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FOOD TECHNOLOGY

The changing of gelatin properties from tra catfish skin (*Pangasianodon hypophthalmus*) by alkaline replacement to enzyme in pretreated process

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ABSTRACT: *The effect of pretreated method to remove the non-collagenous protein by using alkaline and enzyme Alcalase, as well as the temperature and time for extracting on the properties of gelatin from tra catfish skin were investigated. Yields of gelatin extracted at 70 °C for 1h from pretreated skin by enzyme method (16.2%) was significantly higher than that of the sample by alkaline method (12.14%). However, the gel strength of gelatin from skin treated via enzyme Alcalase was lower than gelatin sample pretreated by alkaline while the turbidity values was higher than gelatin from skin pretreated via alkaline. From SDS-PAGE profile, gelatin from skin pretreated by alkaline consisted of two different α- chains in protein pattern while enzymatic gelatin had low molecular weight peptides. The FT-IR spectra showed the lower wavenumber in amide I and III of enzymatic gelatin in compare to alkaline gelatin by the loss of triple helical structure during enzyme treatment. From the results, the using enzyme for pretreated material has potential to replace the alkaline method for gelatin production with purpose to reduce chemical waste caused serious ecological issues.*
Key words: *gelatin, tra catfish skin, pretreated method, alkaline, enzyme Alcalase.*

Alterações das propriedades da gelatina da pele do bagre tra (*Pangasianodon hypophthalmus*) pela substituição alcalina por enzima no processo pré-tratado

RESUMO: *Investigou-se o efeito do método pré-tratado para remoção da proteína não colágena com a utilização da alcalina e da enzima Alcalase, bem como a temperatura e o tempo de extração sobre as propriedades da gelatina da pele do bagre tra. O rendimento da gelatina extraída a 70 °C por 1h da pele pré-tratada pelo método enzimático (16,2%) foi significativamente superior ao da amostra pelo método alcalino (12,14%). No entanto, a força do gel da gelatina da pele tratada com a enzima Alcalase foi menor do que a amostra de gelatina pré-tratada com alcalina, enquanto os valores de turbidez foram maiores do que a gelatina da pele pré-tratada com alcalina. A partir do perfil SDS-PAGE, a gelatina da pele pré-tratada com alcalina consistia em duas cadeias α diferentes no padrão de proteína, enquanto a gelatina enzimática tinha peptídeos de baixo peso molecular. Os espectros de FT-IR mostraram o menor número de onda na amida I e III da gelatina enzimática em comparação com a gelatina alcalina pela perda da estrutura helicoidal tripla durante o tratamento enzimático. Pelos resultados obtidos, a utilização de enzimas para material pré-tratado tem potencial para substituir o método alcalino para produção de gelatina com objetivo de reduzir o desperdício químico causado por sérios problemas ecológicos.*
Palavras-chave: *gelatina, pele de bagre tra, método pré-tratado, alcalino, enzima Alcalase.*

INTRODUCTION

Gelatin is a water-soluble protein generated by partial hydrolysis of collagen from the fibrous structure protein, such as mammalian skin, bones and connective tissues (JOHNSTON-BANKS, 1990). During gelatin production, crude materials are pretreated with weakened acidic or alkaline solutions to destroy a triple-helix structure of collagen and transfer from water-insoluble collagen to water-soluble gelatin. A short pretreatment with dilute acid is typically used for the less covalently cross-linked collagens reported in young animals like skin from young porcine and ossein prepared from the bones of young cattle while a more intense alkaline pretreatment is usually applied for more covalently cross-linked collagens found in older animals like cattle hides and ossein prepared from cattle bones (HAUGH and DRAGET, 2009; JONES, 2004). The alternative pretreatment approach for the production of gelatin from collagen-containing materials is proposed by enzymatic method (ABEDINIA et al., 2017). The advantages of this method are that it does not use chemical compounds

