The Demineralization of *Sardinella gibbosa* Scales Using Hydrochloric Acid and High-Pressure Carbon Dioxide

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Abstract

This research aimed to investigate and compare the demineralization of goldstripe sardinella (GS; *Sardinella gibbosa*) scales, a major by-product in the canned fish industry prevalent in East Africa and Southeast Asia, particularly Thailand. The study focused on conventional and alternative demineralization methods, assessing the yield and quality of the demineralized scales. After removing minerals, fish scales have potential value as an alternative source of collagen and gelatin. For the strong acid treatment using hydrochloric acid (HCl), the effects of HCl concentrations (0.2-1.5 M) and treatment times (30-120 min) on demineralization efficiency were assessed. The results from RSM indicated that HCl concentration was the only treatment factor that significantly affected demineralization yield, HCl concentrations at or above 0.82 M gave a demineralization efficiency of e" 99%, independent of treatment time ($P < 0.0001$). A preliminary investigation into high-pressure carbon dioxide (HPCD) treatment of GS scales (at 10 bar for 1 to 4 h) showed comparatively lower demineralization efficiency. Within the studied parameters, the highest demineralization efficiencies for both methods were 99.89±0.06 and 16.13±1.92%, respectively. SEM images and EDX analysis confirmed the complete removal of minerals (primarily Ca and P) after HCl treatment using HCl 0.85 M for 30 min. Conversely, HPCD-treated scales exhibited altered structure and physical damage.

Keywords: *Sardinella gibbosa,* Demineralization, Hydrochloric acid, High-pressure carbon dioxide, Scanning electron microscope

Introduction

In 2023, the global sardine market reached a volume of 3.6 million US tons and is expected to reach 4.0 million US tons by 2032 (IMARC, 2024). The goldstripe sardinella (GS) species (*Sardinella gibbosa*) is one of the most abundant and widely distributed marine pelagic species in the Indo-West Pacific region, spanning from the East African coast to the Philippines, Indonesia, Northern Australia, and Taiwan (Hue et al., 2018). Thailand is one of the leading countries in sardine production, with an export rate of 13,314 US tons in January-May 2018 (TTIA, 2022).

Fish processing generates by-products, accounting for 20-75% of the total fish weight. These include skin, head, viscera, bones, scales, and contaminants (Batista et al., 2009; Khiari et al., 2017; Bellali et al., 2023). Fish scales are typically removed before filleting and account for approximately 2-5% of the fish weight (Karayannakidis & Zotos, 2016; Zhu et al., 2012). Fish scales have plywood-like structures of closely packed collagen fiber layers stiffened by various minerals (Ikoma et al., 2003). Due to their high collagen content, fish scales have the potential to be used as an alternative source of collagen and gelatin. However, their high mineral content, which can range from 20-60% of the total scale weight depending on the fish species, makes demineralization necessary as a pre-treatment step to remove minerals and increase the purity of extracted gelatin and collagen (Bellali et al., 2016).

Various studies have shown that the demineralization method utilizing either strong acids, like hydrochloric acid (HCl), or weak acids, such as ethylene diamine tetraacetic acid (EDTA) or citric acid ($HOC(CO_2H)(CH_2CO_2H)_2$), is a simple, cost-effective, and efficient approach for treating fish scales (Huang, Zou, & Jiang, 2014). This traditional demineralization method effectively removes minerals, especially calcium and phosphorus, by dissolving calcium phosphate into a solution. The majority of calcium and phosphorus in fish scales exist in the form of hydroxyapatite (HA; $Ca_{10}(PO_4)_6(OH)_2$) and account for

19.64% and 8.43% of total minerals found in fish scales, respectively (Chuaychan, Benjakul, & Kishimura, 2017). Pipatcharoenwong (2008) reported achieving a 99.75% yield in the demineralization of red snapper scales using 1.2 M of HCl and 6 h of reaction time. Feng et al. (2015) also reported a 92.70±1.32% yield in demineralizing *Cyprinus carpio haematopterus* scales using 1 M of HCl and 95 min of reaction time. However, the industrial use of these acids has adverse environmental effects and can affect the quality of collagen, prompting restrictions in some developed countries (Mahmoud et al., 2007; Oechsle et al., 2014; Hasdar et al., 2019).

