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การสกัดผลิตภัณฑ์พลอยได้จากเกล็ดปลานิลและเกล็ดจระเข้

Extraction of By-Products from Nile Tilapia and Siamese Crocodile Scales

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บทคัดย่อ

เกล็ดปลานิลเป็นเศษเหลือจากอุตสาหกรรมการแปรรูปเนื้อปลานิลแล่แช่เย็นแช่แข็ง เกล็ดจระเข้เป็นเศษเหลือที่เกิดจากกระบวนการแปรรูปเครื่องหนังจระเข้ เกล็ดปลาและเกล็ดจระเข้อาจจะมีสารสำคัญที่สามารถใช้เป็นวัตถุดิบในการผลิตผลิตภัณฑ์พลอยได้ที่เป็นประโยชน์ทางอุตสาหกรรมอาหารได้ งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อสกัดผลิตภัณฑ์พลอยได้จากเกล็ดปลานิลและเกล็ดจระเข้ให้เป็นผลิตภัณฑ์เสริมอาหาร การทดลองเริ่มด้วยการวิเคราะห์องค์ประกอบของเกล็ดปลานิลและเกล็ดจระเข้ด้วยเทคนิค Fourier-transform infrared (FTIR) Spectroscopy จากนั้นจึงปรับสภาพเกล็ดด้วยสารเคมี ได้แก่ โซเดียมคลอไรด์ โซเดียมไฮดรอกไซด์ กรดซัลฟิวริก และกรดซิตริก ตามลำดับ ก่อนที่จะนำเกล็ดที่ปรับสภาพด้วยสารเคมีแล้วไปสกัดเจลาตินที่อุณหภูมิ 50 องศาเซลเซียส เป็นเวลา 3 ชั่วโมง และตรวจสอบชนิดคอลลาเจนในเจลาตินที่สกัดได้ด้วย SDS-PAGE ส่วนเกล็ดที่เหลือนำมาสกัดไคติน-ไคโตซานด้วยโซเดียมไฮดรอกไซด์ และกรดไฮโดรคลอริก และวิเคราะห์ระดับของดีอะซิทิเลชันของไคโตซานที่สกัดได้ นอกจากนี้ยังนำสารละลายกรดซัลฟิวริกจากขั้นตอนปรับสภาพเกล็ดด้วยสารเคมีก่อนหน้านี้มาตกตะกอนแคลเซียมด้วยผงโซเดียมคาร์บอเนต และวิเคราะห์ปริมาณแร่ธาตุต่าง ๆ ในผงแคลเซียมที่สกัดได้ จากผลการวิเคราะห์ FTIR พบว่าเกล็ดปลานิลและเกล็ดจระเข้ประกอบด้วยคอลลาเจน ไคติน และแคลเซียม ซึ่งสอดคล้องกับผลการสกัดที่ได้เจลาตินที่เป็นคอลลาเจนชนิดที่ I ไคติน-ไคโตซานที่ได้มีระดับการกำจัดหมู่อะซิทิลสูงกว่าร้อยละ 40 นอกจากนี้เกล็ดปลานิลและเกล็ดจระเข้ยังจะมีปริมาณแร่ธาตุ ได้แก่แคลเซียม ฟอสฟอรัส แมกเนเซียม และเหล็ก โดยที่ผงแคลเซียมจากเกล็ดจระเข้มีปริมาณแคลเซียมสูงถึงร้อยละ 35.96 จึงทำให้เกล็ดปลาและเกล็ดจระเข้เป็นเศษเหลือที่สามารถเพิ่มมูลค่าได้ โดยใช้เป็นวัตถุดิบในการผลิตผลิตภัณฑ์เสริมอาหารที่มีคุณค่าและยังสามารถลดปริมาณขยะที่อาจเป็นมลพิษต่อสิ่งแวดล้อม

คำสำคัญ : แคลเซียม ; ไคโตซาน ; คอลลาเจน ; เจลาติน ; เศษเหลือจากการแปรรูปสัตว์น้ำ

Abstract

Nile tilapia scales are waste from chill / frozen fish fillets processing industries. Crocodile scales are waste from crocodile leather making process. However, valuable substances in the scales of both tilapia and crocodile could be extracted that can be used as raw materials for by-products in food industries. The objective of this study was to extract by-products from Nile tilapia and Siamese crocodile scales for food supplements. The composition of functional groups contained in dried Nile tilapia and Siamese crocodile scales was determined by Fourier-transform infrared (FTIR) Spectroscopy. Tilapia and crocodile scales were treated with sodium chloride, sodium hydroxide, sulfuric acid and citric acid before gelatin extraction at 50 ºC for 3 h then collagen type were evaluated by SDS-PAGE. The residue scales were treated with sodium hydroxide and hydrochloric acid for chitin-chitosan extraction then degree of deacetylation and FTIR Spectroscopy were analyzed. Sodium carbonate was added in sulfuric acid treatment solution for calcium precipitation then minerals contents and FTIR Spectroscopy were analyzed. FTIR result was showed that the scales contained collagen, calcium and chitin. The FTIR result related to extraction of gelatin, chitin-chitosan and calcium powder. Gelatin was collagen type I, chitin-chitosan was degree of deacetylation more than 40% and calcium powder contained calcium, phosphorus, magnesium and iron. Crocodile calcium powder contain high calcium (35.96 %). These extraction of valuable substances for food supplements were value and reduce waste and environment pollution.