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in the pretreatment step (lack of residual chemicals in gelatin) and the resulting gelatin gels show high gel strength. In recent year, utilization enzyme to replace chemical for pre-treating crude materials before collagen or gelatin extraction have increased attention. There are various commercially enzymes that can be used for proteolysis, such as pepsin, Flavourzyme and Alcalase (ABEDINIA et al., 2017; AHMADIFARD et al., 2016). Alcalase is preferred to the other enzymes due to its higher hydrolytic activity (AHMADIFARD et al., 2016). Gelatin had spacious application in the food industry, including gelation in cooking, stabilization, thicknerization, or texturization (GÓMEZ-GUILLÉN et al., 2011; IRWANDI et al., 2009).

In Mekong Delta, Vietnam, tra catfish is the most important freshwater fish species and tra catfish fillet frozen is main exporting product to contribute in the development of economic product with the amount reached to US$2.06 billion in 2019, according to the Vietnam Association of Seafood Exporters and Producers (VASEP). During the filleting process, a huge weight of by-product including skin was discarded, which accounts for 17-22% of total weight (KITTIPHATTANABAWON et al., 2015). Utilization of tra catfish skin as a raw material for gelatin extracting has been represented by SINGH & BENJAKUL. (2017). However, little information is available regarding the effect of pretreatment methods on the properties of gelatin from tra catfish skin. Therefore, this research was conducted to produce gelatin by determining the alteration of gelatin properties from fish skin by different pretreated methods, i.e., alkaline method and enzyme method.

MATERIALS AND METHODS

Fish skin collection and preparation

After collecting fish skin from tra catfish processing companies placed in Can Tho city, Vietnam, skin was washed in chilled water, put in plastic bags, covered with ice and transported to laboratory. Sample was kept at – 20 °C ± 2 °C for storing until conducted experiment.

Pretreated fish skin

Frozen skin was thawed and separated two groups for removing the non-collagenous protein. First group was soaked in NaOH solution at the concentration of 0.1 M with the ratio of skin/ alkaline solution was 1:8 (w/v) for 6 h, the alkaline solution was changed every 3 h and skin were washed thoroughly in cold distilled water until neutral pH. The second group was treated with 0.01% Alcalase enzyme at a ratio of skin to Alcalase enzyme was 1:8 (w/v) for 6h in water bath at 40 °C. After completing the treating process, the inactivated enzymatic was conducted by holding the sample in water bath for 5 minutes at 95 °C.

Gelatin extraction

Two group skins after removing the non-collagenous protein was extracted gelatin following to the method of THUY et al. (2015) with slight adjustments. Two group samples were drained using a cheesecloth and soaked in 7x distilled water (w/v) at various temperatures (60, 70, and 80 °C) for different times (1 h, 2 h and 3 h) with continuous stirring for gelatin extraction. After the extraction of gelatin, the coarse solids were removed by filtration with two layers of cheesecloth and continuously centrifuged at 16,000 x *g* for 30 min at the temperature of 20 °C. The supernatant was collected and dried at 50 °C for 4 h (WTE Binder, Gallenkamp, Germany).

Yield of gelatin extraction

The extraction yield was determined following the method of THUY et al. (2015). Approximately 1 g of the non-collagenous fish skin was weighed, and then extracted in 7x distilled water (w/v) as described above. The supernatant was collected for analyzing the protein content by applying the method of LOWRY et al. (1951) using the protein standard was BSA (bovine serum albumin). The equation for calculating the yield of extracted gelatin (YG) was showed as below:

$$YG(\%) = \frac{\text{Protein concentration (mg/ml) x 7 times of distilled water (ml) x 100\%}}{\text{The weight of fish skin (mg)}}$$

SDS – polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of gelatin from two groups of tra catfish skin was conducted referred to LAEMMLI'S method (1970) with minor modifications. The two group gelatins in the supernatant obtained at 70 °C for 1 h was blended with sample buffer (consisted of Tris-HCl 0.5 M at pH 6.8, with 20% (v/v) glycerol and 10% (w/v) SDS) in the attendance of 10% (v/v) mercaptoethanol at a gelatin/ buffer ratio of 1:2 (v/v). The use of polyacrylamide gel 7.5% for loading about 10 µl samples and the electrophoresis was conducted at a constant current of 20 mA. After that, the gel was staining and distaining. Molecular weight markers

(Bio-Rad Laboratories, Hercules, CA, USA) were used to estimate the molecular weight of proteins.