Consequently, researchers are exploring cost-effective alternative demineralization methods, such as high-pressure carbon dioxide (HPCD) (Yang et al., 2019). When introduced into a reactor, CO_2 reacts with water to form carbonic acid (H_2CO_3), generating hydrogen ions (H^+), thereby reducing pH and increasing acidity to facilitate mineral removal during demineralization. While HPCD has been explored for the demineralization of shrimp shells, it has yet to be previously utilized for fish scales. In a study by Yang et al. in 2019, HPCD was introduced as an innovative fractionation technique to remove minerals from shrimp shell waste. This method involved pre-treatment of hot water at 180 °C for deproteinization and HPCD at 10 bar pressure for demineralization, primarily eliminating calcium carbonate ($CaCO_3$). The results showed that this approach achieved high deproteinization and demineralization efficiencies, exceeding 90%. Moreover, the process was completed within 3 h, resulting in a highly pure chitin product.

To date, no study on demineralization of GS scales has been conducted. This work aimed to assess and compare the yield of demineralization and quality of the treated scales obtained through HCl and HPCD treatments. Response surface methodology (RSM) was also applied to optimize conventional strong acid demineralization of GS scales. Furthermore, treated scales were characterized by scanning electron microscope (SEM) with and without energy dispersive X-ray spectroscopy (EDX) to understand the effects of both demineralization processes on their morphological properties and elemental compositions.

Materials and Methods

Materials

Goldstripe sardinella scales collected from raw fish, with an average size of 20-25 fishes/kg, were supplied by Thai Union Group PCL (Samut Sakhon, Thailand). Fresh scales were washed thoroughly with tap water, and bones and other impurities were separated from the scales before being kept in a polypropylene bag at -20 °C until further use. Hydrochloric acid solution (37% HCl; RCI Labscan Ltd., Bangkok, Thailand) and the stirred reactor (Parr reactor series 4560; Parr Instrument Company, Moline, IL) used in this experiment were supplied by Thai Union Group PCL (Bangkok, Thailand).

Demineralization of Scales Using Hydrochloric acid (HCl)

Cleaned GS scales were demineralized using HCl solutions following the method described by Chuaychan et al. (2016). The studied process parameters included 1) HCl concentrations (0.2, 0.85, and 1.5 M; covering most demineralization conditions commonly used for fish scales (Chuaychan et al., 2016; Feng et al., 2015)); and 2) treatment times (30, 75, and 120 min; time suitable for industrial applications). The ratio of fresh scales to HCl solution used in the experiment was 1:5 (w/v) with constant stirring at room temperature. The conditions for the strong acid treatment are listed in Table 1, with all experiments repeated three times. After the treatment, scales were separated from the used HCl solution by filtering through a 20-mesh sieve, then washed with deionized (DI) water until the pH of the washing water was neutral before being dried using a hot air oven (60 °C, 16 h; series ST-32; Dalle Group, Zhongshan and Lianjiang, China). The dried scales were characterized for their ash contents (AOAC 938.08, 2018), and the yield of demineralization (DM; %) was calculated by comparing the ash contents of the scales before and after demineralization.

For HCl treatment, an on-face central composite design (CCD) of RSM was employed to monitor the effects of two independent variables (HCl concentration and treatment time) on the yield of the demineralization process (DM; %; response or dependent variable). Data obtained were analyzed using Minitab 20.2 (Minitab, LLC, PA, USA) at a type I error (á) 0.05. The results were fitted with the empirical second-order polynomial model, as shown in the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \quad .(1)$$

Where Y is the response, i.e., the yield of demineralization (%); X_1 and X_2 are coded values of HCl concentration (M) and treatment time (min), respectively; b_0 is intercept; b_1 and b_2 are linear effects of HCl concentration and treatment time, respectively; b_{11} and b_{22} are quadratic effects of concentration and treatment time, respectively; b_{12} is an interaction effect

of concentration and treatment time; τ is an effect of block; and b is residual error.

Demineralization of Scales Using High Pressure Carbon Dioxide (HPCD)

The demineralization process using the HPCD method was adapted from Yang et al. (2019). The process was carried out in a commercial stirred reactor at room temperature (25±0.75 °C) with DI water as the solvent in the presence of CO_2 gas in the system. Cleaned scales were loaded into the reactor at a ratio of 5 g of scales to 1 L of water. CO_2 was injected at 10 bar for 1, 2, 3, and 4 h. All experiments were conducted in triplicate. Afterward, the scales were removed from the reactor and washed with DI water until the pH of the washing water was neutral. The dried scales were determined for their ash contents (AOAC 938.08, 2018).

