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Chitin extraction from sardine fish scales

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Abstract

Fish scale is a waste from the canning industry that is abundant in Zamboanga City, Philippine. In this study, the fish scale is converted into a high-value product of chitin through deproteination and demineralization. For deproteination, the concentration of sodium hydroxide and reaction time were varied at constant temperature of 80°C and sample-to-reagent ratio of 1:10. The deproteinized sample undergone demineralization setting concentration of hydrochloric acid and temperature constant while varying the sample-to-reagent ratio and reaction time. Results revealed a deproteination parameters of 4N NaOH, 1:10 sample-to-reagent ratio, 2 hrs reaction time at 80°C and demineralization parameters of 0.75 N HCl, 1:6 sample-to-reagent ratio, 72 hrs reaction time at room temperature produced 63.66(±0.12)% chitin. It is concluded that chitin was successfully extracted from sardine fish scales which is of high-value product in different industries.

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Introduction

Sardines are a small pelagic fish that are important economically in the Philippines. Between 2006 and 2015, the country's overall sardine production was forecasted to be 3.6 million metric tons, with the Zamboanga Peninsula (Region 9) accounting for 58 percent of the national sardine crop. Because of its closeness to the Sulu Sea, a well-known sardine fishing area, Zamboanga City has become the main landing place (83.81% of the catch in the region), establishing the city as the Philippines' sardine capital (Narvaez and Gangan, 2014). The wet market received 20% of the total catch in the region, while the remaining 80% was processed into canned sardines by 12 production units in Zamboanga City.

Sardinella is a genus of tiny pelagic fishes that belongs to the Clupeidae family and is known as sardines collectively. Sardines are normally abundant and thrive in coastal areas and warmer waters, where they cluster to form larger schools. There are roughly 21 species of sardinella recognized around the world (Whitehead, 1985), with 12 commercially important species found in the Philippines, including the world's only freshwater sardine species, the tawilis. The biodiversity of Philippine sardines is one of the highest in the world (Willette *et al.,* 2011), implying that the country has the essential parameters to support a viable habitat and breeding grounds.

Many fish processing companies' effluents and waste material by-products have raised public concern in recent years due to their negative environmental effects. Primarily driven by rising production outputs, these were a direct outcome of increased consumer interest in ready-to-use items, which had a direct impact on the ever-increasing demand for fish-based products (Ferraro *et al.,* 2010). In most processed fisheries resources, only about half of the overall catch (an average of 20 million tons worldwide) is used for actual human consumption, with the remaining 25% is discarded as waste (Rustad, 2003). Fish canning industries generates the second largest source of solid waste and by-product that amounts to 30 – 65% of the fish depending on the specie and type of product produced (AWARENET, 2004).

Heads, innards, scales, skins, bones, offal, and blood are some of the most common waste materials generated by a fish canning facility, and they have a lot of potential as an inexpensive feedstock for extracting high added value compounds, which could boost industry productivity while reducing fish waste management issues.

Fish meal and fish oil are made from almost all of the solid wastes created during the processing of fish. In terms of scale, fish meal is the most profitable non-edible product from fish waste, with global output averaging 5.5 to 7.5 million tons per year (Hardy and Tacon, 2002) and an average market price of $52 to $121/ton (AWARENET, 2004). Fish meal is primarily utilized as an animal feed ingredient, fertilizer, and glue component. Fish oil, on the other hand, can be used in a wide range of edible and non-edible products, including margarine, shortenings, cosmetics, varnishes, drying, and hydraulic oils. Despite the profitability of fish meal and oil extracted from fish waste, there are other high-value compounds that may be extracted from those feedstocks, such as collagen ($15/kg), anti-freeze proteins ($5,566/kg), enzymes ($16,000/kg), and chitin/chitosan ($835/kg) (Ferraro *et al.*,2010). Fish scale is an example of waste from the canning industry that is either directly processed into fishmeal or used to fuel industrial boilers. Per 1000 kilos of fish scaled, around 20 to 40 kilograms of scales are recovered (Arvanitoyannis and Kassaveti, 2008). Recovery of astaxanthin, a high-value chemical, from its seafood industry wastewater is one of its immediate applications (Stepnowski *et al.,* 2004). However, high-value minerals such as hydroxyapatite, collagen, gelatin, and chitin may be also removed from fish scales (Ferraro *et al*., 2010). Although hydroxyapatite is abundantly present in fish scales (50%), challenges are needed to overcome not on the extraction but on the prevalence of its synthetic equivalents. With this, the far more expensive chitin/chitosan presents a possible alternative for high-value material conversion on the fish scale.