Amino acid analysis

One hundred milligrams of gelatin samples were put into a media bottle with the addition of 5 ml oxidation solution with stirring. These bottles were fixed into a refrigerator for 16h at 0 °C. Afterwards, adding 0.84 g of sodium disulfite and 25 ml hydrolysis solution in gelatin samples. Subsequently, the hydrolysis of these gelatin samples was performed in furnace at 110 °C for 23 h. The hydrolytes were derivatizing, diluting by sample diluents and comparing with the amino acids standard. 20 µl of sample were added into the Amino Acid Analyzer system (Biochrom 32+, USA) for amino acid analyzing. The data was recorded in amino acid residues per thousand residues.

Turbidity of gelatin samples

The turbidity of two group skins gelatin were measured by method of CHO et al. (2004) with slight adjustments. Gelatin solution of 0.5% (w/w) was prepared by mixing 0.025 g gelatin powder in 5 mL distilled water with stirring at 60 °C. The absorbance of the solution at 660 nm was determined by using Thermo scientific spectrophotometer, model genesys-30, USA.

Measurement of gel strength

The gel strength was measured referring to method of KITTIPHATTANABAWON et al. (2010) by using TA-XT2 Texture Analyzer Stable Micro Systems (Stable Micro Systems, Surrey, UK) with a load cell of 5 kg. Dissolving gelatin in distilled water at 60 °C for preparing gelatin solution at the concentration of 6.67% (w/v) and moved on sample into a cylindrical mold with 3 cm diameter and 2.5 cm height. The maturation of gelatin gels was performed by storing gelatin solution at 4 °C for 16-18 h. After maturation period, the sample were placed centrally under the 1.27 cm diameter flat-faced cylindrical Teflon@ plunger. The maximum force was expressed as grams (g).

The colour of gelatin samples

The gelatin samples after drying at 60 °C for 4 h were measured the change of color with a Colorimeter PCE-CSM 2. The values of L^* (lightness/brightness), a^* (redness/greenness) and b^* (yellowness/blueness) were recorded. The total difference in color (ΔE^*) was determined according to the equation (SEA-LEAW & BENJAKUL, 2015):

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

where ΔL^*, Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of the white standard (L^*= 93.52, a^* = 0.3 and b^* = 0.57).

FTIR spectra

FTIR analysis of gelatin from two group pretreated skin were analyzed using Omnic 6.0 software (Thermo-Nicolet, Madison, Wisconsin). The spectrum was recorded using Bruker Optics ALPHA FT-IR spectrometer with spectra wavenumber from 4000 to 500 cm^{-1}.

Statistical analysis

All experiments were conducted 6 times and the data were shown as mean ± standard deviation. The mean comparisons were determined basing on the Duncan's multiple range tests. SPSS software was used to perform an analysis (SPSS 16.0 for Windows, USA).