Characterization of Demineralized Scales

The proximate composition of fresh scales, i.e., protein, moisture, ash, carbohydrate, and lipid contents, was estimated according to AOAC (2018). A conversion factor of 6.25 was used to calculate protein content (Tengku-Rozaina, Shu Jeng, & Amiza, 2018).

To characterize their morphologies and mineral compositions, demineralized scales were dehydrated by submersion in a series of ethanol solutions (Absolute ethanol e”99.9% (AR/ACS); RCI Labscan limited, Thailand) with increasing concentration (25%, 50%, 70%, 95%, and 100% v/v). Dried scales were mounted on bronze stubs. For SEM characterization, the scales were coated with gold (Leica EM CPD300, Singapore), while the scales were left uncoated for SEM-EDX (JEOL JSM-IT300, Tokyo, Japan). The specimens were observed on the surface and cross-section with a scanning electron microscope at an acceleration voltage of 15 kV. The SEM was equipped with an electron-dispersive X-ray spectroscope (EDX) to observe a cross-section of the scales and verify their elemental compositions and distributions at 10 kV (Chuaychan, Benjakul, & Kishimura, 2017).

To evaluate the effects of the demineralization process on the quality of demineralized scales, demineralized scales prepared under the chosen conditions were characterized for their hydroxyproline contents and amino acid profile using gas chromatography-mass spectrometry (GC-MS; GC Trace 1310, Thermo Fisher Scientific, MA, USA) and high-performance liquid chromatography (HPLC; Agilent 7890GC/ 7000C GC/MS Triple Quad, Agilent Technologies Inc., CA, USA), respectively (Cho et al., 2015; Hu et al., 2017). The HPLC analysis utilized an SB-C18 column (9.46 mm × 50 mm, Agilent Technologies Inc., CA, USA).

Statistical Analysis

All experiments were performed in triplicates. The obtained data were statistically analyzed using analysis of variance (ANOVA) in SPSS 18 for Windows (SPSS Inc., Chicago, IL, USA) at a confidence level of 95% (á = 0.05). Tukey’s adjustment was used to compare the means. The statistical analysis for RSM of HCl demineralization was described previously (section 2.2).

Results and Discussion

Demineralization of Goldstripe Sardinella Scales Using Hydrochloric Acid (HCl)

The demineralization efficiency of GS scales is shown in Table 1. RSM-CCD was implemented to explore the effects of HCl concentration (independent variable X_1; 0.2-1.5 M) and treatment time (X_2; 30-120 min) on the yield of demineralization (DM; %) of GS scales. The regression equation in Table 2 represents the full model for demineralization yield, incorporating the influence of two independent variables (X_1 and X_2) along with their linear and quadratic interactions. The model had an R^2 of 0.99. According to the model, HCl concentration between 0.2 to 1.5 M and treatment time of 30 to 120 min significantly affected DM ($P < 0.0001$). In contrast, the treatment time effect was insignificant ($P > 0.05$). There were no significant interactions between both process parameters ($P > 0.05$).

From Table 1, the efficiency of HCl treatments increased at increasing HCl concentrations, regardless of treatment time. The use of HCl at low concentration yielded low DM, e.g., at a concentration of 0.2 M and treatment time of 120 min, DM was 12.52±0.78%. On the contrary, > 99% DM was achievable with an HCl concentration of > 0.85 M ($P < 0.0001$). For example, at conditions of 0.85 M HCl and treatment time of 75 min and 1.5 M HCl and 30 min, DM was 99.40±0.44% and 99.85±0.12%, respectively. The outer layer of GS scales primarily consists of HA, which dissolves when exposed to acidic solutions with a pH below 5.5 (the critical pH of HA). As the pH decreases, the yield of demineralization increases (Dawes, 2003; Nasional & Raya, 2013). This dissolution process is accelerated at higher concentrations of HCl as more protons (H^+) are released, leading to a further drop in pH (Feng et al., 2015). Consequently, HA hydrolysis occurs, releasing

