Food Chemistry 197 (2016) 814–820

Contents lists available at ScienceDirect

Food Chemistry

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

Element distribution and morphology of spotted golden goatfish fish scales as affected by demineralisation

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ARTICLE INFO

Article history:
Received 29 April 2015
Received in revised form 21 October 2015
Accepted 10 November 2015
Available online 11 November 2015

Keywords:
Spotted golden goatfish
Morphology
Scales
Demineralization
Element

ABSTRACT

Scales of spotted golden goatfish were subjected to non-collagenous protein removal followed by demineralisation with hydrochloric acid at different concentrations (0.25, 0.5, 0.75 and 1 M) for various times (30, 60 and 90 min). The morphology and element composition/distribution of scales from spotted golden goatfish as influenced by demineralisation conditions were determined. The appropriate demineralisation was pertained using 0.75 M hydrochloric acid for 30 min, in which the ash content was 0.62% (dry weight basis). The scales having non-collagenous protein removal with, and without, subsequent demineralisation were analysed for element content using X-ray fluorescence spectrometer. Images of different scales were determined using scanning electron microscopy (SEM) and scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM–EDX). Based on the images, an external layer rich in inorganic elements was removed. Most of Ca and P were eliminated with the coincidental increases in organic substances (C, N and O) after demineralisation. Demineralisation therefore mainly removed the external layer of scales, which facilitated the further extraction of collagen or gelatin.

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1. Introduction

Fish collagen and gelatin have gained increasing attention since they can be produced from fish processing byproducts, such as skin, scales and bones. They have been consumed widely, irrespective of religion. During dressing and descaling, scales are removed and those scales could be used as collagenous material. Basically, scales are rich in Ca phosphate compounds, such as hydroxyapatite and Ca carbonate. The surface of a fish scale is an osseous layer consisting of randomly oriented collagen fibrils with many hydroxyapatite ($Ca_{10}(O_4)_6(OH)_2$) crystals. Thin layers of the oriented collagen fibrils are piled up to form the fibrillary plate under the osseous layer. This plywood-like structure provides the high mechanical strength of scales (Okuda et al., 2011). Therefore, the removal of Ca from fish scales is a necessary pretreatment for collagen or gelatin production. Demineralisation aims to remove the calcium and other inorganic substances to facilitate the extraction of collagenous components (Waldner, 1977). The inorganic substances in scale can be removed with dilute hydrochloric acid (HCl), whereby the calcium phosphate is dissolved as acid phosphates (Waldner, 1977). Depending on the nature of the material, temperature and acid concentration, the demineralisation time can be varied. HCl concentration is generally used in the range of 2–6%. Acid hydrolysis of the protein should be minimised during demineralisation. High temperature should also be avoided since it can enhance the hydrolysis of proteins, especially under acidic conditions (Waldner, 1977).

Fish scales have plywood-like structures of closely packed collagen fibre layers, reinforced with a mineral phase of calcium-deficient hydroxyapatite (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003). In general, the spatial organisation of collagen fibres is of key importance for the mechanical properties of different connective tissues (Weiner, Traub, & Wagner, 1999). Spotted golden goatfish (*Parupeneus heptacanthus*) has been widely used for frozen fillet manufacturing in Thailand. The scales from this species can serve as alternative raw material for collagen or gelatin extraction, however the proper demineralisation of scales is required. Thus, the better understanding on mineral composition/distribution in scale as affected by demineralisation could bring about the effective extraction of collagen or gelatin from fish scale. Therefore, the aim of this study was to investigate the morphology and element composition/distribution of scales from spotted golden goatfish as influenced by demineralisation.

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http://dx.doi.org/10.1016/j.foodchem.2015.11.044
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2. Materials and methods

2.1. Collection and preparation of spotted golden goatfish scales

Scales of spotted golden goatfish, with an average body weight of 100–120 g/fish, were collected from Kingfisher Holding, Ltd., Songkhla Province, Thailand. The scales were packaged in polyethylene bags and transported in ice with a scale/ice ratio of 1:2 (w/w) to the Department of Food Technology, Prince of Songkla University within 1 h. Upon arrival, scales were washed with chilled water and drained before being packaged in polyethylene bags. The samples were kept at −20 °C and the storage time was not longer than 2 months. Before use, fish scales were thawed using the running water for 20 min.

