MINISTRY OF EDUCATION AND TRAINING
THE UNIVERSITY OF DA NANG

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EXTRACTING, MODIFYING GELATIN FROM SEAFOOD PROCESSING WASTE AND APPLICATION IN FOOD INDUSTRY

Major: Food Technology

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SUMMARY OF TECHNICAL DOCTRINE THESIS

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INTRODUCTION

1. Reasons for choosing the thesis

Gelatin is a protein, widely used in foods, pharmaceuticals, cosmetics, etc. Gelatin is used as a stabilizer, binder, emulsifier and gelling agent. Currently, gelatin is increasingly used, mostly produced from pig skin and cow hide. However, for gelatin obtained from cows or pigs, there is growing concern about infectious diseases and religious matters, so fish processing waste is seen as a potential source of gelatin. The annual production of fish is increasing, while about 50% is used for food, the rest is by-products used for animal feed or for export as raw materials with a very low economic value.The production of gelatin from this waste source is likely to bring high economic value. In spite of that, gelatin produced from fish processing by-products has small molecular weight, low gel strength and viscosity, and limited application. Based on these comments, we chose the research direction of the topic*: "Extracting, modifying gelatin from seafood processing waste and application in food industry".*

2. Research objectives

Development of the technological process to produce and modify gelatin from fish waste; Determination of gelatin's properties before and after modification; Evaluation of gelatin applicability in the food industry.

3. Research content

Analysis of chemical composition to select fish skin materials; Research on the raw material processing methods and the conditions for gelatin extraction, decolorization and deodorization; Research on the conditions for gelatin modification; Determination of gelatin's properties before and after modification; Evaluation of gelatin applicability

4. Scientific significance

Evaluation of the appropriate fish skin processing methods to guarantee the gelatin production quality and efficiency and conditions for gelatin decolorization and deodorization; Evaluation of the conditions for gelatin modification by transglutaminase, caffeic acid, tannic acid and polyphenols to improve gelatin's properties; Provision of information on the gelatin properties, structure and quality before and after modification; Evaluation of gelatin applicability.

5. Practical significance

Improvement of the economic value of the existing fish waste, and reduction of environmental pollution by fish waste; Serving as a basis for the development of the fish waste gelatin production process to substitute gelatin from mammals.

6. Outline of the thesis

The thesis consists of 135 pages, of which there are 33 tables and 53 figures. The Introduction will be 4 pages long, the Conclusion and recommendation of 4 pages, works published of 1 page and reference of 15 pages. The main contents of the thesis will be divided into three chapters as follows: Chapter 1. Overview: 33 pages in length, Chapter 2. Contents and research method: 17 pages long and chapter 3: Results and discussion: 77 pages long.

CHAPTER 1
OVERVIEW

1.1. Overview of collagen and gelatin

Collagen is a fibrous protein, forms a solid framework that supports the body's organs and parts in humans and animals. Collagen has a relatively complex structure, and the simplest structure is collagen molecule or tropocollagen. They are made up of 3 interconnected polypeptide chains (α-chain), known as collagen

triple-helix. This structure is stabilized by hydrogen bonds in each chain and between chains. When heated above 50^0C in a water environment, it results in a local untwisting of the triple helix and forms single polypeptide chain, i.e gelatin is formed. In acidic or alkaline environments, the intrinsic bonds of collagen chains are disrupted, increasing positive or negative charges, leading to mutual repulsion between charges of the same sign, creating a favorable condition for water to move to inside to make collagen be swollen and easily converted to gelatin by heating. Gelatin is derived from the partial hydrolysis of collagen, and it easily absorbs water, is swollen and soluble. The most important function of gelatin is its gelling ability. Its gelling ability is formed by the hydrogen bonds when cooled and evaluated by gel strength (Bloom value). The gel formation ability of gelatin is mainly dependent on molecular weight, amino acid content in gelatin, etc. With the essence of a protein, gelatin is capable of forming viscosity and emulsion, adhesion and able to form films.

1.2. Overview of fish gelatin

Fish gelatin is extracted from skin, scale, bone, etc., but mainly from the skin. Fish gelatin is full of properties such as ability to form viscosity, gel, films, emulsion, etc. similar to mammalian gelatin, but to a lower level. The properties of gelatin are primarily influenced by two main factors: properties of collagen in the fish skin and extraction conditions.

1.3. Overview of modified gelatin

Disadvantages of gelatin from fish skin are low gel strength due to its small molecular weight, short polypeptide chain, low proline and hydroxyproline content, etc. The modification process aims to increase gel strength by forming covalent bonds (cross-linking) between gelatin molecules, to increase size, molecular weight through amine, carboxyl and hydroxyl groups. To create cross-linking between gelatin molecules, the following agents can be

used: physical agents (heat, UV light, irradiation, etc.), chemical agents (glutaraldehyde, phenolic acid, etc.) and biological agents (enzyme). In particular, chemical and biological agents are used extensively in the food industry. The mechanism of cross-linking can be illustrated as follows:

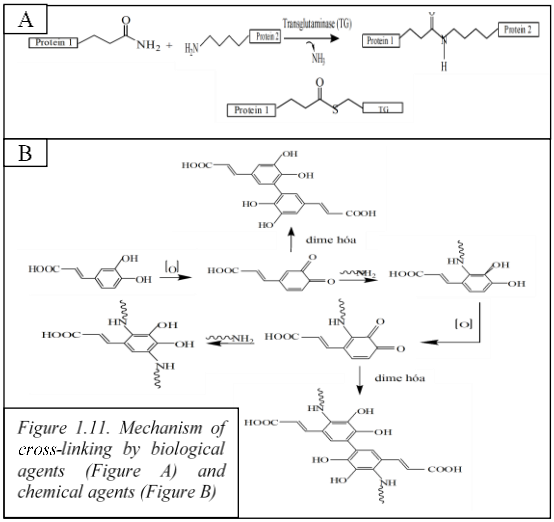

Figure 1.11. Mechanism of cross-linking by biological agents (Figure A) and chemical agents (Figure B)