Table 1. Conditions of strong acid demineralization of the goldstripe sardinella scales as assigned by on-face central composite design of RSM and the corresponding demineralization efficiencies (DM; %)

Run order	Coded value		Actual value		Response; DM (y; %) [1,2]
	HCl concentration (x_1)	Treatment time (x_2)	HCl concentration (x_1; M)	Treatment time (x_2; min)	
1	-1	-1	0.20	30	12.06±1.45[a]
2	0	0	0.85	75	99.66±0.07[b]
3	0	0	0.85	75	99.32±0.70[b]
4	1	1	1.50	120	99.89±0.06[b]
5	-1	1	0.20	120	12.52±0.78[a]
6	0	0	0.85	75	99.37±0.19[b]
7	-1	0	0.20	75	12.13±1.67[a]
8	0	1	0.85	120	99.17±0.43[b]
9	1	0	1.50	75	99.92±0.03[b]
10	0	0	0.85	75	99.05±0.67[b]
11	0	0	0.85	75	99.60±0.28[b]
12	1	-1	1.50	30	99.85±0.12[b]
13	0	-1	0.85	30	98.95±0.02[b]

[1] Different superscript in same column indicates the significant difference ($P < 0.05$).
[2] Mean ± SD values were obtained from 3 replicates (n=3).

Table 2. Parameter estimates of the 2nd order polynomial model for the yield of demineralization by strong acid method

R^2	0.9998				
Adjusted R^2	0.9997				
Predicted R^2	0.9996				
Parameter	**Estimate**	**Standard error**	**T-Value**	**F-Value**	**P-Value**
Constant[1]	99.344	0.147	675.33		<0.0001*
Concentration (x_1)	43.823	0.145	303.00	91809.61	<0.0001*
Time (x_2)	0.121	0.145	0.83	0.70	0.410
Concentration*Concentration (x_1x_1)	-43.186	0.213	-202.58	41040.60	<0.0001*
Time*Time (x_2x_2)	-0.147	0.213	-0.69	0.48	0.496
Concentration*Time (x_1x_2)	-0.107	0.177	-0.60	0.36	0.551
Lack of Fit				3.24	0.021

*Significant at p value of < 0.05, analyzed using a two-tailed test, [1] Constant = Intercept

Table 3. The ash content (%) and yield of demineralization (%) from HPCD process

Conditions[1]	Ash (%)[2]	DM (%)[2]
Raw scales	50.13±0.44[c]	-
10 bar/1 h	40.48±0.14[b]	19.24±0.27[a]
10 bar/2 h	39.78±0.71[b]	20.65±1.41[a]
10 bar/3 h	38.04±0.15[a]	24.12±0.30[b]
10 bar/4 h	37.78±0.15[a]	24.63±0.30[b]

[1]Ratio 5:1 (g/L) [2] Different superscript in same column indicates the significant difference ($P < 0.05$).

ionic minerals and forming soluble calcium ions (Ca^{2+}), phosphate ions (PO_4^{3-}), and hydroxide ions (OH^-) from HA. At an HCl concentration of around e" 0.85 M, there are sufficient H^+ ions to remove e" 99.00% of the minerals within 30 min of treatment time (Table 1), eliminating the need for extended treatment times. Conversely, when demineralizing with HCl at low concentrations, especially those with pH close to the critical pH of HA (pH of around 5.5) within the initial 30 min, the limited H^+ ion content results in a saturation of mineral ions in the solution, raising the pH and reducing demineralization efficiency, even with extended treatment times. Experimental data showed that during the treatment with an HCl concentration of 0.2 M, the pH quickly shifted to around pH 5, indicating decreased demineralization efficiency. In this pH range, the predominating form of phosphate in the acid solution is H_2PO_4 (Al Ghuzaili, Jesil, & Saravanan, 2019; López-Pedrouso et al., 2020).

