

ISOLATION AND CHARACTERIZATION OF BIOACTIVE INGREDIENT COLLAGEN IN JELLYFISH



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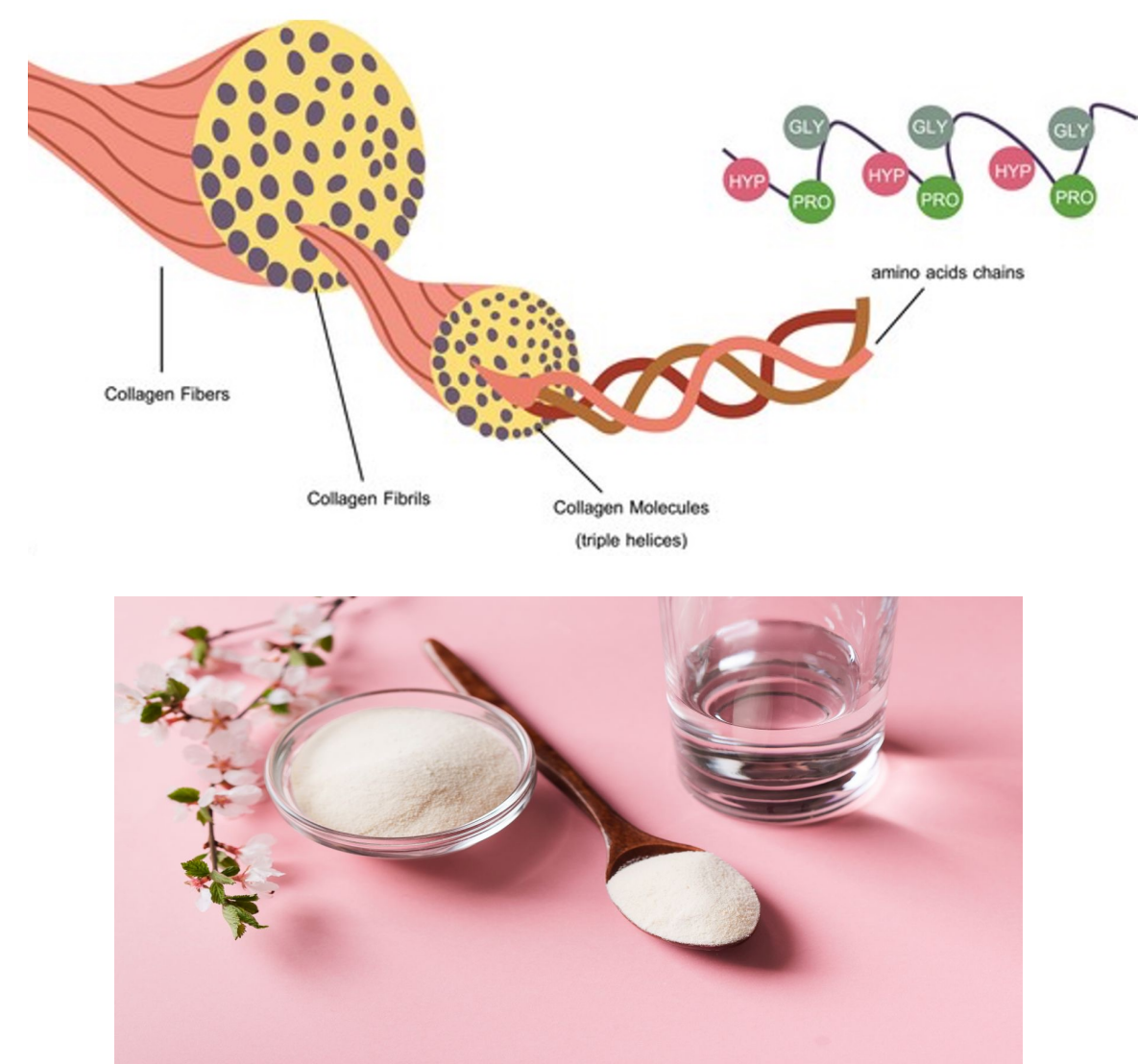
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WHAT IS COLLAGEN?