1.4. Overview of research on gelatin extraction and modification

In the world, studies on gelatin production from fish waste have mostly focused on the production efficiency, while the gel strength (Bloom) has not been paid much attention; Gelatin production from dried fish skin has not caught interest; The use of ultrasonic wave in combination with the material processing has not been studied; Very few studies have been conducted to find the optimal extraction conditions for common fish skin types in Vietnam;

Gelatin decolorization and deodorization have not been studied. Especially, in Vietnam, no scientific study has been conducted on gelatin modification to improve gelatin's functional properties and expand the its scope of application.

CHAPTER 2
RAW MATERIALS AND RESEARCH METHODS

2.1. Raw materials

Main materials include: skin of Catfish, Tuna, Brronze featherback, Salmon, Mackerel and Paradise fish purchased from seafood processing plants in Central and South Vietnam.

2.2. Chemicals

Transglutaminase enzyme provided by Ajinomoto, Janpan; caffeic acid, tannic acid, p-dimethylaminobenzaldehyde, Trinitrobenzensunfonic Acid (TNBS), Chlororamine-T provided by Sigma-Aldrich; Hydroxyproline Standard, thiobarbituric acid supplied by Merck, Germany. In addition, CH_3COOH, $Ca(OH)_2$, NaOH, Na_2SO_3, $Na_2HPO_4.12H_2O$, $NaH_2PO_4.H_2O$, HCl, Glycerol and activated carbon, etc. meet the standards of analysis.

2.3. Research methods

- Physical and chemical methods: moisture determination, pH determination; determination of ash content; determination of gelatin extraction yield; viscosity determination; determination of gel strength of gelatin; determination of cross-linking level; determination of hydroxyproline content; molecular weight determination of gelatin by Polyacrylamide gel electrophoresis; determination of amino acid content by High-Performance Liquid Chromatography HPLC; determination of microstructure of gelatin by Scanning Electron Microscope (SEM); determination of gelatin structure by Fourier-transform infrared spectroscopy (FTIR); determination of the levels of heavy metals by atomic absorption spectrometry; determination of trimethylamine (TMA) content; determination of thiobarbituric acid (TBA); etc.

- Biochemical methods: Determination of protein content by Kjeldahl method; Determination of lipid content by Soxhlet method; Determination of total volatile base nitrogen (TVB-N).

- Microbiological methods: enumeration of total aerobic

microorganisms according to TCVN 4884-1: 2015; enumeration of *Escherichia coli* bacteria according to TCVN 7924-2:2008; enumeration of *Staphylococcus aureus*.

- Sensory evaluation method: evaluation of product quality by scoring tests and tasting tests.

- Optimizing experimental conditions: Experimental conditions are optimized by "Expected function" by Harrington for a multi-factor and multi-objective problem.

CHAPTER 3
RESULTS AND DISCUSSION

3.1. Research on gelatin extraction

3.1.1. Survey of basic chemical composition of fish skins from some species

Carry out analysis of the basic chemical composition of some fish skins, including: protein, lipid, moisture and ash content.

Table 3.1. Basic chemical composition of some fish skins

No.	Fish skin types	Composition				
		Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Collagen (mg/g)
1	Catfish	53.94±0.85^{f}	37.48±0.40^{a}	1.54±0.02^{bc}	0.14±0.00^{d}	278.56±0,13^{b}
2	Tuna	60.46±1.10^{d}	21.1±0.30^{c}	1.40±0.08^{c}	0.16±0.01^{c}	194.68±0,15^{d}
3	Paradise fish	60.54±0.72^{c}	18.75±0.31^{e}	2.58±0.02^{a}	0.23±0.01^{a}	171.24±0,19^{f}
4	Salmon	59.74±1.12^{e}	36.73±0.45^{b}	0.33±0.01^{e}	0.17±0.04^{bc}	296.35±0,14^{a}
5	Brronze featherback	63.40±1.04^{a}	20.92±0.32^{d}	0.63±0.05^{d}	0.18±0.03^{b}	186.63±0,11e
6	Mackerel	61.00±1.06^{b}	21.80±0.24^{d}	1.58±0.06^{b}	0.16±0.02^{c}	189.77±0,18^{c}

Most fish skins have high protein, collagen content and low content of ash and lipid, so they are suitable for gelatin production (except for paradise fish).

3.1.2. Research on raw material treatment

3.1.2.1. Research on treatment of fish skin with acetic acid (acid method

Results of the research show conditions of appropriate acid concentration and treatment time for the best gel strength, viscosity and gelatin extraction yield as follows:

Table 3.2. Results of acid concentration and skin treatment time for the best gel strength, viscosity and gelatin extraction yield

Parameters / Fish skins	Acid concentration, *mM*	Treatment time, *hour*	Gel strength, *gam*	Viscosity, *cP*	Yield, *%*
Catfish	150	2	97.7 ± 0.99^{a}	19.80 ± 1.2^{a}	25.51 ± 0.51^{a}
Mackerel	5	4	81.6 ± 0.8^{c}	15.87 ± 0.22^{d}	26.78 ± 0.32^{b}
Brronze featherback	7,5	4	85.6 ± 0.67^{b}	19.35 ± 0.46^{b}	21.54 ± 0.38^{e}
Salmon	2,5	2	86.3 ± 0.59^{b}	18.43 ± 0.83^{c}	23.63 ± 0.25^{d}
Tuna	7,5	4	60.3 ± 1.18^{d}	8.43 ± 0.38^{e}	27.36 ± 0.29^{a}

The results indicate that the appropriate acid concentration and treatment time for obtaining gelatin of the highest gel strength, viscosity and extraction yield are different depending on the skin type and fluctuate in the range of 2.5mM÷150mM and from 2÷4 hours. Catfish skin needs to be treated at the highest acid concentration (150mM) while Salmon skin only needs to be treated at 2.5mM. When using the acid method, gelatin extraction yield is quite high, but the gel strength and viscosity are quite low.