RESULTS AND DISCUSSION

The yield of gelatin extraction from tra catfish skin

The extraction yield of gelatin from tra catfish skin is showed in table 1. Yields of skin soaked by NaOH ranged from 7.01 to 19.47% and gelatin yields from skin treated by Alcalase enzyme were 10.51 to 23.25%, depending on the temperature and time for extracting. The enhancement of the extraction yield could be observed with the increasing of time and temperature during extracted. This can be explained that the stabilizing hydrogen bonds of collagen are demolished by cooking at a higher extraction time and temperature resulting in the helix-to-coil transition and the conversion of collagen into soluble gelatin (BENJAKUL et al., 2009). Results are agreed to previous researches of THUY et al. (2015) and WANGTUEAI & NOOMHORM. (2009), who reported an increase in gelatin yield from horse mackerel scale and lizard fish scale with enhancing of temperature and time for extracting, respectively. Furthermore, the gelatin yields of enzyme group were significantly higher than gelatin from alkaline group at the similar of extraction temperature and time. This result are supported by ABEDINIA et al. (2017), who reported that gelatin yield from the feet of Pekin duck (*Anas platyrhynchos domestica*) with enzymatic pretreatment was higher than sample treated in alkaline or acidic pretreatment.

Table 1 - The extraction yield (%) of gelatin from tra catfish skin at various extraction temperatures and times.

Temperature (°C)	Time (h)	Enzyme pretreating	Alkaline pretreating
60	1	10.51 ± 1.428^{e}	7.01 ± 0.721^{d}
60	2	17.60 ± 0.849^{d}	14.17 ± 0.976^{b}
60	3	20.87 ± 0.325^{bc}	17.58 ± 0.141^{a}
70	1	16.20 ± 0.283^{d}	12.14 ± 2.04^{c}
70	2	20.27 ± 0.156^{c}	15.36 ± 0.339^{b}
70	3	23.23 ± 1.74^{a}	19.47 ± 0.099^{a}
80	1	17.17 ± 0.438^{d}	13.84 ± 0.283^{bc}
80	2	22.60 ± 0.990^{ab}	18.23 ± 0.552^{a}
80 – 3	3	23.25 ± 1.202^{a}	19.45 ± 0.495^{a}

Data are expressed as mean ± standard deviation (n=3).
Different superscripts in the same column indicate statistical differences ($P < 0.05$).

SDS – PAGE profile

The protein profile of gelatin extracted from skin via alkaline and enzymatic pretreatment at 70 °C for 1 h is presented in figure 1. The SDS – PAGE profile showed the high molecular weight protein, including α and β chains in gelatin from tra catfish skin pretreated by alkaline. The degradation of α and β chains to form a low-molecular-weight peptide in sample pretreated by enzymatic was observed. It could be products of enzymatic hydrolysis of gelatin

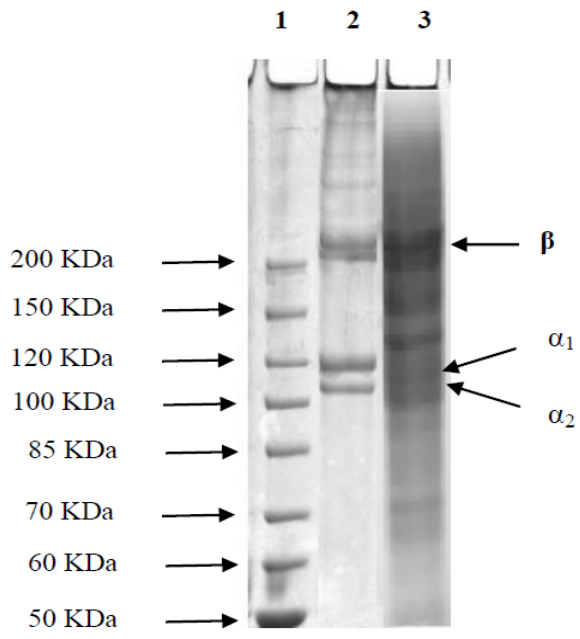

Figure 1 - SDS-PAGE pattern of tra catfish skin gelatin.
Lane 1, high molecular weight marker; lane 2, tra catfish skin pretreated by alkaline; lane 3, tra catfish skin pretreated by enzyme.

components during skin preparation and the sizing of the polypeptide chains was affected by enzyme (ABEDINIA et al., 2017).