Chitin, along with cellulose, is one of the most abundant kinds of renewable polysaccharide polymer.

Chitin is structurally similar to cellulose with the exception that chitin is a β (1→4) linked residue of N-acetyl-2 amino-2-deoxy-D-glucose, whereas cellulose is solely β(1→4) glucose residues. Chitin is an odorless, crystalline white to cream-colored substance with little chemical reactivity and solubility in a wide range of organic solvents. It has varying degrees of acetylation depending on the source, but there is no naturally isolated pure chitin that is 100 percent complete acetylation (Meyers *et al.,* 2008).

Depending on the type of raw material resource, chitin can take on a variety of crystalline polymorph forms. According to several researches (Aranaz *et al.*, 2009; Hayes *et al.*, 2008; Rinaudo, 2006), the forms can be identified as α, β, and γ. The most prevalent and desirable form of chitin for industrial uses is α-chitin, which is mostly obtained from crabs and shrimps. The β-chitin, the second kind of chitin, is recognized for its strong reactivity that found in almost all squid pens and can't be replicated in the lab. α-chitin has parallel and anti-parallel polysaccharide chains that are alternately linked together to form a compact orthorhombic cell, whereas β-chitin has parallel polysaccharide chains that form monoclinic cells (Barikani *et al.*, 2014). On the other hand, because there have been few investigations on γ-chitin, its structure is not well understood. However, it is thought that γ-chitin is simply a mixture of the α-chitin and β-chitin structures (Aranaz *et al.*, 2009).

Chitin is produced mostly by arthropods such as insects and crabs. Fungi, nematodes, mollusks, and diatoms may biosynthesize chitin to some extent, and vertebrates (fish and amphibians) can produce it endogenously (Tang *et al.,* 2015). However, spent shell offal from crab, shrimp, and krill processing is the most prevalent industrial source of chitin, accounting for 37,000 tons per year (Chang, 2007). Chitin content varies according on the species, water habitat, maturity stage, and harvesting conditions (Hayes *et al.,* 2008). Recent research has found chitin in the scales of *Labeo rohita* (Iqbal *et al.*, 2011; Muslim *et al.,* 2013; Suneeta *et al.*, 2016), carp or *Cyprinius carpio* (Zaku *et al.*, 2011), and nile tilapia or *Oreochromis niloticus* (Boarin Alcalde and Graciano Fonseca, 2016). However, there was no published evidence at the time of writing about chitin recovery as a high-value chemical from sardine scales, which are a common fish residue in Zamboanga City.

Material and methods

Reagents, collection, and preparation of samples

AR-grade chemicals (NaOH, HCl) were purchased from reputable chemical suppliers and were used as received. High purity distilled water were also used for solution preparations and appropriate analytical procedures were employed for preparation of solutions and reagents. Fish scales were obtained from MEGA Fishing Corporation located in Zamboanga City via purposive sampling technique. Approximately, 5 sacks of 25-Kg sack container were collected at random collection day that depend on the need of the experiment. The collected raw sardine scales free of bones and other foreign materials were repeatedly washed with hot water at approximately 95˚C to remove the remnant flesh residues and oils adhering to the scales. After the washing process the sardine scales were oven dried at 105˚C, pulverized with an analytical mill, and sieved through 150 microns sieve for particle size consistency.