3.1.2.2. Research on treatment of fish skin with liquid lime (alkaline method)

Table 3.3. Results of appropriate lime content and treatment time for obtaining gelatin of the best gel strength, viscosity and yield

Paremeters / Fish skin	Content, *g/l*	Time, *day*	Gel strength, *gram*	Viscosity, *cP*	Yield, *%*
Catfish	20	5	251.3 ± 1.86^{a}	33.1 ± 0.71^{a}	22.41 ± 0.70^{c}
Mackerel	30	3	106.3 ± 1.36^{d}	21.72 ± 0.63^{c}	23.27 ± 1.19^{b}
Brronzefeatherback	20	3	114.3 ± 1.40^{c}	21.3 ± 0.79^{c}	19.45 ± 0.54^{d}
Salmon	9	0,5	154.2 ± 1.93^{b}	24.2 ± 1.11^{b}	24.32 ± 0.53^{ab}
Tuna	20	3	65.3 ± 1.23^{e}	19.57 ± 0.80^{d}	25.06 ± 0.76^{a}

The conditions for obtaining gelatin of the highest gel strength, viscosity, and extraction yield by the alkaline method are mainly at

lime content of 20÷30 g/l and treatment time of 3÷5 days. Salmon skin is treated with lime content of 9g/l and duration of 0.5 days. When using the alkaline method, extraction yield is lower, but gel strength and viscosity is higher than acid method.

3.1.2.3. Research on treatment of fish skin with liquid lime and acid solution respectively (alkaline-acid method)

To evaluate the effect of fish skin treatment with liquid lime and acid solutions, we select the appropriate acid concentration and lime content based on the results stated in Tables 3.2 and 3.3.

Table 3.4. Results of skin treatment time in liquid lime, acid solution to obtain gelatin of the highest gel strength and viscosity

Parameters / Fish skin	Lime soaking period, *day*	Acidic soaking period, *hour*	Gel strength, *gam*	Viscosity, *cP*	Yield, *%*
Catfish	2	2	235.6 ± 1.5^{a}	32.40 ± 1.33^{a}	21.49 ± 0.81^{c}
Mackerel	1	3	110.6 ± 1.12^{d}	22.44 ± 1.63^{c}	24.38 ± 0.89^{ab}
Brronze featherback	2	3	120.3 ± 1.53^{b}	22.63 ± 1.42^{c}	21.04 ± 0.21^{c}
Salmon	2 hours	1.5	198.4 ± 1.96^{c}	29.21 ± 0.85^{b}	23.35 ± 0.62^{b}
Tuna	1.5	2	102.8 ± 1.02^{e}	20.40 ± 0.97^{c}	25.43 ± 1.02^{a}

The results of Table 3.4 show that the treatment time of fish skin in liquid lime decreases by 50% compared to the alkaline method, treatment time in acid solution slightly decrease compared to the acid method. Its extraction yield is equivalent to the alkaline method but lower than the acid method. Viscosity and gel strength are higher than those of the acid and alkaline methods. In particular, gel strength and viscosity of catfish skin are not much different from those of the alkaline method. The above results represent that: Gelatin extracted from catfish skin has the highest gel strength, and Gelatin from Tuna skin has the lowest gel strength, but both types of fish above have quite high yield and large skin output. Skin of catfish and Tuna are the subject of further research.

3.1.2.4. Research on gelatin extraction from dried skin material

To reduce storage costs of raw materials compared to frozen fish skins, we have investigated the possibility of gelatin extraction

from dried fish skins (Catfish and Tuna). Investigation of the gelatin extraction process is conducted under three methods like frozen skins. The results indicate that dried fish skin is also suitable for production of gelatin with gel strength, viscosity of gelatin solution, extraction yield similar to those of frozen fish skin. However, it takes longer skin treatment time or requires to treat skin in acid solution, liquid lime with a higher concentration than frozen fish skin.

3.1.2.5. Research on treatment of fish skin with the support of ultrasonic waves

For the purpose of shortening the period of fish skin treatment, we have conducted skin treatment in liquid lime combined with ultrasound. The effects of ultrasonic waves mainly depend on: amplitude, wave cycle and effect time of ultrasonic waves.

Results of ultrasonic conditions to obtain gelatin of the highest quality and yield: Catfish: amplitude: 90%; period: 0.9s; time of ultrasound: 90 minutes; gel strength: 251.3 gram; viscosity: 31.35 cP; yield: 23.97%; Tuna: 80%; period: 0.8s; time of ultrasound: 90 minutes; gel strength: 103.6 gram; viscosity: 23.51 cP; yield: 25.6%. Based on the results, it is found that when treating fish skin in liquid lime combined with ultrasonic waves, fish skin treatment time is much lower, but gel strength, viscosity and yield of gelatin obtained are equivalent to the case without using ultrasonic waves.