Amino acid composition

The amino acid profile of tra catfish skin gelatin after pretreated by alkaline and enzyme extracted at 70 °C for 1 h is presented in table 2. The most abundant amino acid in gelatin from tra catfish skin pretreated by alkaline and enzyme was glycine, which amount of more than 30% in total amino acids (346 and 341 residues/1000 residues). Proline and hydroxyproline contents (imino acid) of gelatin sample with alkaline and enzyme pretreatment (198 and 200 residues/1000 residues, respectively) were slightly lower than those of clown feather back skin (207 residues/1000 residues) (KITTIPHATANABAWON et al., 2015) and mammalian gelatin (216-225 residues/1000 residues) (AVENA-BUSTILLOS et al., 2006). Imino acid, especially hydroxyproline, plays an essential role in the triple helical structure of gelatin and affected in gel formation by acting as H-donor, in which hydrogen bond could be created with conterminous chain possessing H-acceptor (BENJAKUL et al., 2012).

Turbidity of gelatin samples

Turbidity of gelatin samples treated in alkaline and enzyme at different extracting conditions is shown in table 3. All gelatin pretreated by enzyme

Table 2 - Amino acid compositions of two groups gelatin from tra catfish skins. Residues/1000 residues.

Amino acid	Enzyme pretreated	Alkaline pretreated
Aspartic acid	46	46
Threonine	26	27
Serine	32	33
Glutamic acid	76	75
Glycine	341	346
Alanine	91	91
Valine	25	24
Cysteine	2	2
Methionine	10	10
Tryptophan	ND	ND
Isoleucine	7	6
Leucine	29	29
Tyrosine	5	5
Phenylalanine	16	16
Hydrolysine	7	6
Lysine	29	29
Histidine	5	5
Arginine	56	56
Hydroxyproline	89	87
Proline	111	111
Imino acids*	200	198

Data are expressed as mean (n=3).
"ND" means not detected.

Table 3 - Gel strength (g) and turbidity value (NTU) of gelatin from tra catfish skin at various extraction temperatures and times.

Temperature (°C)	Time (h)	----------Gel strength (g)----------		----------Turbidity value (NTU)----------	
		Enzyme pretreated	Alkaline pretreated	Enzyme pretreated	Alkaline pretreated
60	1	140.8 ± 14.20^{ef}	140.2 ± 6.45^{de}	337±4.04^{g}	307±5.69^{f}
60	2	149.6 ± 10.59^{de}	137.1 ± 3.52^{e}	381±2.08^{e}	340±12.42^{e}
60	3	134.0 ± 10.25^{f}	121.0 ± 11.76^{f}	416±6.02^{c}	395±9.45^{bc}
70	1	208 .7± 5.12^{a}	176.0 ± 11.62^{b}	353±3.79^{f}	330±11.01^{e}
70	2	184.8 ± 5.02^{b}	172.2 ± 13.64^{b}	404±8.54^{d}	358±6.51^{d}
70	3	156.5 ± 2.67^{cd}	163.0 ± 6.14^{bc}	429±3.06^{b}	405±8.62^{b}
80	1	168.3 ± 4.55^{c}	192.8 ± 2.72^{a}	396±4.73^{e}	387±6.25^{c}
80	2	156.2 ± 4.11^{cd}	165.5 ± 5.80^{bc}	434±4.00^{b}	402±6.43^{b}
80	3	122.9 ± 8.09^{f}	154.2 ± 2.67^{cd}	471±3.51^{a}	441±7.37^{a}

Data are expressed as mean ± standard deviation (n=3).
Different superscripts in the same column indicate statistical differences ($P < 0.05$).

were shown the higher turbidity values than sample pretreated in alkaline at the similar extracted temperature and time. AHMAD et al. (2019) and MONTERO et al. (2002) expressed complete depression of β and α chain in sample pretreated by enzyme, resulting in the attendance of more aggregates of high molecular weight in gelatin. This might be due to the increasing of turbidity values in gelatin pretreated by enzyme.