Fish scales deproteinization

The procedure for deproteinization and demineralization process was adapted from the study of the study of Chang and Tsai (1997) and Kumari *et al.* (2016), with some modification. The deproteinization process was carried over, utilizing 20.0g of dried fish scales powder. Two concentrations of NaOH were selected for this study at 1N and 4N with a fixed ratio of 1:10 of dried fish scales powder in grams per mL NaOH. The fish scales sample with the NaOH were placed in a round bottom-flask then attached with a reflux condenser, heated with constant stirring at chosen temperature of 80°C for 2-hour and 6-hour periods respectively. After the reflux process, the samples were filtered and washed with distilled water until the pH became neutral. Then, the deproteinized sample were oven-dried at 105°C for 12 hours. The percent protein content of the deproteinized samples were evaluated by Kjeldahl method using the AOAC 981.10.

Fish scales demineralization.

For demineralization, 5.0g of deproteinized fish scale were added with 0.75N HCl at selected ratio of 1:6 and 1:12 of grams of deproteinized fish scale per mL of HCl. The mixtures were stirred at ambient room temperature (approximately 25˚C) for chosen stirring time of 6 hours and 24 hours to dissolve the calcium carbonate. The product was filtered and washed with distilled water until the mixture becomes neutral. Then the demineralized material was oven dried at 105˚C for 6 hours. The potential calcium content of the demineralized product was measured with atomic absorption spectrometer using AOAC 985.35 and 999.10. The isolated chitin content of the powdered sardine scales was computed from the weight difference of the initial dry raw material and the resulting weight after the treatment.

Results and discussion

The isolation of chitin from the fish scales of *Sardinella longiceps* (Tamban) undergone stepwise reaction of deproteination (DP) and demineralization (DM). These two major steps remove small amounts of pigments and lipids which improve the purity of chitin (Younes and Rinaudo, 2015).

Table 1 shows four (4) deproteination parameters each with constant heating temperature (80°C) and sample (g) to reagent (mL) ratio with varying concentration of sodium hydroxide (1.0 N and 4.0 N) and reaction time (2 hrs and 6 hrs). These protein denaturation parameters are within the parameters employed in the study of Tokatli and Demirdoven (2018) and Poeloengasihhernawan *et al*. (2009).

Table 1. Protein Denaturation Parameters.

Experiment Run	Temperature (˚C)	sample (g) to reagent (mL) ratio	Concentration NaOH	Reaction Time	Percent Protein Content (%)	Percent Yield Deproteinization (%)
DP1	80	1:10	1.0 N	2 hours	0.160(±0.030)	59.96
DP2	80	1:10	4.0 N	2 hours	0.057(±0.012)	59.80
DP3	80	1:10	1.0 N	6 hours	0.090(±0.026)	58.94
DP4	80	1:10	4.0 N	6 hours	0.313(±0.196)	57.56

At constant heating temperature and sample to alkali solution of 1:10, the experimental runs of DP1 and DP3 obtained % protein content of 0.160(±0.030) and 0.090(±0.026), respectively. On the other hand, DP2 and DP4 generated 0.057(±0.012) and 0.313(±0.196) %protein content. The chosen best deproteination parameters are that of experimental run of DP2 that resulted to low %protein content with %deproteinized yield of 59.80. As stated in previous studies, prolonged alkali treatment leads depolymerization, deacetylation (Fernandez-Kim, 2004), and aldol condensation products (Toan *et al.*, 2006).

The deproteinized fish scales sample undergone demineralization at constant concentration of hydrochloric acid (0.75 N) and reaction temperature (25 °C) with varying sample-to-reagent ratio (1:6 and 1:12) and reaction time (6 hrs and 24 hrs). As shown in Table 2, the sample-to-reagent ratio had minor effect on the demineralization of deproteinized fish scales at constant reaction time. Moreover, a significant difference was observed for the %demineralization yield (DM1, 48.78% and DM2, 23.70%) with varied sample-to-reagent ratio. In this study, the best demineralization parameters were 0.75 N HCl, 25 °C, 1:6 (sample-to-reagent ratio) and reaction time of 24 hrs that resulted to lower %Ca Content (13.40%) and higher %demineralized yield (62.91%). As compared to other literature, the percent yield of chitin after the deproteination and demineralization (62.91%) is much higher than the %chitin from shrimp of 10.13% (Tokatli and Demirdöven, 2018).