3.1.3. Research on the extraction process

The extraction conditions for gelatin production with highest quality and efficiency are as follows: Catfish: temperature: 60^0C, duration: 8 hours, solid/liquid ratio: 1/5(w/v), gel strength: 246.8 g, viscosity: 33.84 cP, and efficiency: 22.9%. Tuna: temperature: 55^0C, duration: 7 hours, solid/liquid ratio: 1/5(w/v), gel strength: 102.8 g, viscosity: 20.84 cP, and efficiency: 25.64%.

3.1.4. Research on cleaning of gelatin

Gelatin solution after extraction is decolorized and deodorized

by fine-grained activated carbon for the best performance over charcoal of larger grains and sand.

Table 3.8. Conditions of gelatin deodorization and decolorization by activated carbon (AC)

Condition / Type of gelatin	Rate of AC , % (w/v)	Time, *minute*	Temperature, ^{0}C
Catfish	1.5	45	45
Tuna	2	75	45

After deodorization and decolorization, obtained getalin is bright white like gelatin products on the market and has characteristic aroma of gelatin.

3.1.5. Determination of some characteristics of finished gelatin

Determine the gelatin characteristics of the four samples as follows GNDDT: gelatin from Tuna skin; GNDDS: gelatin from Tuna skin with the support of ultrasonic waves ; GTRAT: gelatin from Catfish skin and GTRAS: gelatin from Catfish skin the support of ultrasonic waves.

3.1.5.1. Molecular weight determination of Gelatin

Molecular weight of gelatin is determined by Polyacrylamide gel electrophoresis of the four gelatin samples as shown above with marker (MK).

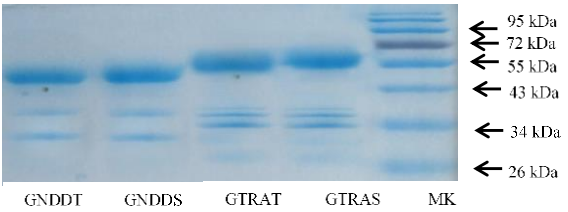

Figure 3.12. Molecular weight distribution of gelatin

Molecular weight of getalin from Tuna skin is mainly around 43÷55 kDa and that from Catfish skin is mainly about 55÷72 kDa. In particular, the molecular weight of gelatin with or without the support of ultrasonic waves is equivalent on both types of fish skin

material.

3.1.5.2. Analysis of gelatin structure by scanning electron microscope (SEM)

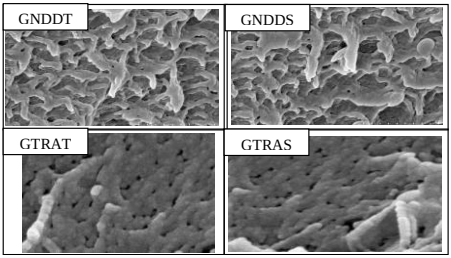

Figure 3.13. Microstructure of gelatin extracted from skin of Tuna and catfish

Getalin from skin of Tuna (GNDDT, GNDDS) and skin of Catfish (GTRAT, GTRAS) clearly differ in gel network structure. Gelatin from Catfish skin has a dense and tight protein structure, and gaps between the protein fibers which are smaller and less than gelatin from Tuna skin. Gelatins with and without ultrasonic waves have similar structure.

3.1.5.3. Analysis of Fourier transform infrared (FTIR) spectroscopy of gelatin

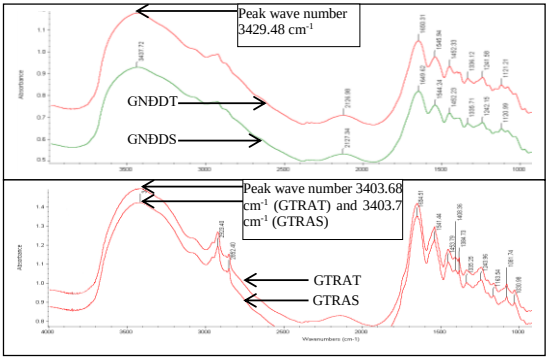

Figure 3.14. Fourier transform infrared spectroscopy of gelatin

Fourier transform infrared spectroscopy of gelatin show that,

peak waves number 1121.9÷1649.6 cm^{-1}(Tuna) and 1030.9÷1654.5 cm^{-1} (Catfish) represent the amide I, amide II and amide III region. Peak waves number 2127.3 cm^{-1} (Tuna) and 2652.4 cm^{-1}, 2923.4 cm^{-1} (Catfish) represent the amide A band; Peak waves number 3429.5cm^{-1}(Tuna) and 3403.7cm^{-1} (Catfish) represent the amide B band. Fish skin gelatin from both tuna and catfish has basic links of a gelatin and there are negligible differences between gelatin processed with and without ultrasonic support.

3.1.5.4. Amino acid composition

Conduct analysis of the amino acid composition of the above four gelatin samples. The results show that all four types of gelatin have the basic amino acid composition of fish gelatin. The important amino acids such as proline, hydroxyproline, etc. in gelatin extracted from Catfish are higher than those of gelatin extracted from Tuna. Amino acid composition in gelatin with and without the support of ultrasonic waves is similar, but cysteine is present in gelatin with ultrasound support and absent in gelatin samples without ultrasound support.

3.1.6. Evaluation of gelatin quality indicator

Quality standards of finished gelatin are evaluated based on QCVN 4-21: 2011/BYT. Gelatin products were obtained under the condition that the contents of heavy metals, microbiological indicators, pH and basic composition of lipid, protein, ash, moisture, etc. are within the allowable limits for gelatin food set out by the Ministry of Health.

3.1.7. Research on storage of gelatin

- At normal temperature: gelatin stored in HDPE bags with a duration of more than 120 days still ensures quality, which shouldt be stored in LDPE bags for a long time.