The results obtained in this study agreed with the study conducted by Feng et al. (2015) on *Cyprinus carpio haematopterus* scales using HCl solution (0.6

to 1.4 M; treatment time of 30 to 150 min), which showed that 92.70±1.32% DM could be achieved at 1 M HCl and treatment time of 95 min. DM also no longer increased when the concentration exceeded 1 M. In another study by Chuaychan et al. (2016) on DM of spotted golden goatfish scales using HCl solution (0.2 to 1 M; treatment time of 30 to 90 min), it was found that DM of 98.73±0.53% could be obtained with the treatment at e" 0.75 M for 30 min. This indicated that the demineralization of fish scales with HCl should be conducted at high acid concentrations to meet high process efficiency. It was possible to lower the treatment time to less than 30 min, which was the minimal treatment time often selected by most studies (Chuaychan et al., 2016; Feng et al., 2015), and still be able to reach DM of e" 99% (Table 1).

As no previous studies have included the demineralization of sardine scales using a treatment time of less than 30 min, additional demineralization of GS scales with 1.5 M HCl was conducted, varying treatment times from 5 to 25 min in 5 min increments. The regression equation for DM as a function of treatment time (x) is displayed in Equation 2. The equation had an R^2 of 0.95:

$$DM_{1.5M\ HCl} = 0.0023x^2 + 0.0352x + 96.559 \quad \text{.....(2)}$$

Where X is Treatment Time (min)

Based on Equation 2, the demineralization with 1.5 M HCl for only 5 min yielded approximately 96.83±0.27% DM, and the efficiency significantly increased with increasing treatment time.

Apart from being influenced by the acid concentration and treatment time, the efficiency of acid demineralization is significantly influenced by the fish species and type. Fish exhibit substantial variations in chemical composition, impurities, and external characteristics like scale size and thickness that can impact the efficacy of acid penetration (Dawes, 2003; Caruso, Floris, Serangeli, & Di Paola, 2020).

Demineralization of Goldstripe Sardinella Scales using High-Pressure Carbon Dioxide (HPCD)

The potential use of HPCD for demineralization of goldstripe sardinella scales was investigated using a commercial high-pressure reactor. Table 3 shows DM of HPCD at 10 bar for up to 4 h of treatment time. There were slight increases in DM (%) as treatment time increased ($P < 0.05$). The highest DM, 24.63±0.30%, was obtained at 10 bar for 4 h, significantly lower than those achieved with the HCl method.

The exposure of fish scales to CO_2 (in the form of carbonic acid (H_2CO_3)) initiates HA dissolution, releasing Ca^{2+}, PO_4^{3-}, and OH^- from fish scales into the solution. The acidity of the H_2CO_3 solution is directly proportional to the concentration of H^+ ions, affecting the efficiency of demineralization. In conventional acid demineralization using HCl, a solution with a low pH (pH < 0) was observed at a high concentration, indicating an elevated concentration of H^+ ions in the solution. On the other hand, in an HPCD system, the pH reduction is limited (from around 6 to 3.5), signifying a lower concentration of H^+ ions in the solution due to the constraints of CO_2 solubility and saturation of ionic minerals. In this system, CO_2 primarily exists in the gas phase (~99%), with only about 1% dissolving in the aqueous phase, thus maintaining a pH above 3 (Knoche, 1980).

Furthermore, during the demineralization process, the limited presence of H^+ ions in the solution allows various mineral ions to remain, leading to an increase in the overall pH and a reduction in demineralization efficiency. Moreover, the low acidity of carbonic acid is attributed to its limited H^+ dissociation ability, which is evident in its two-step dissociation (with pKa values of 6.35 and 10.33, respectively). In comparison, HCl, having a single dissociation step with a pKa of < 0, exhibits complete dissociation, resulting in a pronounced release of H^+ ions. This difference implies a more efficient demineralization with HCl due to its substantial release of H^+ ions.

HA dissolution also creates a pH buffering system within the mixture (Mbembela, Ngarashi, & Nyamuryekung'e, 2023). During the process, PO_4^{3-} reacts with H^+ in the solution to form dihydrogen phosphate ($H_2PO_4^-$), which acts as an acid, while HPO_4^{2-} acts as a conjugate base capable of accepting H^+ and thus maintains the pH at around 5 in HPCD system (Tanner, 2009). The rise of pH from around 3.5 to ~5 was observed during treatments. This resulted in low demineralization efficiency, even with extended treatment time (Vanderzee & Zeman, 2018).

Comparatively, this early work on HPCD demineralization of SG scales showed lower DM than that of HPCD-treated shrimp shells in the study by Yang et al. (2019). This difference could be attributed to variations in raw materials and pre-treatment methods. Minerals in fish scales predominantly exist in the form of HA (Ashwitha, Thamizharasan, & Bhatt, 2020), while shrimp shells primarily consist of calcium carbonate (Yang et al., 2019).