- Collagen is the most abundant component of the extracellular matrix of all connective tissues such as skins, tendons, ligaments and bones.
- It represents about one-fourth of the total protein content in most animals.
- It is a fibrous protein formed by three polypeptide α -chains, arranged as a triple helix enfolded around each other.
- Each one is composed of a set of amino acid with repeating motif of Gly-X-Y, where X and Y are predominantly, proline (Pro) and hydroxyproline (Hyp).
- These building blocks combine to form collagen fibrils.

TYPES OF COLLAGEN

Types of Collagen	Source	Major Roles/Functions
I	Fish Bovine, Egg Bone broth	Supports youthful looking skin
II	Bone broth Chicken	Helps builds & maintain gut lining. Supports joints & digestive health. Promotes immune function
III	Fish Bovine Egg	Supports skin & bone health Part of arterial walls & important for heart health



SOURCE OF COLLAGEN

- Over the years, bovine and porcine have been used as common source of collagen
- However, collagen from bovine and porcine poses the **risk of transmitting disease** such as spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE) and food and mouth disease (FMD) which limited their use.
- Besides, the usage of bovine and porcine collagen is related with **cultural and religious considerations** (for Muslims, Jews and Hindus).
- Jellyfish is a particularly attractive marine source for collagen for a variety of reasons.
- Some species of jellyfish have been used as traditional food and medicine suggesting good biocompatibility of jellyfish material.
- Jellyfish has shown to be rich in minerals and proteins and collagen is a major protein in jellyfish.



METHODOLOGY

i) DESALTING THE JELLYFISH

- Wash the salted jellyfish with clean water for more than 5 minutes
- Change into another bucket of clean water and soak for 1h. Repeat for 5 times.
- Soak in clean water overnight (24h)
- Filter the jellyfish, cut into small pieces and kept at 4 °C



ii) ACID SOLUBLE COLLAGEN (ASC)

Soaked and stirred the jellyfish in 0.1 M NaOH for 48 hours (1:10 w/v) at 4 °C. The medium was changed once a day. Washed the residue with distilled water until pH 7.



0.5 M acetic acid was added into the residue and crashed into smaller pieces (1:10 w/v) (around 5 min) by using homogenizer. Soaked the mixture for 72 hours at 4 °C.



Filter the mixture by using cheesecloth.



Solid NaCl salt was added into the collagen extract until 0.9 M concentration achieved and stirred overnight at 4 °C.



The medium was centrifuged for 4, 000 g for 30 min at 4 °C and remove the salt solution and collect the collagen.



The collagen was redissolved with 0.5 M acetic acid (1:10 w/v), stirred for 1 hour and centrifuged for 4, 000 g, 30 min, 4 °C, remove the solution and collect the collagen. Repeat all steps with 0.1M and distilled water.