Keywords : calcium, chitosan, collagen, gelatin, fish processing waste

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Introduction

Fish scales contain a large amount of protein, mainly as collagen, which accounts for about 50 - 60% and calcium is present in the range of 16–59 % of the total weight. Calcium in fish scales is in the form of calcium salts as calcium hydroxyapatite, which binds the collagen fibers together. Calcium covers the surface of collagen present in the tissues and can influence the process and quality of collagen extraction from fish scales (Singh *et al.*, 2021). Decalcification of fish scale is a process of calcium removal with EDTA, hydrochloric acid and citric acid, which can improve collagen yield (Huang *et al.*, 2015). Tilapia scales can be used to produce collagen (El-Rashidy *et al.*, 2015; Chen *et al.*, 2016; Huang *et al.*, 2016) or gelatin (Ngo *et al.*, 2010; Weng *et al.*, 2014; Weng and Zheng, 2015; Liu *et al.*, 2019). Thailand exports tilapia fillets, as the main freshwater fish species. Tilapia fillet processing generates 800 tons of waste; head, bone, intestine, skin and scales (Tohmadlae *et al.*, 2019). Chitosan is the deacetylated derivative of chitin, which is one of the most abundant polysaccharides (Ramasamy and Shanmugam, 2015). Crustacean waste from the shells of shrimp, crab, crayfish and krill are composed of chitin, which forms a complex with proteins. Fish scales, shrimp and crab shells are composed of different components such as 15-50% protein, 30-50% minerals, and 15-30% chitin (Younes *et al.*, 2014; Hajji *et al.*, 2015). Crocodile farming is a very profitable business and could be a multimillion dollar industry to Thailand. Crocodile's skin is dominantly rare and expensive when converted to shoes, handbags, belts, wallets, jackets and other leather crafts (Adan, 2000). Meat was seen as a by-product for this industry, nevertheless lately it started to be an important economic source. Crocodile meat can be marketed as a healthy food enrich with unsaturated fatty acid profile (Tosun, 2013). Processed crocodile meat is a delicacy in some countries. The meat can also be canned for export to Hong Kong, Japan and other Asian and European countries. Blood for production of pharmaceutical products. Oil derived from its flesh also has a big market abroad. The teeth, head and bones of crocodile are turned into jewelry, unique souvenir items or decorative products. The bones can also be processed into animal feed (Hllaing, 2019; Adan, 2000). However there was no report on utilization of crocodile scale.

Tilapia and crocodile scales, meanwhile, remain as waste from processing and would be land pollution. Crocodile scale may have chemical composition to be functional ingredient as well as fish scale. The objective of this study was to extract by-products from Nile tilapia and Siamese crocodile scales for food supplements.

Methods

Materials

Nile tilapia (*Oreochromis niloticus*) scales were obtained from Grobest Marine Co., Ltd., Bangkok, Thailand. Siamese crocodile (*Crocodylus siamensis*) scales were obtained from a CITES-registered crocodile farm

in Samutsakorn Province, Thailand. Both materials were transported to the Department of Fishery Products, Kasetsart University, Bangkok. Fresh scales were cleaned with tapped water and dried by hot air oven at 60 °C for 3 h then packed in polyethylene bags and stored at room temperature until use.

Functional group in composition of scales

Dried scales were crushed into a powder by using kitchen blender. Functional group in composition of dried scales was determined by using Fourier-transform infrared (FTIR) Spectroscopy (Bruker EQUINOX 55, Ettlingen, Germany) as described by Sae-Leaw *et al.* (2016). The spectra in the infrared region were recorded in the region from 400 to 4000 cm^{-1}.

Chemical treatment

Dried scales were treated with 1.5 % NaCl, 0.2 %NaOH, 0.2 %H_2SO_4 and 1 %citric acid to remove blood, mucus, soluble proteins, lipid, non-collagen substances and fishy odor according to Tohmadlae *et al.* (2019). Treated scales and sulfuric acid treatment solution were collected for extraction of gelatin and calcium powder, respectively.

Extraction of gelatin

Gelatin was extracted from treated scales with hot water at 50 ºC for 3 h, then the filtered solution was evaporated at 50 ºC, followed by oven drying at 50 ºC for 16 h according to Tohmadlae *et al.* (2019). Yield and protein patterns of gelatin were determined. The residue scales were collected for extraction of chitin and chitosan.