- At cold temperature: gelatin stored in HDPE or LDPE bags are guaranteed quality for a period of more than 120 days.

3.1.8. Proposal of gelatin extraction process from fish skin

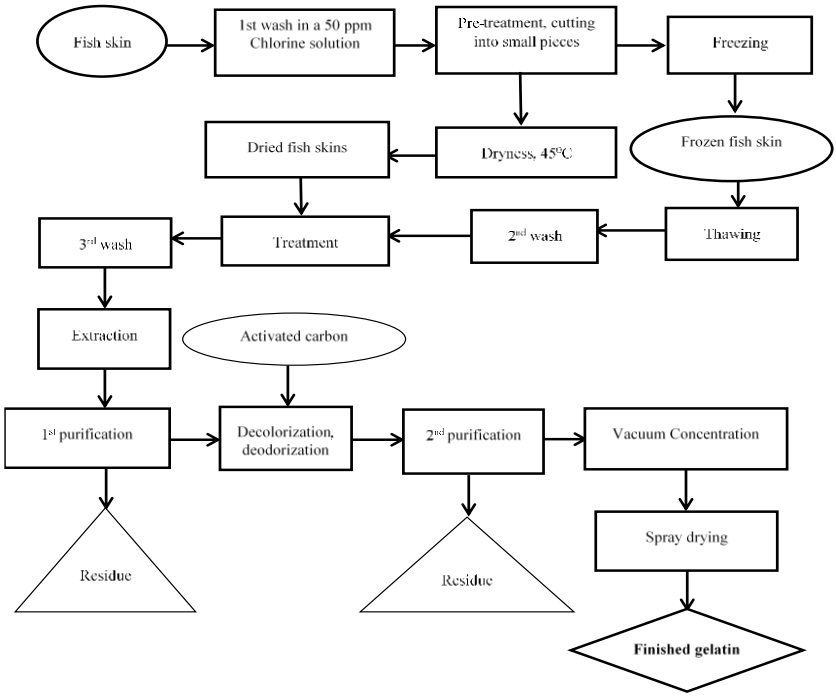

Figure 3.16. Process of gelatin extraction

3.2. Research on modification of gelatin from Tuna skin

3.2.1. Research on gelatin modification by enzyme transglutaminase, caffeic acid and tannic acid

3.2.1.1. Research on denaturing conditions

Conduct a survey of factors temperature, time, content of denaturing agent, gelatin concentration affecting gel strength. The results of denaturing conditions are appropriate for gelatin to have the highest gel strength as follows:

Table 3.15. The most suitable conditions for gelatin modification by

transglutaminase enzyme, caffeic acid and tannic acid

Type of denaturing agent	*Content of agent, mg/g*	*Time, minute*	*Gelatin-concentration, %*	*Tempo-rary, ^0C*
Transglutaminase	25	80	18	40
Caffeic acid	15	90	15	40
Acid tannic	25	60	20	40

3.2.1.4. Research on the change in gelatin molecular weight

Gelatin molecular weight is determined on 4 samples: GPE: modification gelatin with transglutaminase; GPC: modification gelatin with caffeic acid; GPT: modification gelatin with tannic acid with marker sample with the known molecular weight (MK).

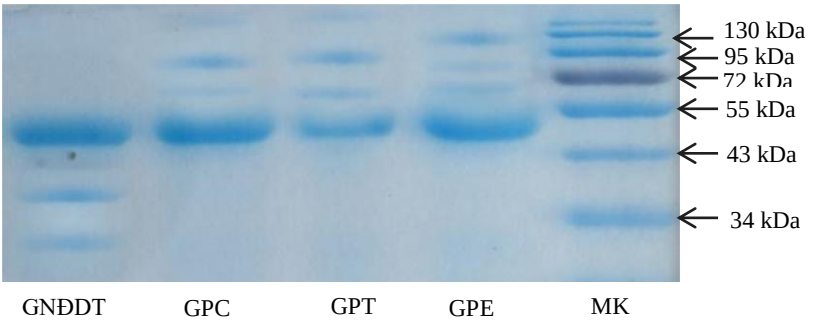

Figure 3.20. Electrophoretic images of modified gelatin and the marker sample

After modification, the gelatin molecule weight increased, GC, GT and GE samples showed additional streaks in 55÷95 kDa. In addition, GE sample also showed a streak in 95÷130 kDa. At the same time, there is vanishing of protein streaks with a low molecular weight of 26÷43 kDa in GNĐDT samples after modification .

3.2.1.5. Research on *gelatin structure by scanning electron microscope (SEM)*

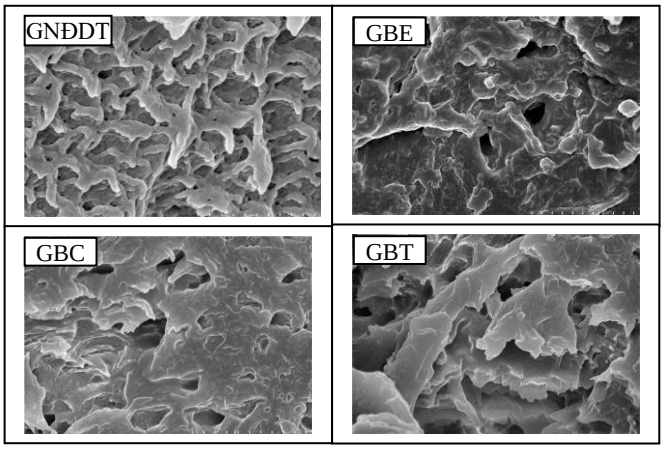

Figure 3.21. Microstructure of gelatin before and after modification

The gelatinous fiber structures after modification with transglutaminase (GBC), caffeic acid (GBC) and tannic acid (GBT) are more dense, fiber dimension is coarser than undenatured gelatin (GNDDT). In which, transglutaminase-modified gelatin (GBE) and caffeic acid (GBC) for denser gel network structure than denatured gelatin with tannic acid (GBT).