This study further explored the effect of reaction time to lower the %Ca by varying from 24 hours with an increment of 12 hrs to achieve the 1% basis to generate quality chitin from the sardine fish scales. Table 3 revealed that a reaction time of 72 hrs gave the lowest %Ca content of 0.0379 with %chitin yield of 63.66(± 0.12).

Table 2. Protein Demineralization Parameters.

Experiment Run	Concentration HCl	Temperature °C	sample (g) to reagent (mL) ratio	Reaction Time	Percent Calcium Content (%)	Percent Yield Demineralization (%)
DM1	0.75 N	25	1:6	6 hours	27.77(±1.16)	48.78
DM2	0.75 N	25	1:12	6 hours	27.11(±0.21)	23.70
DM3	0.75 N	25	1:6	24 hours	13.40(±0.68)	62.91
DM4	0.75 N	25	1:12	24 hours	13.57(±0.30)	24.65

Table 3. Calcium Content at Improved Reaction Time for DM3.

Reaction Time	% Calcium	%Chitin Yield
24 hours	11.72	-
36 hours	5.78	-
48 hours	4.90	-
72 hours	0.0379	63.66(± 0.12)

The FT-IR spectra of chitin produced (Fig. 1) from sardine fish scales was observed and evaluated to that of typical chitin. The FT-IR spectra revealed an absorption band in 3285cm^{-1}, indicating the vibrational mode peculiar to N-H stretching of amide functional group. The faint secondary amide stretch bands at roughly 1633cm^{-1} was also found. Another significant band at 1542cm^{-1} could be also attributed N-H bending and C-N stretching of the amide functional group. The CH_2 ending and CH_3 deformation in the structure of chitin can be observed by the peak formation at at 1466cm^{-1}, whereas C-O asymmetric stretch in phase ring (saccharide ring) could be the reason for the band observed at 1027cm^{-1}. This shows that FT-IR spectrum from the obtained product of deproteinization and demineralization of sardine fish scales was successfully transformed into chitin. Chitin can be chemically modified through deacetylation (Aguilor *et al.*, 2022) to form chitosan which is a potent fish preservative (Siddique *et al.*, 2020).

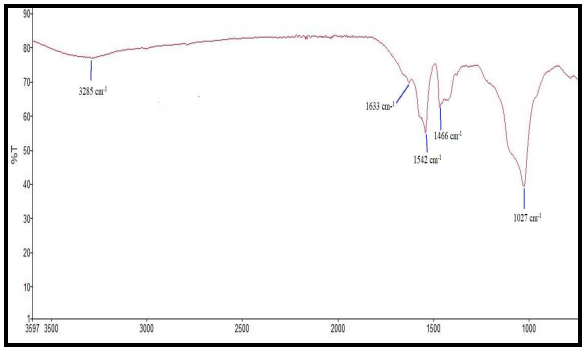

Fig. 1. FT-IR spectra of the isolated chitin from sardine fish scales.

Conclusions

This work established the deproteinization and demineralization of sardine scales, a common fish residue in Zamboanga City, Philippines into chitin as a high-value chemical. The chitin production was done using 4.0N NaOH, 80°C, 2 hrs reaction time and 1:10 ratio for deproteination and 0.75N HCl, 25°C, 24 hrs and 1:6 ratio (g:mL) for demineralization. At 72 hrs demineralization, the chitin yield was 63.66 (±0.12)%. These results showed that fish scales from *Sardinella longiceps* (Tamban) which are abundant in the Philippines can be successfully converted into chitin, a value-added product for use in different fields as medical, cosmetics and food industries among others.

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