Yield of gelatin

Yield of extracted gelatin was calculated from the formula:

Yield (%) = (dried weight of gelatin/ dried weight of scales) x 100

Protein patterns of gelatin

Protein patterns of gelatin were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), with 7.5% separating gel and 4% stacking gel according to the method described by Laemmli (1970). A 50% (w/v) gelatin solution was prepared. A 100-µl gelatin solution was mixed with 450 µl of H_2O, 450 µl of 10% SDS and 2 g of urea before mixing with sample buffer (0.5 M Tris-HCl, pH 6.8, containing 10% SDS (w/v), glycerol, 0.5% bromophenol blue, 2-mercaptoethanol) at a ratio of 1:1 (v/v). The mixtures were incubated at 90 °C for 30 minutes and centrifuged at 6,000xg for 30 min to remove insoluble debris. The loading volume of each sample was 10 µl per well. Electrophoresis was performed at a constant voltage of 180 V by using Mini-Protein®II Electrophoresis cell (Bio-Rad Laboratories Ltd, Thailand). After electrophoresis, the gel was stained with 0.1% (w/v) Coomassie blue R-250 in 40% (v/v) methanol, 10% (v/v) acetic acid and 50% (v/v) water, and then de-stained with

40% (v/v) methanol, 10% (v/v) acetic acid and 50% (v/v) water. Precision Plus Protein standard (New England BioLabs Inc., USA) was used to estimate the molecular weight of proteins.

Extraction of chitin and chitosan

After gelatin extraction, the residue scales were treated with 4.2% NaOH, 52% HCl and 58% NaOH, respectively according to Veerapan (2011). The extract was oven drying at 50 ºC for 6 h before crushing into a powder by using kitchen blender. Yield, degree of deacetylation and functional group of chitin and chitosan were determined.

Yield of chitin and chitosan

Yield of extracted chitin and chitosan was calculated from the formula:

Yield (%) = (dried weight of chitin and chitosan powder/ dried weight of scales) x 100

Determination of the degree of deacetylation of chitin and chitosan

Determination of the degree of deacetylation was according to methods by Kumari (2015). Dried product (0.5 g) was dissolved in 25 ml of 0.1 M HCl solution. The solution was then topped up to 100 ml with distilled water and calculated amounts of KCl were added to adjust the ionic strength to 0.1. The titrate was a solution of 0.05M NaOH. A pH meter was used for pH measurements under continuous stirring. The titrate was added until the pH value reached 2.0. The NaOH was then added stepwise and the pH values of the solution were recorded and a curve with two inflection points was obtained. The difference of NaOH solution volumes between these points corresponds to the acid consumed for calcification of the amine groups of chitosan and allows the determination of DD% (degree of deacetylation) of the chitosan. The DD% was calculated from the formula:

$$DD\% = (1-161Q)/(1+42Q)$$

where $Q = N\Delta V/m$

ΔV is the volume of NaOH consumed between the two inflection points (in l), N is the concentration of NaOH (in mol/l, in this investigation 0.05 mol/l) and m is the dry weight of chitin/chitosan (in g).

Determination of functional group of chitin-chitosan

Functional group of dried chitin-chitosan was determined by using FTIR Spectroscopy (Bruker EQUINOX 55, Ettlingen, Germany) as described by Sae-Leaw *et al*. (2016). The spectra in the infrared region were recorded in the region from 400 to 4000 cm^{-1}.

Extraction of calcium powder

Sulfuric acid treatment solution was collected. Calcium was precipitated by adding powdered sodium carbonate into the acid solution until a white precipitate was formed. The precipitate was washed with distilled water before oven drying at 105°C for 1 h. Yield, functional groups and mineral content of the calcium powder were determined.

Yield of calcium powder

Yield of calcium powder was calculated from the formula:

Yield (%) = (dried weight of calcium powder / dried weight of scales) x 100

Determination of functional group of calcium powder

Functional group of calcium powder was determined by using FTIR Spectroscopy (Bruker EQUINOX 55, Ettlingen, Germany) as described by Sae-Leaw *et al*. (2016). The spectra in the infrared region were recorded in the region from 400 to 4000 cm^{-1}.

Determination of mineral content

An inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin Elmer Optima 8300, USA) was used for determination of P, Fe, Mg, Ca and K in the calcium powder according to methods of Feist and Mikula (2014). The wavelengths used for P, Fe, Mg, Ca and K detection were 213.617, 238.204, 285.200, 317.933 and 766.500 nm, respectively.

Color analysis

The color of the calcium powder was determined using colorimeter (Minolta CM-3500d, Japan). L* (lightness), a* (redness) and b* (yellowness) values were determined. To measure the total difference in color (ΔE^*), the following equation was used : $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

Where ΔL^*, Δa^* and Δb^* are the differences between the corresponding color parameters of the sample and those of the white standard.