2.2. Removal of non-collagenous proteins of spotted golden goatfish scales

Spotted golden goatfish scales were subjected to non-collagenous protein removal, according to the method described by Weng, Zheng, and Su (2014), with a slight modification. Scales were firstly suspended in 0.1 M NaOH for 6 h at the ratio of 1:10 (w/v) with continuous stirring at 25 °C. The solution was changed every 3 h. Treated scales were washed with distilled water until wash water became neutral or faintly alkaline.

2.3. Demineralisation of spotted golden goatfish scales under different conditions

Demineralisation was performed according to the method described by Weng et al. (2014) with a slight modification. The prepared scales were soaked in HCl of different concentrations (0.25, 0.5, 0.75 and 1 M) for various times (30, 60 and 90 min) at the scale/solution ratio of 1:5 (w/v) with continuous stirring at 25 °C. After being demineralised, the scales were washed until the neutral or faintly acidic pH of wash water was obtained.

2.4. Determination of ash content

The resulting scales obtained from different demineralisation conditions were determined for ash content (AOAC, 2000). The scales demineralised under the appropriate condition, showing the lowest ash content, were further analysed.

2.5. Analyses of scales without and with different pretreatments

Different scales, including (1) original scales, (2) scales with non-collagenous protein removal and (3) scales having non-collagenous protein removal followed by the selected demineralisation (using 0.75 M HCl for 30 min), were subjected to analyses.

2.5.1. Scanning electron microscopy

Scanning electron microscopy was used according to the method described by Lin, Wei, Olevsky, and Meyers (2011) with slight modification. Microstructure of scale samples was visualised using a scanning electron microscopy (SEM). Scale was fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h. The samples were rinsed with distilled water for 1 h and dehydrated in ethanol with a serial concentration of 50%, 70%, 80%, 90% and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 20 kV.

2.5.2. Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM–EDX)

Scanning electron microscopy with energy dispersive X-ray spectroscopy was used as per the method of Lin et al. (2011) with slight modification. Scale samples were also visualised using a field emission scanning electron microscope (FEI-XL30, FEI Company, Hillsboro, OR, USA) equipped with electron-dispersive X-ray spectroscope (EDX). The samples were gold coated and observed with secondary electron mode at a 10 kV accelerating voltage. Elemental analysis was conducted on the cross section of the scale by EDX to verify the element content and distribution.

2.5.3. X-ray fluorescence spectrometer

X-ray fluorescence spectrometer was used as described by Lin et al. (2011) with slight modification. Elemental contents of different scale samples were determined by a X-ray fluorescence spectrometer (PW2400-Sequential WXRF Spectrometer, Philips, Eindhoven, The Netherlands). The scale was heated at high temperature (950 °C) for 2 h. The residues (0.5–1 g) were analysed using a flow and scintillation detector (PW2400-Sequential WXRF Spectrometer, Philips, Eindhoven, The Netherlands). Start angles (°2T) were 12–130 and end angles (°2T) were 18.6–146.98. kV of 24–60 and mA of 40–100 were used.

2.6. Statistical analysis

Demineralisation was carried out in triplicate. All analyses were performed in duplicate, except for ash analysis, which was conducted in triplicate. Data were subjected to analysis of variance and mean comparison was done using Duncan's multiple range test. Analysis was performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Ash contents of scales demineralised under different conditions

Ash contents of spotted golden goatfish scale demineralised using different HCl concentrations and soaking times are shown in Table 1. Ash contents of scales generally decreased with increasing HCl concentrations ($p < 0.05$), regardless of soaking time. However, when scales were treated with a 0.5 HCl solution, the lower ash content was found as the soaking time of 1.5 h was used

Table 1
Ash contents of spotted golden goatfish scale demineralised under different conditions.

HCl concentration (M)	Soaking time (h)	Ash (%, dry weight basis)
Control		$48.69 \pm 0.76^{*,a}$
0.25	0.5	41.60 ± 1.68^{b}
	1	39.95 ± 0.71^{b}
	1.5	39.12 ± 1.52^{b}
0.5	0.5	23.12 ± 1.02^{c}
	1	22.17 ± 1.24^{c}
	1.5	14.70 ± 0.93^{d}
0.75	0.5	0.62 ± 0.53^{e}
	1	0.56 ± 1.04^{e}
	1.5	0.19 ± 0.72^{e}
1	0.5	0.17 ± 0.78^{e}
	1	0.11 ± 1.01^{e}
	1.5	0.08 ± 0.82^{e}

Different superscripts in the same column indicate the significant difference ($p < 0.05$).
[*] Values and mean ± SD ($n = 3$).

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