3.2.1.6. Determination of cross-linking level

To confirm getalin modification with the used agents is due to the formation of cross-linkages, we investigate cross-linking level of gelatin by quantifying the number of free amino groups present in the side chain of the gelatin consisting of the above samples. After modification, cross-linking levels of the samples are 22.5% (GBE), 20.8 (GBC) and 16.4% (GBT).

3.2.1.7. Analysis of infrared spectra of post-modification gelatin

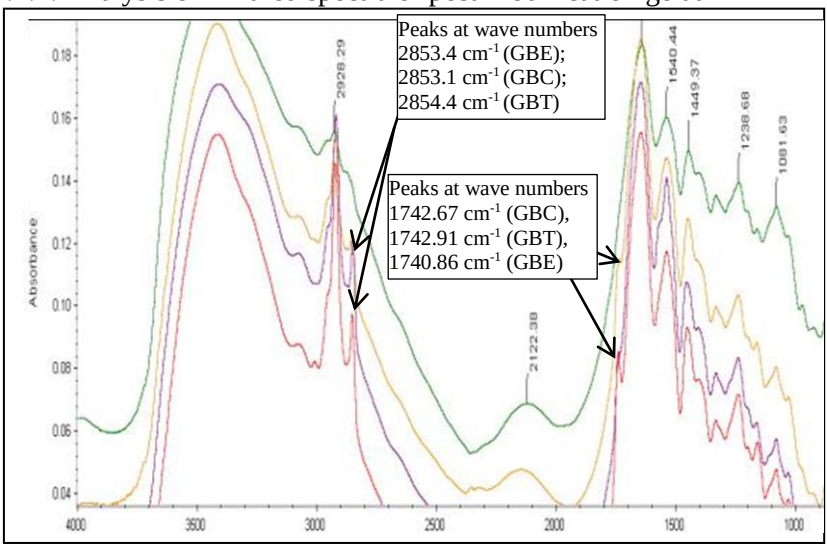

Figure 3.23. Gelatin infrared spectra (FTIR)

In addition to the peaks with the same wavenumber as which of GNDDT, GBC, GBT and GBE also have peaks at wavenumber 1742.67 cm^{-1}, 1742.91 cm^{-1} and 1740.86 cm^{-1} at amide I, respectively, due to the oscillation C=O associated with CN linkage and peak at wave number 2853.4 cm^{-1} (GBE); 2853.1 cm^{-1} (GBC); 2854.4 cm^{-1} (GBT) in amide B, due to the asymmetric oscillation of the C-H linkage as well as–NH^{+}_{3} group.

3.2.2. Research on gelatin modification using polyphenols in green tea

3.2.2.1. Research on determination of denaturing conditions

Investigate the factors that affect the modification process such as temperature, time, polyphenol content, gelatin concentration to gel strength, viscosity, cross-linking level etc. of gelatin: polyphenol content: 20 mg/g; time: 40 minutes; temperature: 40^{0}C,

gelatin concentration: 20%; gel strength: 116.4 gram; cross-linking level: 15.7%. When denaturing with polyphenols, after drying, the gelatin is not or less dissolved in water, which proves gelatin is hydrophobic.

3.2.2.5. Research on changes in molecular weight after modification

Gelatin molecular mass was determined by SDS-PAGE electrophoresis on the polyphenol-modified gelatin sample (GBP), the control sample (GNDDT) and the marker sample (MK).

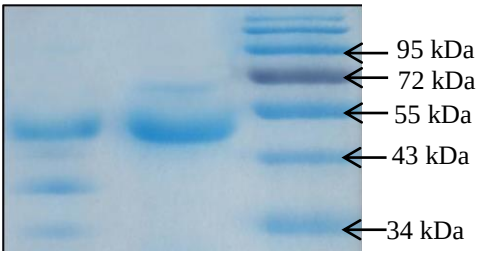

Figure 3.25. Electrophoretic images of modified gelatin and the marker sample

The result of electrophoresis shows that a GP sample appears additional streaks in 55÷75 kDa, and also streaks in 34÷43 kDa of GNDDT sample are vanished. This indicates an increase in gelatin molecular weight after modification.

3.2.2.6. Determination of gelatin structures

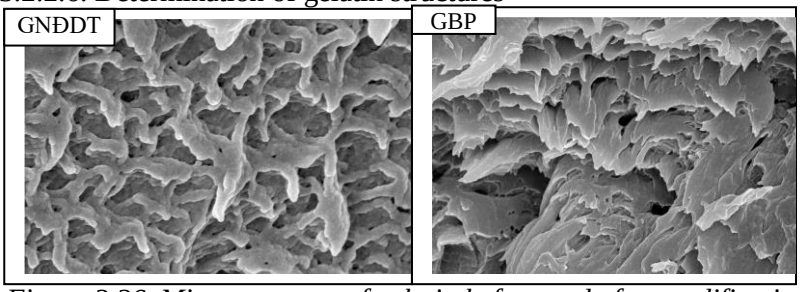

Figure 3.26. Microstructure of gelatin before and after modification

After modification, gelatin no longer had a clear fiber structure as in natural gelatin; polyphenols seemed to have covered the gelatin fibers, thickening the fiber structure