Results

Composition of scales

The FTIR spectrum of Nile tilapia (A) and Siamese crocodile (B) scale powder obtained after drying and crushing were illustrated in Figure 1. Different peaks were detected as amide group (C=O ···HN) at wave number 1652, 1653 cm^{-1} (amide I); 1560, 1539 cm^{-1} (amide II); 1243, 1239 cm^{-1} (amide III) and 2932, 2926 cm^{-1} (amide B),

carbonate group (CO_3^{2-}) at 1032, 1105 cm^{-1}, hydroxyl group (OH^-) at 3450, 3297 cm^{-1}, phosphate group (PO_4^{3-}) at 562 $cm^{-1}$and hydroxymethyl group (CH_2OH) at 1454, 1447 cm^{-1}, respectively.

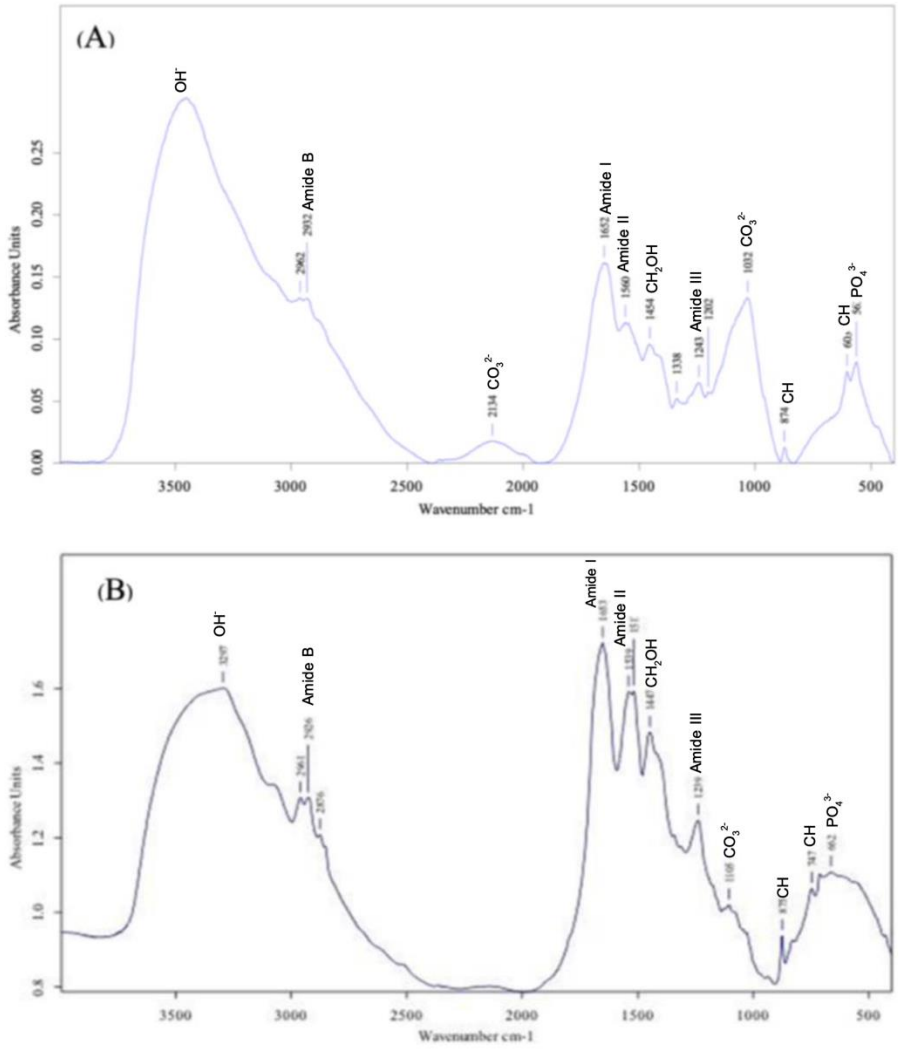

Figure 1 FTIR spectrum of scales from Nile Tilapia (A) and Siamese crocodile (B).

Gelatin from scales

The extracted gelatin yield was 3.67±0.11% for tilapia and 0.64±0.18% for crocodile scales, and gelatin solution was set when kept at 4 °C. Nile tilapia gelatin solution was clear, colorless and no fishy odor whereas

crocodile gelatin was yellow, and had a fishy odor. Protein patterns of gelatin were shown in Figure 2. Gelatin contained α-chains as the major component. A β-component (α-chain dimers) was also noticeable.