Characterization of Demineralized Scales

Fish scales of different fish types and/or species have different chemical compositions (Pipatcharoenwong, 2012; Cao et al., 2017) and

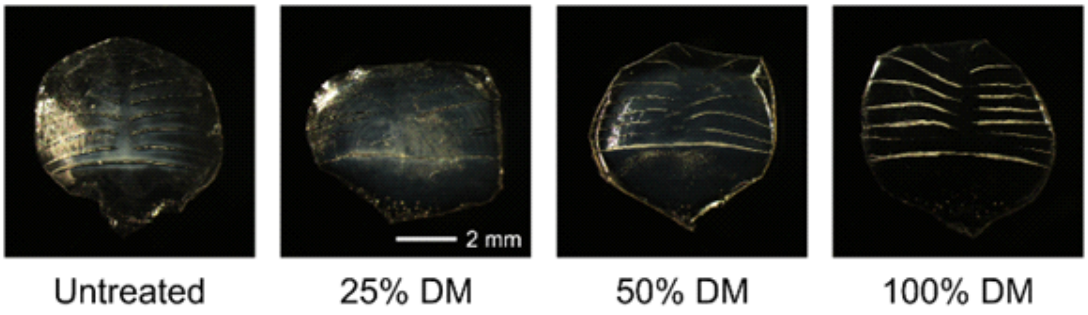

Figure 1. Appearances of goldstripe sardinella scales before and after HCl treatments.

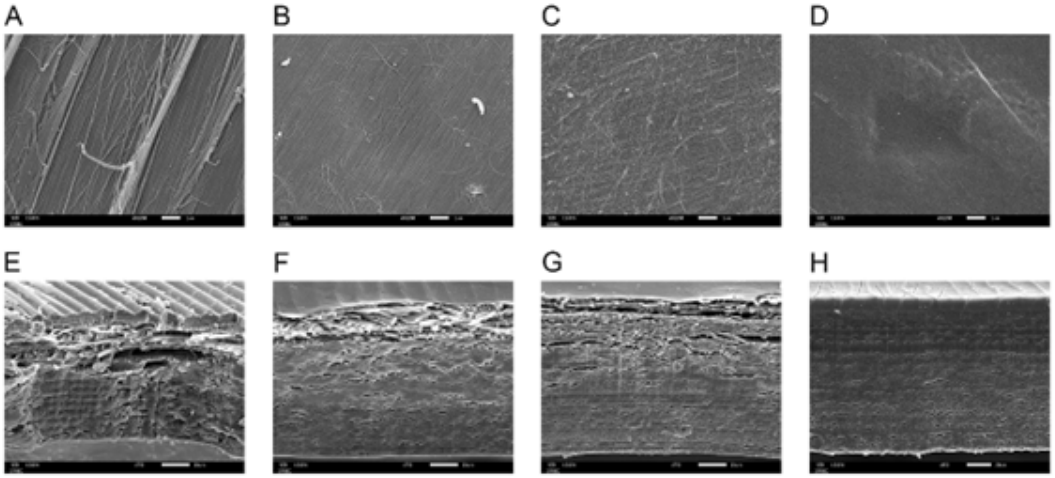

Figure 2. SEM images of surface (scale bar of A to D; 1 µm) and cross section (scale bar of E to H; 20 µm) of goldstripe sardinella scales with different degrees of demineralization by HCl; from left to right column, untreated scales (A and E); treated scales with 25% DM (B and F); treated scales with 50% DM (C and G); and treated scales with 100% DM (D and H).