Gel strength of two group gelatins from tra catfish skin

Gel strength of two group gelatins extracted at various extraction temperatures for different times is presented in table 3. The increasing of gel strength from two group gelatins was observed at the range of extraction from 60 to 70 ºC and the gel strength degradation could be observed with the increasing of extracted heating higher than 80 °C and extracted time extended more than 2 h). It could be explained that protein structure was destroyed to form protein fragments and lead to the decline of gelling ability with the increasing of extraction temperature and time (NORMAND et al., 2000). Nevertheless, the gel strength of gelatin pretreated by enzyme was higher than gelatin sample pretreated by alkaline at the similar of extraction condition. Low strength of gelatin from the skin pretreated by enzyme caused intense cleavage of collagen chains due to over-hydrolysis leading to form a shorter chains, resulting in the higher gel strength of gelatin from skin pretreated by alkaline in comparison to sample via enzyme pretreatment (NORZIAH et al., 2014).

Colour of gelatin

The total difference in color (ΔE^*) of gelatin powder at various temperature and extraction time from tra catfish skin under two different conditions for pretreated are shown in table 4. The results were reported that there was no significantly difference of ΔE^* from gelatins via alkaline and enzyme pretreatment at the similar extraction condition ($P > 0.05$). However, the increase on ΔE^* could be observed with the enhancement of temperature and time for extracting. It has been noticed that Maillard reaction might occur to a higher extent (LERTITTIKUL et al., 2007) with the extending extraction time. Among all gelatins from skin of tra catfish, the sample extracted at 70 ºC for 1 h showed the lowest total difference in color value (ΔE^*) with the highest lightness (L^*- values). These results indicated that the condition of pretreated skin was not affected to color of tra catfish skin gelatin.

FTIR spectroscopy of two group gelatins

FTIR spectra of extracted gelatin at 70 ºC for 1 h from tra catfish skin via alkaline and enzyme pretreatment are showed in figure 2. The similar patterns in FTIR spectrum could be observed between gelatin from skin was pretreated by alkaline and enzyme. The spectra of amide I (1657 and 1639

Table 4 - Colour of gelatin from tra catfish skin pretreated by enzyme and alkaline at various extraction temperatures and times.

Temperature (°C)	Time (h)	L*	a*	b*	ΔE
----------Gelatin from skin pretreated by enzyme----------					
60	1	80.72 ± 0.566^{a}	4.29±0.290^{a}	19.42±0.454^{a}	22.47±0.058^{e}
60	2	79.59 ± 0.246^{b}	4.46±0.125^{a}	19.80±0.160^{a}	23.40±0.100^{cd}
60	3	76.11 ± 0.439^{c}	3.45±0.026^{bc}	17.23±0.228^{c}	23.73±0.208^{c}
70	1	79.82 ± 0.358^{ab}	3.75±0.115^{bc}	19.13±0.810^{ab}	22.63±0.833^{de}
70	2	76.83 ± 0.649^{c}	3.77±0.044^{b}	18.27±0.422^{b}	23.97±0.674^{c}
70	3	72.96 ± 0.854^{e}	3.34±0.185^{d}	15.62±0.355^{d}	25.13±0.709^{b}
80	1	76.44 ± 0.962^{c}	4.47±0.245^{a}	18.90±0.776^{ab}	24.83±0.231^{b}
80	2	74.53 ± 0.283^{d}	3.41±0.314^{cd}	14.71±0.567^{d}	23.37±0.058^{cd}
80	3	71.37 ± 0.346^{f}	3.16±0.130^{d}	14.99±0.422^{d}	26.13±0.404^{a}
Temperature (°C)	Time (h)	L*	a*	b*	ΔE
----------Gelatin from skin pretreated by alkaline----------					
60	1	82.49 ± 0.503^{a}	3.28±0.147^{c}	17.63±0.202^{b}	19.80±0.173^{e}
60	2	79.78 ± 0.673^{b}	3.80±0.097^{b}	17.79±0.306^{b}	21.67±0.473^{d}
60	3	78.29 ± 0.593^{c}	3.73±0.120^{b}	18.04±0.476^{b}	22.80±0.520^{c}
70	1	80.01 ± 0.382^{b}	3.79±0.060^{b}	17.82±0.37^{b}	21.53±0.503^{d}
70	2	76.83 ± 0.820^{d}	3.84±0.053^{b}	18.78±0.061^{a}	24.33±0.551^{b}
70	3	75.95 ± 0.805^{de}	4.51±0.412^{a}	19.14±0.517^{a}	25.33±0.950^{b}
80	1	79.11 ± 0.370^{bc}	3.51±0.049^{bc}	17.63±0.229^{b}	21.90±0.361^{cd}
80	2	75.62 ± 0.976^{e}	3.82±0.220^{b}	18.05±0.228^{b}	24.70±0.794^{b}
80	3	71.93 ± 0.311^{f}	4.39±0.045^{a}	18.77±0.186^{a}	28.00±0.346^{a}