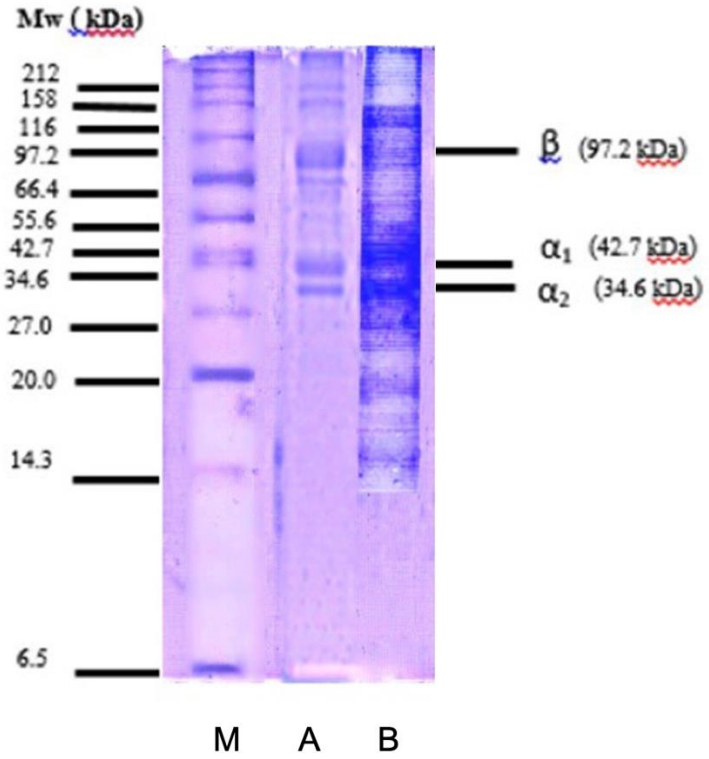

Figure 2 Protein MW distributions of gelatin from Nile tilapia (A) and Siamese crocodile (B) scales compared with protein marker (M).

Chitin and chitosan from scales

The extracted chitin and chitosan yield from tilapia and crocodile scales were 32.32±0.12 % and 85.57±0.25%, respectively; degree of deacetylation were 59.63±0.13 %and 42.37±1.28%, respectively. The FTIR spectrum of Nile tilapia and Siamese crocodile chitin and chitosan were illustrated in Figure 3, were similarly to scale. The peaks were detected amide I at wave number 1637-1640cm^{-1}, amide II at 1534 cm^{-1} , amide III at 1241 cm^{-1}, amide B at 3280 cm^{-1}, carbonate group at 1106-1035 cm^{-1}, hydroxyl group at 3280-3453 cm^{-1}, phosphate group at 541-567 cm^{-1} and hydroxymethyl group at 1444-1465 cm^{-1}. The dominant peaks were hydroxide group in both of tilapia and crocodile scales.

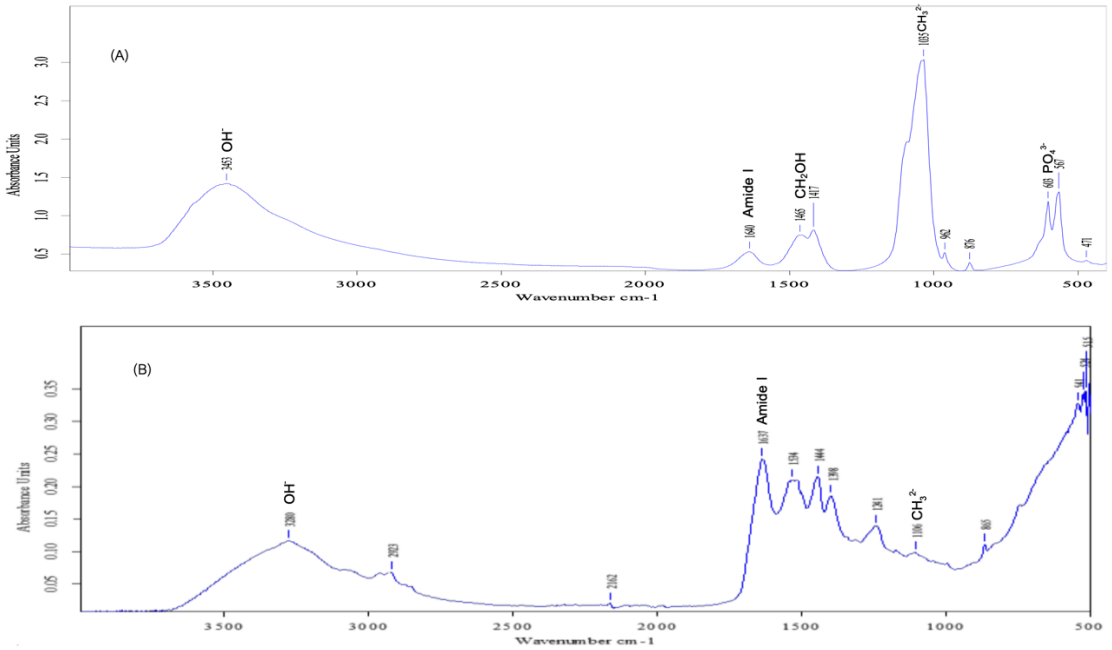

Figure 3 FTIR spectrum of chitin and chitosan from Nile tilapia (A) and Siamese crocodile (B) scales.