RESULTS & DISCUSSION

i) STRUCTURAL ANALYSIS

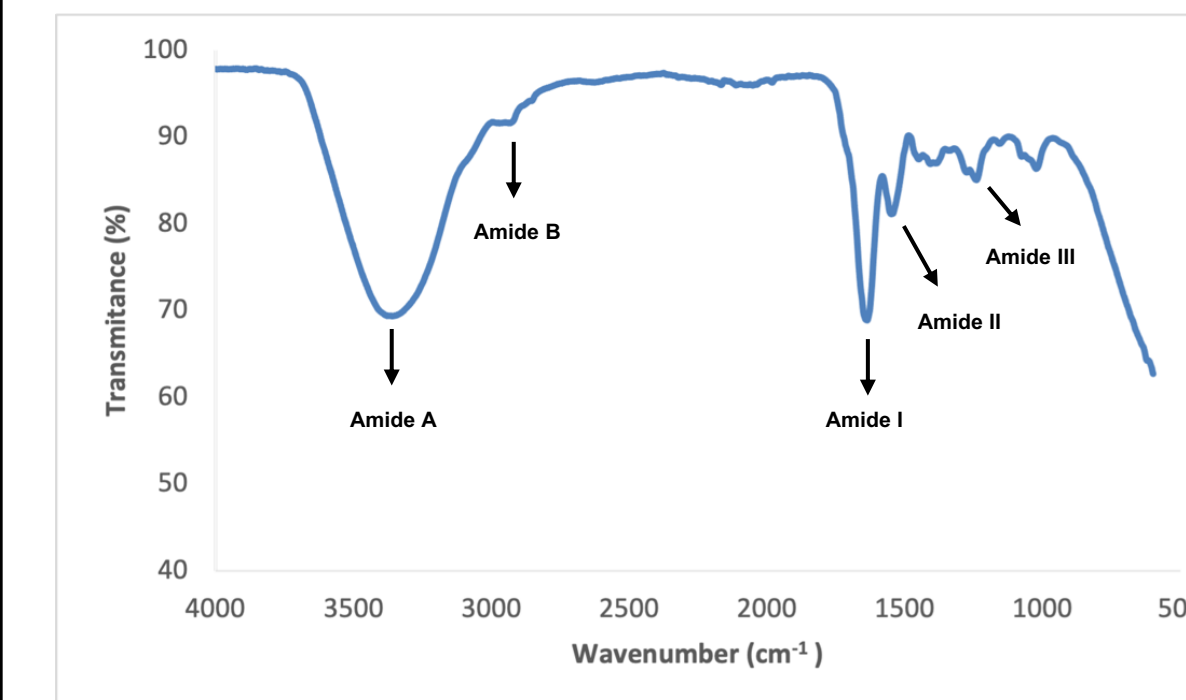


Figure 1 The FTIR spectra of collagen jellyfish

	Wavenumber (cm ⁻¹)	Assignments
Amide A	3375	N-H stretching
Amide B	2951	CH ₂ and NH ₃ ⁺
Amide I	1647	C=O stretching, N=H bending
Amide II	1557	N-H bending, C-N stretching
Amide III	1251	C-N stretching, N-H deformation

- There are five important bands observed in collagen spectra which are Amide A, Amide B, Amide I, Amide II and Amide III.
- Based on the spectra, it was confirmed the structural of collagen from jellyfish.

ii) ZETA POTENTIAL

- A sharp decreased of zeta potential of collagen was observed from pH 2 until it reached at pH 9
- This suggested that collagen jellyfish is not stable in alkaline solution.

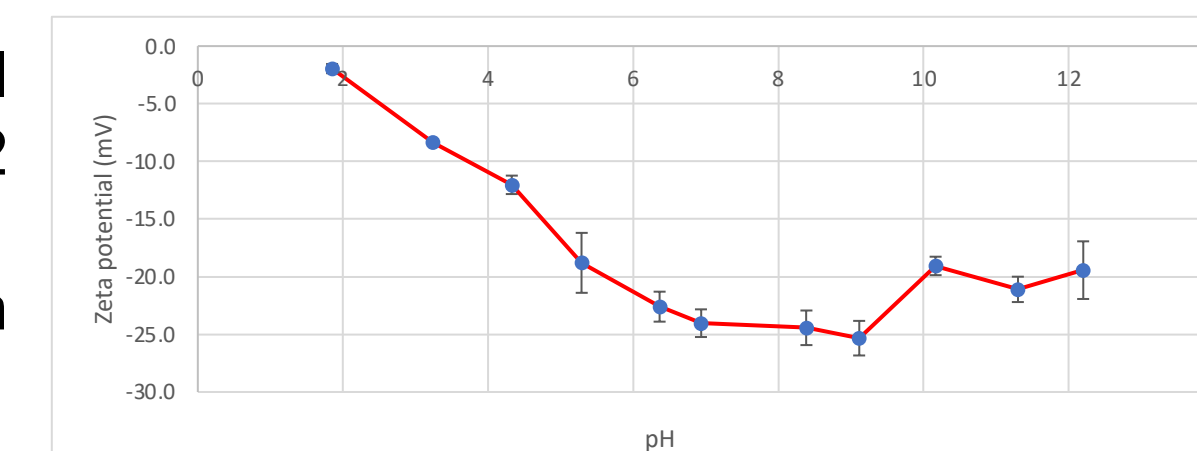


Figure 2 The Zeta potential of collagen jellyfish

iii) SDS-PAGE ANALYSIS

- Collagen jellyfish consisted of α chain (α 1) and β chains (with molecular mass values of >240 and ~140 kDa, respectively, which referred to type II collagen