3.2.2.7. Determination of infrared spectra (FTIR) of modified gelatin

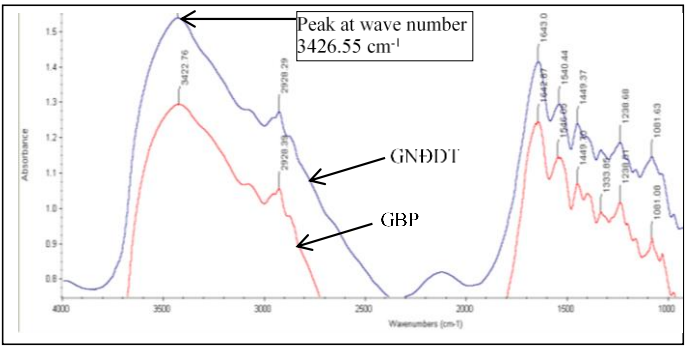

Figure 3.27. Infrared spectra of gelatin before and after modification

Infrared spectra show a decrease in absorption intensity of the GBP sample (at wave no. 3422.76 cm^{-1}) compared to the GNDDT sample (at wave no. 3426.55 cm^{-1}) in amide B region. That shows interaction of the -NH3 group and interaction of hydrophobic group between peptide chains with polyphenols.

3.2.2.8. Proposal of gelatin modification process

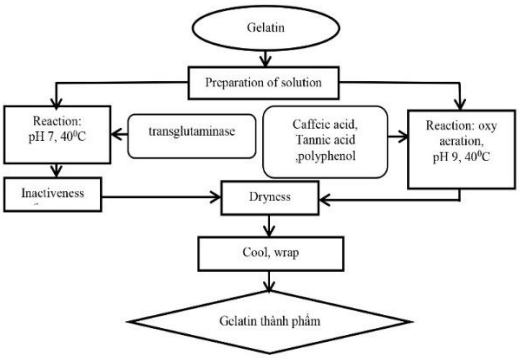

Figure 3.29. Gelatin modification process

3.3. Gelatin application

3.3.1. Evaluation of the applicability as a gel-forming agent for marshmallow cream in chocopie production

Use gelatin from Catfish skin with a gel strength of 250 g as a

gelling agent for marshmallow cream at Biscafun Quang Ngai confectionery factory. Factory comments when using gelatin: State: fine; Color: white; Flavor: Characteristic; The state of gelatin when immersed in water: good swelling; Meltness (50^0C): Rapid; The toughness of marshmallow cream: good toughness; Elasticity of marshmallow cream: good elasticity; Sponge of marshmallow cream: sponge; Marshmallow cream structure: stable.

3.3.2. Application in the production of orange marshmallows candy

Four samples of candy made with the same formula including sucrose 1000g, starch syrup 250g, citric acid 5g, orange flavor 1.2g and gelatin 150g (4 types of gelatin: catfish gelatin (sample 1), transglutaminase-modified tuna gelatin (sample 2), marketed pork gelatin (sample 3) and unmodified tuna gelatin (sample 4). The analysis results indicated that samples 1, 2, and 3 had the same physicochemical status which met the requirements of TCVN 5908:2009 and the marshmallow products were accepted by consumers. Sample 4 failed to meet the quality requirements according to TCVN 5908:2009 and was unaccepted by consumers.

3.3.3. Application as anthocyanin microcover material

Use transglutaminase-modified getalin with Bloom 250g as anthocyanin-based natural pigment microcover material. Proceed microcoating as follows: microcoated material (anthocyanin)/ coating material (gelatin + maltodextrin) = 1/4. The performance of microcover is 87.98% and the sample stored in PE bag at room temperature. Monitor the loss of anthocyanin content after 100 days, the remaining anthocyanin content 88.32% while the sample does not be microcoated, the remaining anthocyanin content 76.78%.

3.2.4. Evaluation of the applicability of modified gelatin with polyphenols in green tea as the film for beef preservation

3.2.4.1. Research on production of film

In order to create film with satisfactory thickness, water vapor permeability, mechanical properties, etc. we investigate the effect of:

gelatin concentration, glycerol content. Results obtained: gelatin after modification with polyphernols, transformed to concentration of 3%, added with 20% glycerol (compared to gelatin) creates the best film. The film with moderate thickness, low permeability, relatively high mechanical properties should be suitable for preserving meat.

3.2.4.2. Research on preserving beef with gelatin film

Conduct gelatin film coating on beef by dipping the meat into prepared gelatin with a gelatin concentration of 3%, 20% glycerol. However, after dipping and draining, the formed gelatin film is too thin, not enough to cover the meat surface. Therefore, we chose a higher gelatin concentration: 9%; 12% and 15% with 20% glycerol content (compared to gelatin). After dipping in gelatin solution, draining, placing meat in tray, covered with PE film and cooled to $4 \div 5^0$C. Check sample: Beef was not dipped in gelatin solution but only covered with PE film and cooled $4 \div 5^0$C. Monitor changes in sensory, physicochemical standards (volatile base nitrogen, thiobarbituric acid, moisture content, pH) and microbiological criteria (total aerobic microorganisms, E. coli, *Staphylococcus aureus*) for 5 days of preservation. Results: Meat samples coated with gelatin film combined with PE film have sensory, physicochemical and microbiological indicators within the permitted limits (according to TCVN 7046: 2009) and guarantee to be used as food for 4 days of preservation. Of these, 12% and 15% gelatin samples has similar quality criteria and are better than the 9% gelatin sample. At the same time, meat sample covered only with PE film ensures sensory, physical and microbiological indicators for 3 days of preservation.

CONCLUSION AND RECOMMENDATION

A. Conclusion

1/ The appropriate conditions for fish skin processing for 5 types of frozen fish skin and 2 types of dried fish skin to obtain gelatin with the best gel-forming ability were identified.