Calcium powder from scales

The calcium extract was white coarse powder as shown in Figure 4. The calcium powder yield from tilapia and crocodile scales were 11.84±0.07% and 8.92±0.83%, respectively. Calcium powder of tilapia and crocodile had mineral contents of calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg) and iron (Fe), respectively as shown in Table 1.

Table 1 Mineral content in calcium powder from Tilapia and Crocodile scale

Source of calcium powder	Mineral content				
	% Calcium	% Phosphorus	% Potassium	% Magnesium	Iron ($mg.kg^{-1}$)
Tilapia scale	12.83	0.28	0.04	0.38	52.00
Crocodile scale	35.96	0.10	0.025	0.74	<33.33

The FTIR spectrum of Nile Tilapia (A) and Siamese crocodile (B) calcium powder were illustrated in Figure 5, were similarly to spectrum of fish and crocodile scales, The peaks were detected amide I at wave number 1622-1625 cm^{-1}, amide II at 1512 cm^{-1} , amide III at 11159 cm^{-1}, amide B at 2925 cm^{-1}, carbonate group

at 1105-1115 cm^{-1}, hydroxyl group at 3283-3419, cm^{-1}, phosphate group at 670 cm^{-1} and hydroxymethyl group at 1443 cm^{-1}. The dominant peaks were carbonate group.

(A) (B)

Figure 4 Calcium powder from Nile tilapia (A) and Siamese crocodile (B) scales.

Dicussion

Composition of scales

FTIR is a powerful technique that can be used to evaluate collagen structure of fish scales (Silva *et al.*, 2014). FTIR spectrum of collagen possess five major adsorption bands in the amide band region, including 1600-1700 cm^{-1} (amide I), 1540-1600 cm^{-1} (amide II), 1220-1320 cm^{-1} (amide III), 3304-3315 cm^{-1} (amide A), and 2922-2940 cm^{-1} (amide B) (Muyonga *et al.*, 2004; Kittiphattanabawon *et al.*, 2010; Zhang *et al.*, 2019). In the present study, FTIR spectrum of both scales of Nile tilapia and Siamese crocodile exhibited the characteristic peaks of amide I, II, III and amide B, were similar to the spectrum exhibited by other collagens (Jackson *et al.*, 1995; Martins *et al.*, 2018).

FTIR spectrum of tilapia and crocodile scale were detected phosphate group (PO_4^{3-}), hydroxyl group (OH^-) and hydroxymethyl group (CH_2OH) were in the range of phosphate group (PO_4^{3-}) at 576 - 568 cm^{-1}; hydroxyl group (OH^-) at 3200 - 3572 (Panda *et al.*, 2014); hydroxymethyl group (CH_2OH) at 1350 - 1520 $cm^{-1}$and carbonyl group (CO_3^{2-}) at 1000 – 1100 cm^{-1}(Zhang *et al.*, 2019).

The amide I vibration mode is primarily a C=O stretching vibration associated with the C-N stretch, C-C-N deformation and in-plane NH bending modes (Bandekar, 1992). The amide III arises from the combination of peaks between C-N stretching vibrations and N-H deformation from amide linkages. Absorption arising from wagging vibrations of CH_2 groups of glycine backbone and proline side-chains also contribute to amide III (Jackson *et al.*,

1995). The complete removal of peptides in the calcium powder then amide II, A and B are detected in the ranges of 1541-1548, 3304-3315 and 2922-2940 cm^{-1}, respectively (Muyonga *et al.,* 2004; Kittiphattanabawon *et al*., 2010).

Collagen was triple-helical structure which has a single interstand $N\text{-}H_{(Gly)}\cdots OC_{(Xaa)}$, where Xaa can be proline, hydroxyproline and any amino acid. (Shoulders and Raines, 2009). Chitin and chitosan are a family of linear polysaccharides consisting of varying amounts of β (1 - 4) linked residues of N-acetyl-2 amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose residues (Aranaz *et al.*, 2009). Chitin-chitosan structure have various functional group as amide group (C=O ···HN), hydroxyl group (OH), hydroxymethyl group (CH_2OH), acetyl group ($C=O\cdots CH_3$) and others. Calcium organic salts are tricalcium citrate, calcium lactate, calcium lactate gluconate, calcium gluconate and inorganic salts like calcium chloride, calcium carbonate and calcium phosphate (Trailokya *et al.*, 2017). FTIR results indicated that tilapia and crocodile scale consisted of collagen, chitin-chitosan and calcium.