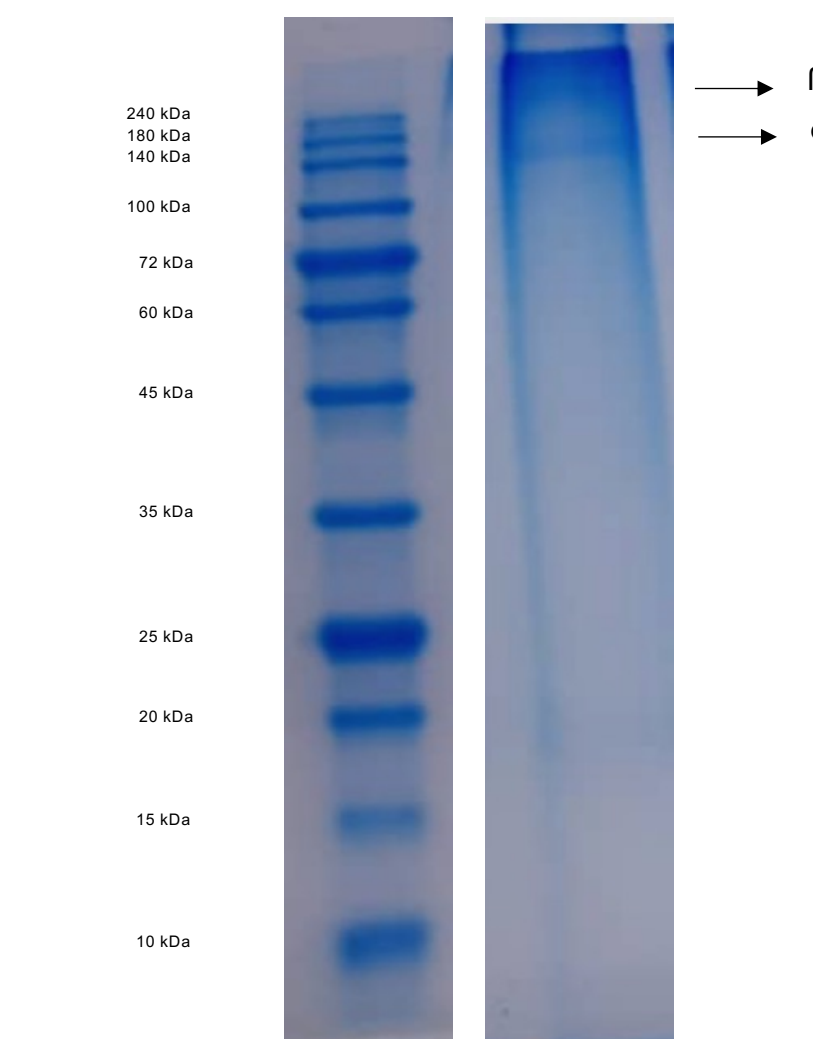


Figure 3 The SDS-PAGE analysis of collagen jellyfish

iv) AMINO ACID PROFILING

Amino acid compound	Compound content (%)	Amino acid compound	Compound content (%)
Hydroxyproline	5.966	Proline	7.337
Aspartic Acid	9.989	Cysteine	2.199
Serine	3.473	Tyrosine	1.888
Glutamic Acid	13.787	Valine	3.814
Glycine	15.940	Methionine	1.657
Histidine	0.956	Lysine	5.409
Arginine	7.794	Isoleucine	3.035
Threonine	3.430	Leucine	4.077
Alanine	7.627	Phenylalanine	1.820
		Total amount amino acid	100.00

Table 1 The Amino Acid analysis of collagen jellyfish

- Glycine (Gly) was the major amino acid in jellyfish collagen with 15/100 residues. This result is in the good agreement with the basic structure of collagen which consists of Gly-X-Y amino acid model in which Gly is the highest content of amino acid in collagen jellyfish.

v) THERMAL ANALYSIS

- The thermal stability of jellyfish collagen, $T_{max} = 32.11$ °C, $\Delta H = 34.25$ J/g
- The collagen from jellyfish undergoes second at third decomposition at 179.4 °C and 242.08 °C, respectively.