Data are expressed as mean ± standard deviation (n=3).
Different superscripts in the same column indicate statistical differences ($P < 0.05$).

cm^{-1}) in two groups gelatin from tra catfish skin via alkaline and enzyme pretreatment, respectively. Amide I band, wavenumber from 1600 to 1700 cm^{-1}, plays most important role for the secondary structure of protein with stretching vibrations of carbonyl groups in peptides (NIKOO et al., 2014). However, the wavenumber range of 1660 cm^{-1} to 1650 cm^{-1}, typical for the α-helical structures, while the β-sheet structures was presented from 1640 cm^{-1} to 1620 cm^{-1}. The differences in wavenumber in amide I of two group gelatins were significantly correlated to the different conformations of polypeptide chains. In addition, amide III has been also associated with the disruption of triple-helix structure of collagen due to transfer of collagen to gelatin (MUYONGA et al., 2004). The wave number of amide III from two group gelatins (alkaline and enzyme pretreatment) was 1241 and 1239 cm^{-1}. The spectra of amide I and amide III from tra catfish skin gelatin pretreated by enzyme showed lower wavelength number than gelatin group pretreated by alkaline. The result was in agreement of KITTIPHATTANABAWON et al. (2012), who reported that the denaturation of α-helix by uncoupling of intermolecular cross-links and disruption of intra molecular bonding has related with the increase of wavelength number in amide I and III.

