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Extraction optimization and properties of collagen from yellowfin tuna (*Thunnus albacares*) dorsal skin

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Abstract

Yellowfin tuna (*Thunnus albacares*) dorsal skin obtained from tuna processing was used as a source of fish collagen. Optimization of collagen extraction was investigated by using a central composite design of response surface methodology. The optimal conditions were $X_1 = 0.92$ N (NaOH concentration), $X_2 = 24$ h (NaOH treatment time), $X_3 = 0.98\%$ (w/v) (pepsin concentration) and $X_4 = 23.5$ h (digestion time). The predicted collagen content under optimal conditions was 26.7%, and the actual experimental collagen content was 27.1%. The properties of yellowfin tuna dorsal skin collagen were characterized by amino acid analysis, SDS-PAGE, FT-IR, solubility and viscosity. The yellowfin tuna dorsal skin collagen had a 20.5% imino acid content. Electrophoresis revealed two different α (α_1 and α_2) chains, β-component and γ-component. FT-IR showed regions of amides A, I, II and III were 3427, 1651, 1544 and 1240 cm^{-1}, respectively. Collagen solubility sharply decreased at over pH 4.0 and it was relatively low in the range of pH 5.0–9.0. Collagen solubility continuously decreased with increasing salt concentrations up to 4% and was little changed at higher concentrations. The viscosity of the collagen solution decreased at a constant rate until 32 °C and then decreased at a slower rate from 33 to 50 °C.

Keywords: Collagen; Yellowfin tuna (*Thunnus albacares*); Optimization; Response surface methodology; Physicochemical property

1. Introduction

Collagen, one of the most abundant animal derived proteins, is the precursor of gelatin which is widely applied to commercial products. Collagen accounts for about 30% of the total protein of most organisms, and has 19 variants designated type I–XIX. Collagen can be found in all animal parts, but is especially concentrated in skin-associated tissues and bones (Nakamura, Iwamoto, Ono, Nishimura, & Tabata, 2003). Collagen is also a major component of extracellular matrices and has the function of improving strength and resistance in tissues (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003). Collagen is widely and diversely used in food, medicine, cosmetics and cell cultures; the consumption of collagen has increased with the development of new industrial applications. Collagen used in commercial products is mainly obtained from cows and pigs, but mammalian diseases such as bovine spongiform encephalopathy (BSE) and foot/mouth diseases present safety problems because of the risk of transferring the diseases to humans. Because of this problem, there is a need to develop new and safer sources of collagen to prevent collagen shortages as consumption increases in the future.

The search for new collagen sources has resulted in studies of the functional properties of marine source collagens: i.e. skins of salt and fresh water fish (Ikoma et al., 2003), shark skins (Yoshimura, Terashima, Hozan, & Shirai, 2000), brownstripe red snapper skin (Jongjareonrak, Benjakul, Visessanguan, Nagai, & Tanaka, 2005), skins and bones of bigeye snapper (Kittiphattanabawon, Benjakul, Visessanguan, Nagai, & Tanaka, 2005), squid skin (Kolodziejska, Sikorski, & Niecikowska, 1999), skins of young and adult Nile perch (Muyonga, Cole, & Duodu, 2004a, b) and outer skins of the paper nautilus (Nagai & Suzuki, 2002). These collagens are extracted from such byproducts as skins, bones and fins during processing of

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the above fishes. The amount of byproduct produced during fish processing can be as high as 75% of the total weight of the fish, a very high percentage. Some of the byproducts are used in animal feed, but much of it is discarded and is a source of environmental contamination. The amount of hazardous waste produced from fish processing has tended to increase annually (Shahide, 1994). Therefore, utilization of fish waste to produce useful ingredients such as collagen, chondroitin sulfate and gelatin has significant indirect environmental benefits.

Large quantities of yellowfin tuna are commercially used in canned products and as sasimi, a delicate-tasting raw fish product popular in Korea and Japan. The use of yellowfin tuna as sasimi is increasing in several other countries, with an annual worldwide production of 3,400,000 MT (2001 World Capture Production of FAO Fisheries Department). Therefore, if high-quality collagen can be extracted form yellowfin tuna byproducts, it would be an economically important source of disease-free collagen and substantially reduce the amount of pollution from waste products.