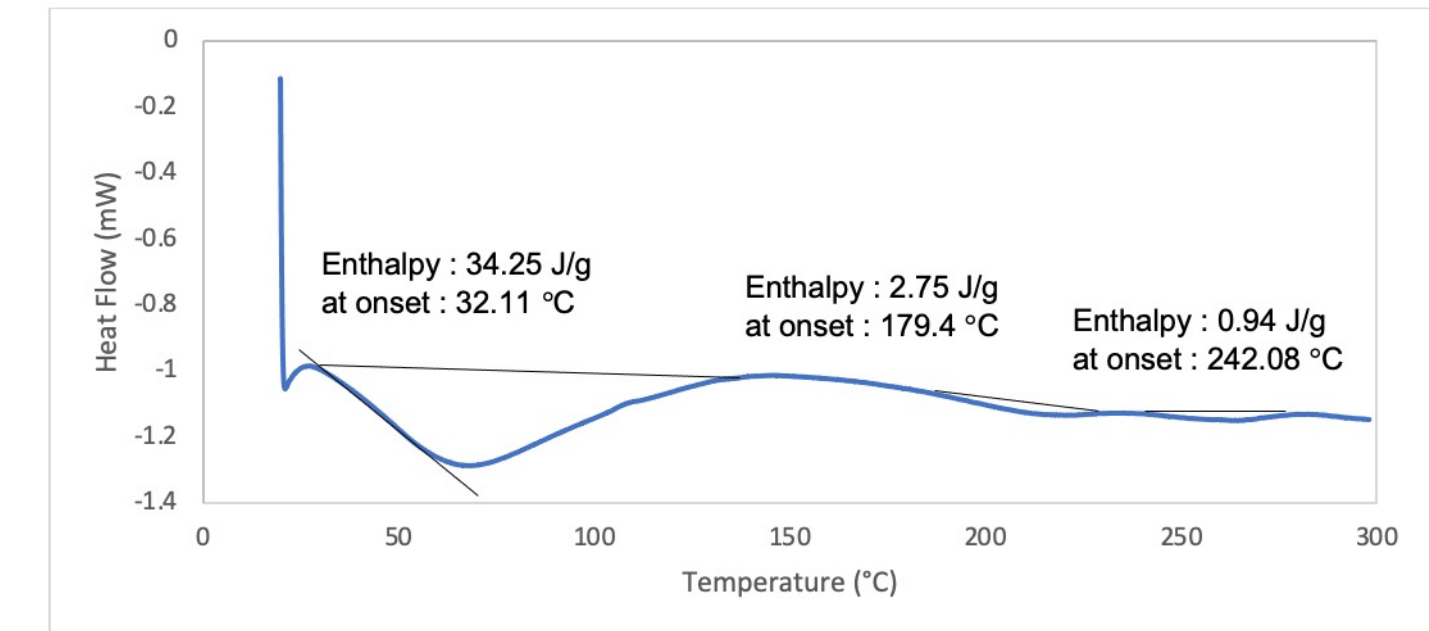


Figure 4 DSC thermogram of collagen jellyfish

CONCLUSION

- Acid Soluble Collagen (ASC)** was successfully **extracted** from commercial jellyfish with a maximum **yield** of **20%** (based on wet weight).
- The structure of the jellyfish collagen shows five important band of collagen which are Amide A, Amide B, Amide I, Amide II and Amide III.
- The zeta potential of the jellyfish collagen was stable up to pH 9 and after that, the collagen starts to decompose
- The transition temperature (T_{max}) of jellyfish collagen was higher than that of some other marine sources of collagen
- The amino acid composition of jellyfish collagen was identical with standard type II collagen from chicken feet
- The findings indicated that this jellyfish species may be a valuable source of type II collagen that can be used as an alternative to land-based sources.

FUTURE WORK

- Extraction of jellyfish collagen by using enzyme soluble collagen to produce collagen peptide for better solubility
- Characterizations: solubility, color, odor, moisture, ash, fat and protein
- Antioxidant and antimicrobial test

ACKNOWLEDGEMENT

- Institute of Bioproduct Development, School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia
- MM Cosmetic Sdn. Bhd. under research contract grant (R.J130000.7651.4C559)

