ribbon fish (*Lepturacanthus savel*). The effects of enzyme concentration (X_1), acid pretreatment time (X_2), extraction temperature (X_3) and extraction time (X_4) on gelatin yield (Y_1) and gel strength (Y_2) were investigated. The optimum conditions for gelatin extraction were found to be: bromelain concentration, 0.3 g/L; acid pretreatment time, 1.5 h; extraction temperature, 41 °C and extraction time, 5 h. Under these optimum conditions, yield of gelatin obtained was 18.3±1.1% and gel strength was 62.9±1.7 g. The yield of gelatin was found to increase by almost 50% compared with that obtained from gelatin extraction without addition of bromelain. The physicochemical properties of the extracted fish gelatin were determined and compared with those of commercial fish, porcine and bovine gelatin. The ash content, viscosity and amount of imino acid (hydroxyproline and proline) for extracted fish gelatin were 2.4%, 1.9±0.05 mPa s and 144.8/1000 amino residues, respectively. This study shows the potential of surimi processing wastes as an alternate source for fish gelatin production.

1. Introduction

Gelatin is a water soluble biopolymer derived from thermal denaturation of collagen. Being the most abundant protein constituent in the vertebrate body, collagen is distributed within animal skins, bones, tendons and vessels (Brinckmann, 2005). In the manufacturing of gelatin, raw materials are pretreated with either acid or alkaline to allow the swelling of collagen in order to increase the efficiency of gelatin extraction during thermal hydrolysis. The resultant gelatin may be classified into two types: Acid pretreated gelatin is classified as Type A gelatin with an isoelectric point between 7 and 9; and alkali pretreated gelatin as Type B gelatin with an isoelectric point of about 5 (Eastoe & Leach, 1977). Pretreatment with a mild acid does not hydrolyze the amide nitrogen of glutamine and asparagine, thus, yielding type A gelatin with an isoelectric point that might be as high as 9.4. If a more severe acid solvent is used during the pretreatment process, the isoelectric point of the resulting gelatin ranges from 6 to 8, which is similar to that of a collagen molecule in the raw material (Eastoe & Leach, 1977). In alkaline pretreatment process, the alkali progressively hydrolyses the amide groups of asparagine and glutamine side chains of collagen leading to a reduction in the number of amide groups. As a result, there is a net negative charge on the collagen molecule due to number of carboxyl groups remaining on gelatin molecule, thus resulting in a lower isoelectric point (Malafaya, Silva, & Reis, 2007; Young, Wong, Tabata, & Mikos, 2005). During thermal hydrolysis extraction step, the triple helices of the collagen molecule undergo a

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helix-to-coil deformation to produce loosely coiled gelatin polypeptide chains (See, Hong, Ng, Wan Aida, & Babji, 2010). In the industry, the thermal extraction step is usually carried out in several steps with gradually increasing temperatures starting from 50 to 60 °C at about 5 to 10 °C temperature increment, and then until boiling temperatures. Gelatins collected at lower extraction temperatures are those with minimal degradation while those collected at higher extraction temperatures have wider molecular weight distribution. Finally, the gelatinous solution is subjected to filtration, evaporation, deionization, drying and grinding (Hinterwaldner, 1977).

Gelatin is applied extensively in the food industry for the production of confectionaries, dairy products, bakery products, meat products and canned foods. Gelatin is used to improve the properties and the melt-in-the-mouth sensation of these products. The pharmaceutical industry uses gelatin as one of the main ingredients to manufacture pharmaceutical capsules, tablet coating, emulsions and cosmetics. Gelatin also plays an important role in the manufacturing of cohesive glues as well as in the making of films for the photographic industry (Djagny, Wang, & Xu, 2001; Gomez-Guillen et al., 2002; Hendriks, Lesser, Stewart, & Nishimura, 1984). Gelatin produced from raw materials such as porcine and bovine skins and bones (Hinterwaldner, 1977; Ward & Courts, 1977) are rejected by Muslims, Hindus and Jews due to religious issues while other consumers are concerned about food safety ever since the outbreak of Bovine Spongiform Encephalopathy (BSE or mad cow disease). To overcome these problems, fish gelatin, one of the alternatives to porcine and bovine gelatin, is gaining interest recently from gelatin manufacturers and consumers (Haug, Draget, & Smidsrød, 2004; Pranoto, Lee, & Park, 2007).