In order to optimize collagen extraction, response surface methodology (RSM; Box & Wilson, 1951) was utilized. The basic principle of RSM is the evaluation of the relationship between the predicted values of the dependent variable and the conditions of dependent variables (Edwards & Jutan, 1997). Cho, Gu, and Kim (2005) previously reported that gelatin extraction was optimized from yellowfin tuna skin by using RSM. They demonstrated that the RSM was very effective for investigating the optimum extraction conditions for producing gelatin.

The objectives of this study were to determine the optimum conditions for extracting collagen from yellowfin tuna dorsal skin using RSM, and to investigate the chemical properties of the extracted collage by amino acid analysis, SDS-polyacrylamide gel electrophoresis (PAGE), Fourier transform infrared spectroscopy (FT-IR), solubility and viscosity.

2. Materials and methods

2.1. Materials

Collagen was extracted by using a slightly modified method of Ogawa et al. (2004). Frozen yellowfin tuna (*Thunnus albacares*) dorsal skin was provided by Doo-Young Fisheries Co. (Busan, Korea). The tuna skins were washed in order to remove the attached meats and scales. The washed skins were chopped (IS-12S chopper, Ilshin Co., Incheon, Korea) and stored at −18 °C until used. Chemical properties of the yellowfin tuna skin collagen were compared with a mammalian collagen extracted from calf skins (EC No. 232-697-4, Type I; Sigma, USA). All reagents used in this study were analytical grade.

2.2. Extraction of collagen from yellowfin tuna

The cleaned skins were treated with five volumes (v/w) of alkali solution (0.5–1.3 N NaOH) at 9 °C in a shaking incubator at 200 rpm (HB-201SF, Hanbaek Scientific Co., Korea) for 12–36 h. NaOH treatment removed non-collagen proteins and subcutaneous tissues after they were swollen. After the alkali treatment, the skins were neutralized with 6 N HCl and washed. For pepsin digestion, 20 volumes (v/w) of pepsin solution (0.6–1.4% (w/v)) in an HCl solution (pH 2.0) was added to the alkali-treated skin samples in a shaking incubator at 9 °C and 200 rpm for 12–36 h. The upper phase was vacuum-filtered through filter paper (5A 110 mm, Advantec, Japan).

The final concentration of the filtered sample was adjusted to 5% with 20% NaCl. The sample solution was centrifuged at 10,000*g* at 4 °C. The centrifuged solution was discarded. Ten-fold distilled water was added to the precipitate, mixed and centrifuged. Three or four subsequent centrifugations were carried out. Total precipitates were collected, neutralized and washed. The extracted collagen was freeze-dried for low-heat denaturation and used for experiments.

2.3. Experimental design and analysis of data

A central composite design (CCD; Box & Wilson, 1951) was used for the optimization of collagen extraction from yellowfin tuna dorsal skins. CCD in the experimental design consisted of 2^4 factorial points, eight axial points ($\alpha = 2$) and three replicates of the central point (Table 2). Processing of collagen included two important continuous steps: alkali treatment and pepsin hydrolysis. Concentration of NaOH (N, X_1), alkali treatment time (h, X_2), pepsin concentration (% (w/v), X_3) and pepsin digestion time (h, X_4) were chosen as independent variables in five different ranges. The ranges and center point values of four independent variables were based on the results of preliminary experiments (Table 1). Collagen content (Y, %) was selected for the dependent variable. The basic model equation is shown in the following formula:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_1^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j, \qquad (1)$$

where Y is the dependent variable (collagen contents, %), β_0 is a constant, β_i, β_{ii}, β_{ij} are regression coefficients and X_i, X_j are levels of the independent variables.

Table 1
Experimental range and values of the independent variables in the central composite design for collagen processing from yellowfin tuna (*Thunnus albacares*) dorsal skin

Independent variables	Symbol	Range and levels				
		−2	−1	0	+1	+2
Concentration of NaOH (N)	X_1	0.5	0.7	0.9	1.1	1.3
Treatment time (h)	X_2	12	18	24	30	36
Pepsin concentration (%, w/v)	X_3	0.6	0.8	1	1.2	1.4
Hydrolysis time (h)	X_4	12	18	24	30	36