morphological structures (Bräger et al., 2017). In this study, it was found that GS scales contained 52.60±0.67% of protein, 51.64±0.14% of minerals, and 0.74±0.18% of fat on a dry basis, while scales of *Sardina pilchardus* were composed of 36.25±2.3 % protein, and 50.23±0.47% minerals on a dry basis (Bellali et al., 2023). Figure 1 shows the general appearance of GS scales before and after solid acid demineralization and the progression of demineralization of the scales at different DM (%). Untreated GS scales initially had low translucency due to their high mineral content, exhibiting a whitish center (Chuaychan et al., 2016). Demineralization began around the edges, gradually progressing toward the center. As demineralization progressed (reaching 50% DM), the central region became more translucent while the edges turned transparent. This change is due to the natural thinness of the scales' edges and the accumulation of calcium phosphate in the outer layers, making calcium extraction easier (Pipatcharoenwong, 2008; Verbenas & Alma, 1958; Ogawa, Ura, & Takagi, 2010). At 100% DM, the entire scale became fully transparent (Figure 1). Eliminating minerals from scales in an acidic solution causes the disruption of non-covalent bonds in collagen molecules, loosening the protein structure. This facilitates water penetration, causing swelling and breaking intra- and intermolecular bonds. Upon drying, scales show reduced thickness and altered morphologies, appearing noticeably thinner and more transparent (Gómez-Guillœn & Montero, 2001; Pipatcharoenwong, 2008). Observations of HPCD-treated scales frequently revealed noticeable alterations in appearance (Figure not shown), likely attributed to the application of pressure. The applied pressure (10 bar) led to structural compression and physical damage (Hazen, 2009; Gagnon, Beavers, & Clearfield, 2013). These visual observations aligned consistently with the findings obtained from SEM images (Figure 2) and EDX analysis (Figure 3).

Figures 2 and 3 show the morphological structures and elemental compositions of untreated GS scales, HCl-treated scales at approximately 25, 50, and 100% DM, and HPCD-treated scales (~20% DM; Figure 3). SEM and SEM-EDX images of the untreated scale