In surimi processing, fish meat is separated from other parts of the fish and used for further processing. The surimi processing by-products, comprising of fish skins, bones and scales, could reach 50–60% of the total catch (Arvanitoyannis & Kassaveti, 2008; Morrissey, Lin, & Ismond, 2005). These by-products, sometimes called fish wastes, are conventionally turned into fishmeal for animal feed but usually are discarded. However, if the disposal of these fish wastes is not properly carried out with proper waste treatment protocols, it may result in serious environmental problem. As a rich source of naturally occurring collagen, fish wastes can be utilized as raw materials to produce value-added ingredients such as gelatin. Studies have been carried out to characterize and to improve the properties of gelatin extracted from fish waste of different fish species, such as gray triggerfish skin (Jellouli et al., 2011), skin and bone of tiger-toothed croaker and pink perch (Koli et al., 2012), unicorn leatherjacket skin (Ahmad & Benjakul, 2011), walleye Pollock skin (Yan, Li, Zhao, & Yi, 2011), red tilapia skin, walking catfish skin and striped catfish skin (Jamilah, Tan, Umi Hartina, & Azizah, 2011), carp skin (Duan, Zhang, Du, Yao, & Konno, 2009), blue shark skin (Limpisophon, Tanaka, Weng, Abe, & Osako, 2009) and NZ hoki skin (Mohtar, Perera, & Quek, 2011).

Relatively new pretreatment processes using enzymes are less time consuming and can produce a higher gelatin yield with higher purity. The effectiveness of converting collagen to gelatin is however, dependent on the degree of cross linking in the raw materials (Galea, Dalrymple, Kuypers, & Blakeley, 2000). Studies have been done on gelatin extraction using pepsin or proctase, isolated from *Aspergillus niger*, from bovine skins (Chomarat, Robert, Seris, & Kern, 1994) and using pepsin and protease inhibitor from bigeye snapper skin (Nalinanon, Benjakul, Visessanguan, & Kishimura, 2008). However, most of these reports have focused on gelatin extraction from sources such as bovine and fish skins using acids and bases, with very few reports using enzymes in the pretreatment steps. To the best of our knowledge, bromelain, a proteolytic enzyme has not been used in any gelatin extraction methods. This study aims to produce fish gelatin from under-utilized surimi processing by-products of ribbon fish species (*Trichiurus lepturus*), using extraction methods aided by bromelain. This work describes the use of response surface methodology (RSM) approach to optimize the gelatin extraction conditions. In addition, the physicochemical properties and rheological properties of the extracted fish gelatin were also determined and compared with commercial fish, bovine and porcine gelatin preparations were made.

2. Materials and methods

2.1. Materials and chemicals

Surimi processing wastes were collected from a local surimi processing plant in Perak, Malaysia. The fish waste materials are by-products from the surimi processing plant, collected after the mincing stage i.e. after removal of head and guts (dressing step) and separation of fish meat from the bones and skins (meat separator step). The fish wastes (mainly fish skins, bones, scales and remaining bits of fish meat) were packed in 5 kg blocks and kept frozen after being produced at the surimi processing plant and were immediately transported frozen to the laboratory. Upon arrival of samples, they were partially thawed and homogenized before repacking in 300 g portion in plastic bags and stored at −20 °C until further use. Bromelain was purchased from Merck Group (Germany). Commercial gelatin preparations such as fish gelatin (provided by Croda Colloid Ltd, UK), bovine gelatin (Halalgel Sdn. Bhd., Malaysia) and porcine gelatin (Gelnex Gelatin, Brazil) were obtained. For amino acid analysis by high performance liquid chromatography, borate buffer (pH 10.2), o-phthalaldehyde (OPA) and 9- fluorenylmethyl chloroformate (FMOC) were purchased from Agilent Technologies (USA). For electrophoretic analysis, sodium dodecyl sulfate (SDS), β-mercaptoethanol, N,N,N′,N′-tetramethylethylenediamine (TEMED), and Bio-safe Coomassie Brilliant Blue were purchased from Bio-Rad Laboratories (USA). HPLC grade acetonitrile was from Fisher Scientific (UK) and HPLC grade methanol was from Merck (Germany). Other chemicals used were of analytical grade.

2.2. Gelatin extraction procedures

Gelatin was extracted from fish wastes following the methods described by Nalinanon et al. (2008) with slight modifications. Calcium hydroxide and citric acid are used in the extraction process based on our previous work (Norziah, Al-Hassan, Khairulnizam, Mordi, & Norita, 2009) and also