- Catfish skin: soaking lime (20g / l) for 5 days;
- Tuna skin: soak in lime (20g / l) for 1.5 days, then soak in acetic acid (7.5mM) for 2 hours;
- Mackerel skin: soaked in lime (30g / l) for 1 day, then soaked in acetic acid (5mM) for 3 hours;
- Brronze featherback skin: soaked in lime (20g / l) for 2 days, then soaked in acetic acid (7.5mM) for 3 hours;
- Salmon skin: soaked in lime: (9g / l) for 2 hours, then soaked in acetic acid (2.5mM) for 1.5 hours;

The best Bloom of fish skin gelatin per fish may be arranged in descending order as follows: Catfish (251.3 g) > Salmon (198.4 g) > Bronze featherback (120.3 g) > Mackerel (110.6 g) > Tuna (102.8 g).

2/ Appropriate ultrasonic amplitude, cycle and duration to assist the Catfish and Tuna skin immersion in lime to shorten the immersion duration while creating gelatin with gel strength and production efficiency similar to which from non-ultrasonic method were identified.

Suitable ultrasound conditions are as follows:

- Catfish skin: amplitude: 90%; period: 0.9 seconds; Time: 90 minutes;
- Tuna Skin: Amplitude: 80%; period: 0.8 seconds; Time: 90 minutes;

3/ The conditions for gelatin decolorization and deodorization by fine activated charcoal were identified as follows: Tuna skin gelatin: charcoal content: 2% (w/v), temperature: 45^0C, and duration: 75 mins; Catfish skin gelatin: charcoal content: 1.5% (w/v),

temperature: 45^0C, and duration: 45 mins. After decolorization, gelatin no longer smells fishy.

4/ Content of modification factors, gelatin content, temperature and duration in 4 modification methods: using transglutaminase, caffeic acid, tannic acid and green tea polyphenols were determined to increase the gel strength of modified gelatin compared to the original gelatin.

The best denaturing conditions for each specific type of agent are as follows:

- Enzyme transglutaminase: Content: 25 mg / g; Time: 80 minutes; gelatin concentration: 18%; temperature: 40^0C; Bloom level increased by 149.5%.

- Caffeic acid: Content: 15 mg / g; Time: 90 minutes; gelatin concentration: 15%; temperature: 40^0C; Bloom increased by 65.3%.

- Tannic acid: Content: 25 mg / g; Duration: 60 minutes; gelatin concentration: 20%; temperature: 40^0C; Bloom level increased by 30.2%.

- Green tea polyphenols: Content: 20 mg / g; Duration: 40 minutes; gelatin concentration: 20%; temperature: 40^0C. Bloom level increased by 17.2%.

5/ Changes in molecular mass, gel structure, level of cross-linking, formation of new links in modified gelatin compared to natural gelatin were determined by SDS electrophoresis, SEM microstructure analysis, UV-VIS and infrared spectroscopy.

6/ The applicability of Catfish skin gelatin in the production of chocopie and marshmallows was evaluated. At the same time, packaging beef using green tea polyphenol-modified Tuna skin gelatin gel was empirically confirmed to prolong the storage compared to the storage time of the control sample in cold storage. Meanwhile, transglutaminase-modified gelatin as anthocyanin microcapsules was determined to be able to limit color loss during storage.

B. New contribution of the thesis

1/ For the first time in Vietnam, it was determined the systematic conditions for gelatin acquisition system on 5 common fish skin in Vietnam, in which the process of obtaining gelatin technology has the highest Bloom from Catfish and Tuna skin. In particular, the possibility of ultrasound application has been evaluated to shorten the processing time of fish skin during gelatin acquisition.

2/ Proposal of the Tuna skin gelatin modification process to improve the gel forming ability and the change in the hydrophilicity by 4 agents: transglutaminase enzyme, caffeic acid, tannic acid and green tea polyphenols to extend the application of gelatin.

3/ Scientific information on the differences in molecular mass, gel structure and infrared spectrum of Tuna skin gelatin before and after modification, this enriches the scientific database on the amino acid composition and the molecular mass of Catfish and Tuna skin gelatin.

C. Recommendations for Follow-Up studies

1/ Research on the gelatin production method using high pressure to reduce the immersion duration and increase the production efficiency. **2/** Research on the optimization of gelatin concentration and drying processes to guarantee the quality and the economic efficiency. **3/** Research on the gelatin modification using other agents such as Genipin and Ferulic acid to improve the functional properties of fish skin gelatin on a larger scale. **4/** Application of modified gelatin in the industrial-scale production of other food products.

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1. Chau Thanh Hien, Đang Minh Nhat, Tran Thi Xo, Cao Thi Ngoc Xinh (2014), "Methods of gelatin extraction from Salmon fish skins", *The Journal of Science and Technology*, Vietnamese Academy of Sciences and Technology, vol. 52 - No. 5C, pp. 393-399.

2. Chau Thanh Hien, Đang Minh Nhat (2015), "Effect of various factors on the extraction yield and quality of gelatin obtained from Tuna skins", *The Journal of Science and Technology*, Vietnamese Academy of Sciences and Technology, vol. 53 - No. 4B, pp. 38-43.

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5. Chau Thanh Hien, Đang Minh Nhat (2016) "Deodoration and decoloration of gelatin extracted from Tuna skin", *Journal of Science and Technology*, Da Nang University, vol. 11- No. 108, pp. 61-64.

6. Chau Thanh Hien, Đang Minh Nhat (2017) "Application of gelatin modified by green tea polyphenol for food coating", *Proceedings of the national seminar*, pp. 63-69.