Fish collagen extraction and application

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Collagen

- What is collagen?
  - **Collagen**: the main protein of connective tissue in animals and the most abundant protein in mammals, making up about 25% to 35% of the whole-body protein content. Collagen constitutes 1% to 2% of muscle tissue, and accounts for 6% of the weight of strong, tendinious muscles.

- What is it used for?
  - Food: structure additive, ...
  - Pharmaceutical: soft and hard capsule, artificial skin,...
  - Cosmetic: repair rough skin, wrinkle-resistant,...
Main sources

- Pig skins or cow skins and bones: low cost of the final product
Why do we shift to collagen extraction from fish?

- Major advantage of marine collagen sources is that they are not associated with the risk of bovine spongiform encephalopathy (BSE) outbreaks.
- Fish collagen is acceptable for Islam and with a minimum restriction for Judaism.
- Fish skin is a major by-product of the fish-processing industry, causing wastage and pollution, and could provide a valuable source of collagen.
- More suitable for human skin than collagen from livestock and poultry.
Extraction methods

- **Chemical methods:**
  - Solvents for removing non-collagenous proteins, defatting, deordoring: salt, dilute acid, alcohol, detergents, $\text{H}_2\text{O}_2$...
  - Chemicals for deashing: EDTA (for pre-treating bone)
  - Solvent for extracting collagen: delute acide (acid acetic, acide lactic), enzyme (pepsin), bacteria Bacillus, yeast. **Temperature: 0-4°C**
Our researching

1. Pre-treatment steps:
2. Extracting acid-soluble collagen
3. Purify collagen

Source: Min Zhang, Wentao Liu, Guoying Li, (2008), Sichuan University, China
Source: Inwoo Bae, (2007), Nagasaki University, Japan
Source: Takeshi Nagai, (1999), Kyushu University, Japan
Extraction methods

- Supercritical extraction method by using CO2 as solvent

*Environmental improvement and reduced product contamination*
Advantages

- Easy to control dissolving power of supercritical fluid (SCF) by controlling T, P.
- Easy to recover SCF by decompressing pressure.
- Impossible to separate the precipitate from extracts by centrifugation.
- Non-toxic solvent
- Applicable to extract the thermally decomposed compounds
Disadvantages

- Require high pressure.
- Investing elaborate equipment and high capital.
- High technical worker.
ANALYSE METHODS

1. Sodium dodecyl sulphate polyacrylamine gel electrophoresis (SDS-PAGE) to determine the forms of collagen (α-chain, β-chain)
2. SDS-PAGE to determine Peptide mapping
3. Determination of hydroxyproline content
4. Determination of denaturation temperature
5. Viscosity of collagen solution
6. Effect pH and NaCl on Collagen solubility
7. FT-IR
8. SEM to determine structure of collagen

Source: Boyl R. Switzer,(1991),University of North Carolina, USA.
Methods to be applied

- Data-base set up, investigation of structure, characteristics and application capability of collagen
- Investigation of collagen concentration in skin and skeleton of catfish (basa fish).
- Choice of suitable solvent for collagen extraction
- Implementation of extraction process for high yield and selection of collagen extraction.
- Analysis and evaluation of extracted collagen:
RBE Plan

Supervising system of master’s program

Project team:
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Dr. Mai Thanh Phong
Prof. Dr. Phan Dinh Tuan
Dr. Doan Huu Luc
KS. Nguyen Thi Nguyen

Le Thi Thu Huong, PhD. student

Ngo Hong Bao Chau
Master student

Nguyen Thi Diem Phuong
Undergraduate student
RBE Plan

- For the leader of the laboratory:
  - Supervising master students from the first day of the program until they finish it.
  - Guiding/supervising master students so that they can create one academic paper based on the joint research under SUPREM-HCMUT.
  - Giving regular lab meetings where master students are required to report their research progress.
RBE Plan

- For master students:
  - Must create their master thesis based on the research outcomes.
  - Requested to write an academic paper as the first author and based on the Key Issues given.
- Substantial goals:
  - One PhD, one master student and 2 undergraduate students involve in the research work along with lab management and team leader.
  - One master thesis will be created on July 2010.
  - At least two papers conducted by master student will be submitted.
RBE Plan

- Actions to achieve the substantial goal:
  - Assigning a supervisor to the master student
  - Organizing regular seminars within the research group to transform the content of some lectures to research based seminars
  - Desk arrangement in the Lab for students
  - Assigning a research assistant to master students
  - Planning the research project taking the publication into consideration
  - Frequent research discussions will be once a week
References

- Boyl R. Switzer, (1991), *Dertermination of hydroxyproline*, University of North Carolina, USA.
- Min Zhang, Wentao Liu, Guoying Li, (2008), *Isolation and characterisation of collagen from the skin of largefin longbarbel catfish*, Sichuan University, Chin.
- Inwoo Bae, (2007), *Biochemical properties of acid-soluble collagens extracted from the skin of underutilised fishes*, Nagasaki University, Japan.