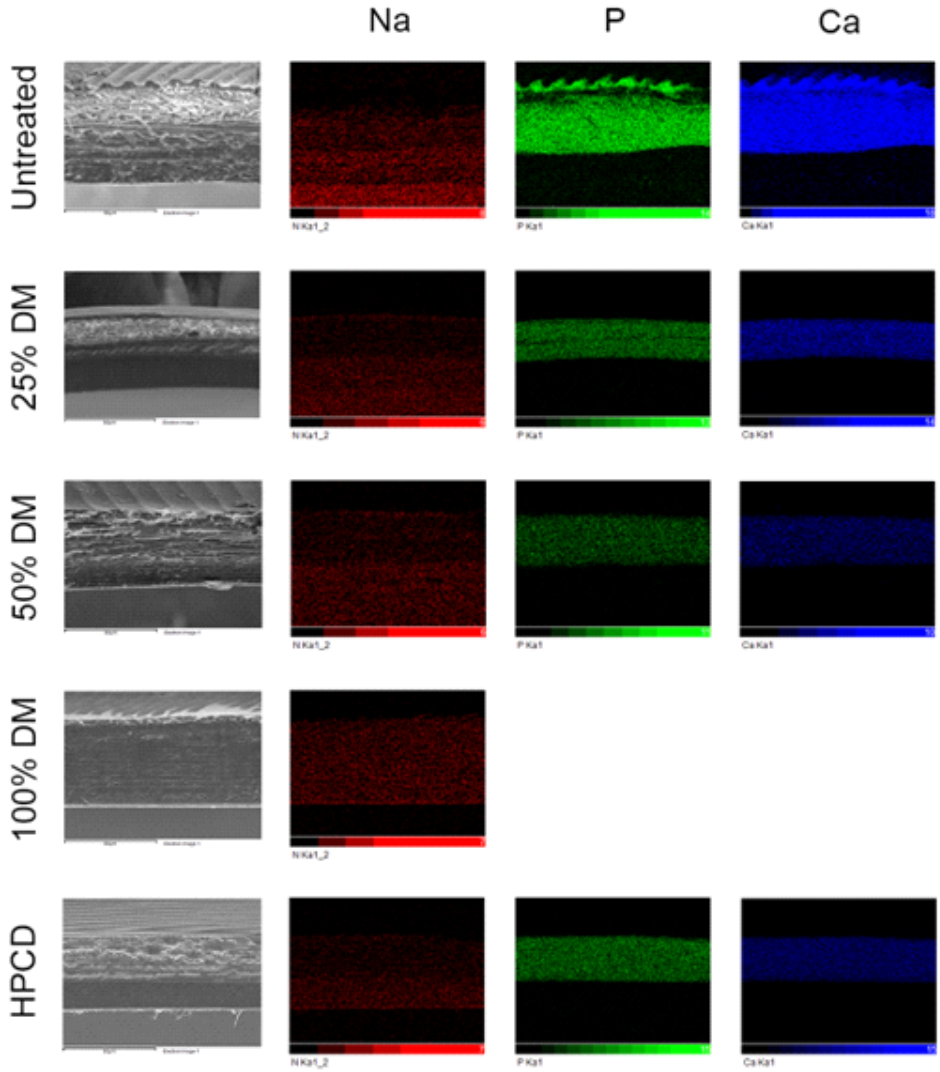

Figure 3. SEM-EDX images of cross section of goldstripe sardinella scales with 0, 25, 50, and 100% DM prepared by HCl treatment; and scales with ~20% DM prepared by HPCD method.

(Figures 2 and 3) showed dense mineral distribution (mainly Ca and P) within an external layer of the scales, particularly prominent on the rough side (distinguished by a noticeable convex line) (Ceseña et al., 2011; Chuaychan, Benjakul, & Nuthong, 2016; Torres et al., 2007). Conversely, the inner layer was rich in nitrogen (N), indicating the presence of protein or collagen type I (Chuaychan, Benjakul, & Nuthong, 2016). The elemental composition analysis of raw GS scales indicated a predominance of calcium (2.40%), phosphorus (1.44%), and magnesium (0.06%) on a dry basis, primarily existing as HA (Caruso et al., 2020). Figure 3 demonstrates the gradual removal of minerals from the external layers of the scales at 25 and 50% DM, with the central regions retaining higher mineral content. These findings were consistent with the corresponding treated scales in Figure 1. All minerals were removed at 100% DM (Figure 3), leaving behind only protein, resulting in a more densely packed structure (Figures 2 and 3). The HPCD-treated scales (~20% DM) contained 1.70% calcium and 1.19% phosphorus on a dry basis, showing no significant difference from HCl-treated scales with 25% DM.

Several studies have reported a significant reduction in hydroxyproline content, indicating a decline in collagen quality, after demineralization of fish scales using HCl (Bellali et al., 2016; Feng et al., 2015; Wang et al., 2014). However, collagen solubility depends on several factors, including acid concentration, process time, and substrate-to-solution ratio (Chuaychan, Benjakul, & Nuthong, 2016). To assess the effects of the demineralization process on the qualities of GS

scales, untreated and treated scales with 25 and 100% DM were analyzed for their amino acid contents. The primary amino acids in raw GS scales included glycine, L-arginine, proline, L-alanine, and hydroxyproline, with traces of cystine and tyrosine. Glycine is the predominant amino acid in fish scales, while hydroxyproline and proline indicate the collagen content (Chinh et al., 2019). The amino acid profile of raw GS scales was similar to that of scales with 25% DM but slightly different from that of scales with 100% DM. The predominant amino acids in fish scales treated with HPCD were glycine, proline, and hydroxyproline.

The GS scales were prepared under different conditions to explore the impact of HCl treatment parameters on demineralized scale quality, achieving similar DM (%) at approximately 95.40%, i.e., 1.5M HCl for 28 min and 0.85M HCl for 75 min. The collagen contents recovered from both demineralized GS scales were found to be 82.70% and 84.80% on a wet basis, suggesting that the use of a high concentration of HCl did not result in significant collagen loss.

Conclusions

The initial comparative analysis between the demineralization process using HCl and environmentally friendly HPCD indicated that HCl treatment was superior as a pre-treatment for collagen extraction from GS scales. The RSM model demonstrated that achieving a demineralization yield of e" 99% was feasible with an HCl concentration of e" 0.82 M. At the same time, HPCD, even at 10 bar, resulted in lower overall DM, marginally increasing with extended treatment time. For both methods, pH was the primary factor influencing the demineralization efficiency. SEM and SEM-EDX analyses confirmed the abundance of major minerals, calcium and phosphorus in the external layer of GS scales. Increasing HCl concentration facilitated the removal of these minerals, while HPCD induced structural alterations, potentially complicating the extraction process. Further research is crucial to optimize the effectiveness of HPCD demineralization. Suggested investigations include pre-treatment methods like surface enhancement through grinding and exploring additional factors, such as increased CO_2 pressure, temperature variation, and extended treatment time.

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Supplementary Materials

Supplementary materials is not available for this article

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