Gelatin from scales

Yield of tilapia scale gelatin (3.67±0.11%) was higher than crocodile gelatin (0.64±0.18%). The present result, Nile tilapia scale gave lower gelatin yield than tilapia scale (Huang *et al*, 2016; Martin *et al.*, 2018) due to different chemical treatment. This chemical treatment gave Nile tilapia gelatin solution was clear, colorless and no fishy odor. Sodium chloride removed blood, mucus, soluble proteins and fishy odor; sodium hydroxide removed basic soluble proteins and non-collagen substances; sulfuric acid removed acidic soluble proteins and mineral; citric acid removed fishy odor from scales (Tohmadlae *et al.*, 2019). Collagen is acidic soluble protein. Acid treatment can dissolve some collagen from scale and effected to gelatin yield. Crocodile scale is bigger and thicker than tilapia scale. Chemical solution could not pass through the tissue meanwhile protein and others could not dissolve in the solution. Crocodile gelatin was yellow, and had a fishy odor.

Gelatin from both of scales contained α1 and α2 chains, and was characterized as type I collagen (Duan *et al.*, 2009; Benjakul *et al.*, 2010; Sukkwai *et al.*, 2010; Weng and Wu, 2015; Huang *et al.*, 2016). During gelatin extraction, the conversion of collagen to gelatin with varying molecular mass takes place, due to the cleavage of inter-chain cross-links (Zhou *et al.*, 2006). Conversion of collagen to gelatin modifies it solubility, making it water soluble. It is readily soluble in hot water, swells in cold water. It is colorless to yellowish, tasteless, transparent to slightly translucent. The setting point is the temperature where the softened gel starts hardening. Gelatin set when cold 15 °C or lower and at temperature of 30-40 °C, the gel melts to solution depend on material source (Mahmood *et al.*, 2016). Gelatin solution from tilapia and crocodile scale were also set at 4 °C and melt at room temperature. These gelatin can be used to gel in various products.

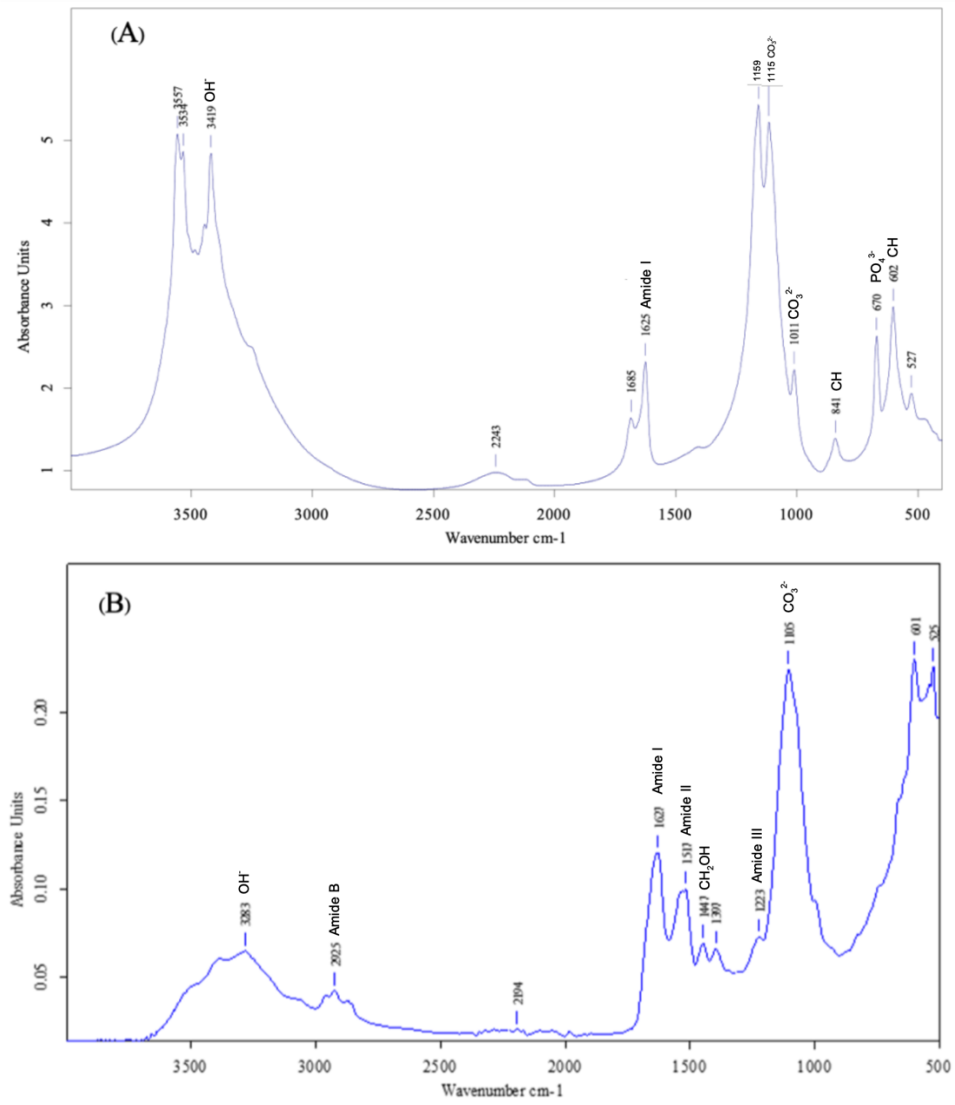

Figure 5 FTIR spectrum of calcium powder from Nile tilapia (A) and Siamese crocodile (B) scales.