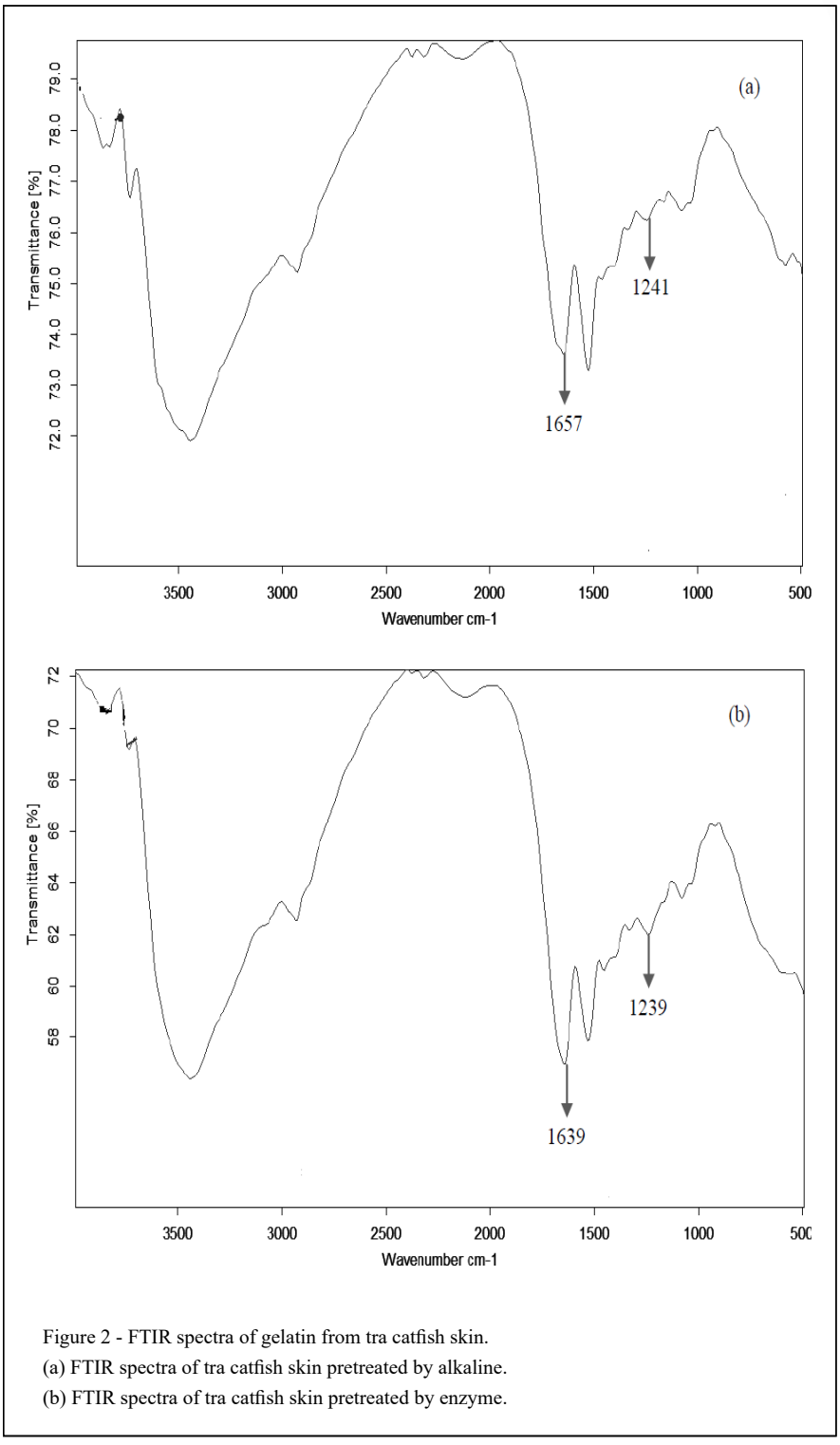

Figure 2 - FTIR spectra of gelatin from tra catfish skin.
(a) FTIR spectra of tra catfish skin pretreated by alkaline.
(b) FTIR spectra of tra catfish skin pretreated by enzyme.

FTIR spectra showed that the method to remove the non-collagenous protein in tra catfish skin (by using alkaline and enzyme) could be affected to the gelatin structure.

CONCLUSION

The characteristic of tra catfish skin gelatin were affected by pretreatment method to remove the non-collagenous protein, as well as gelatin extraction temperature and time. Gelatin from skin pretreated via Alcalase enzyme showed higher extraction yield than gelatin from skin pretreated by alkaline. The turbidity and gel strength of gelatin were affected with treatment method and extraction condition. The skin pretreated via enzyme resulted in a deterioration of protein patterns with to form a shorten peptide chain. The pretreatment methods have influence the properties of the resulting gelatin. Thus, the skin of tra catfish could be applied as a raw material for extracting gelatin and pretreatment method can be adjusted following to the purposive target.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

We authors of the article entitled "The changing of gelatin properties from tra catfish skin (*Pangasianodon hypophthalmus*) by the replacement alkaline to enzyme in pretreated process" declared, for all due purposes, the project that gave rise to the data of the same has not been submitted for evaluation to the Ethics Committee of the Can Tho University, but we are aware of the content of the Brazilian resolutions of the National Council for Control of Animal Experimentation (CONCEA) <http://www.mct.gov.br/index.php/content/view/310553.html> if it involves animals. Thus, the authors assume full responsibility for the presented data and are available for possible questions, should they be required by the competente authorities.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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