Chitin and chitosan from scales

Yield of tilapia chitin-chitosan (32.32±0.12 %) was lower than crocodile (85.57±0.25%) was higher than alkali process tilapia chitin (24%) (Boarin-Alcalde and Graciano-Fonseca, 2016) meanwhile degree of deacetylation of tilapia (59.63±0.13 %) was higher than crocodile (42.37±1.28%) due to different of material and extraction procedure. Thermal treatments of chitin under strong aqueous alkali are usually needed to give partially deacetylated chitin and degree of deacetylation lower than 30%. Usually, sodium or potassium hydroxides are used

at a concentration of 30-50% at high temperature (100ºC) (Aranaz *et al.*, 2009). Present results, degree of deacetylation in tilapia and crocodile scale chitin-chitosan was in range of typical chitin-chitosan.

The FTIR spectrum of chitin and chitosan from tilapia and crocodile scales shown a pattern similar to Yasmeen *et al.* (2016). Some of the structure of chitin does not change to a function group from the acetamido group (-$NHCOCH_3$) to be the amino group (-NH_2), resulting in a level of mass removal Acetyl below 50%. The FTIR spectrum of tilapia and crocodile chitin and chitosan were similarly to scale. The peaks were detected amide I, amide II, amide III and amide B, major functional group of collagen and carbonate group and phosphate group, major functional group of calcium salts. Even through the dominant peaks were hydroxide group in both of tilapia and crocodile scales. Collagen and calcium salt contaminated in chitin-chitosan proportion from tilapia higher than crocodile.

Calcium powder from scales

Fish scales are rich in minerals (Ali *et al.*, 2017). The source of calcium in fish scales is in the form of "calcium salts," which cover the surface of collagen present in the tissues. The mineral content of these calcium sources is present in the range of 16–59 % (Singh *et al.*, 2021). Tilapia and crocodile scales were treated by chemical treatment. The scales were treated with sulfuric acid. Minerals and collagen would be found in sulfuric acid treatment solution. These results related to FTIR spectrum of calcium powder from tilapia and crocodile scales as shown in Figure 5 that found amide groups of collagen (amide I, amide II, amide III and amide B). Spectrum of hydroxyl group was found in calcium powder that indicated calcium powder was contaminated with collagen and chitin-chitosan.

Amino acids as well as lipids that might undergo oxidation. Maillard reaction with an amino group of free amino acids, peptides or proteins in the calcium powder, particularly during the drying process. This process can augment the development of a yellow color in the calcium powder (Benjakul *et al*., 2017). The calcium powder from both tilapia and crocodiles were white color due to protein and lipid were removed in chemical treatment.

Yield of calcium powder from tilapia scale was higher than crocodile, anywhere crocodile calcium powder contain calcium content higher than tilapia. Calcium content in crocodile scale (35.96%) was closed to fish bone that displayed a high presence of calcium with other components such as sodium, chloride, magnesium and potassium (Singh *et al*., 2021).

Tilapia scale consists of extracellular matrix that contains Type I collagen arranged in three dimensions and also calcium-deficient hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (Ikoma *et al*., 2003; Cimdina and Borodajenko, 2012. Peaks corresponding to hydroxyapatite were detected at 1011, 602 and 527 cm^{-1}, and hydrogenophosphate was

detected at 841 cm^{-1}, similar to findings of Raynaud *et al.* (2002). The bands relate to the bending vibration of phosphate (PO_4^{3-}, O-P) (Chakraborty *et al.*, 2013). The peak at 602 cm^{-1}indicates OH ions present in the lattice structure, as reported by Piccirillo *et al.* (2013). The peak at 3419 cm^{-1}corresponds to absorbed hydrate, andindicates the stretching vibration of lattice OH^- ions (Chakraborty and Chowdhury, 2013). This result reconfirms the presence of a hydroxyl group in hydroxyapatite. The peak at 1115 cm^{-1}, which is characteristic of β-tricalcium phosphate, is similar to results of Habelitz *et al*. (2001). In present study, Nile tilapia calcium powder was detected at 527, 602, 841, 1101, 1115 and 3419 cm^{-1} whereas crocodile was detected some peaks in same wavenumber as shown in Figure 5. This result indicated Nile tilapia calcium powder consisted of calcium-deficient hydroxyapatite that different from crocodile.

Conclusions

The scales of both tilapia and crocodiles could be extracted gelatin, chitin-chitosan and biocalcium with similar properties to different aquatic animal by-products. These resulting lend to industrial waste treatment and valued food supplement development.

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