VALORIZATION OF MALABAR GROUPER *Epinephelus malabaricus* SKIN WASTE: PHYSICOCHEMICAL CHARACTERIZATION OF GELATIN AND ACE INHIBITORY ACTIVITY OF ITS PEPTIDES

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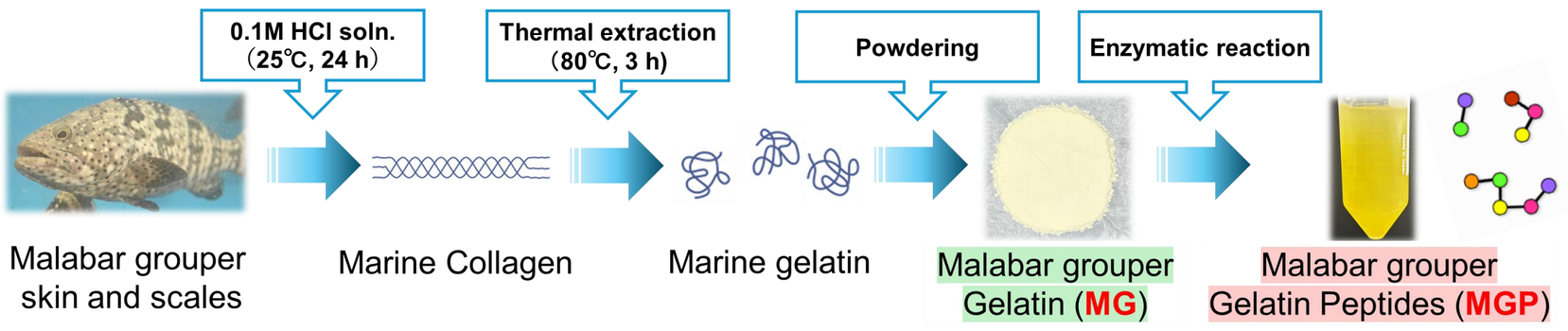
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Introduction

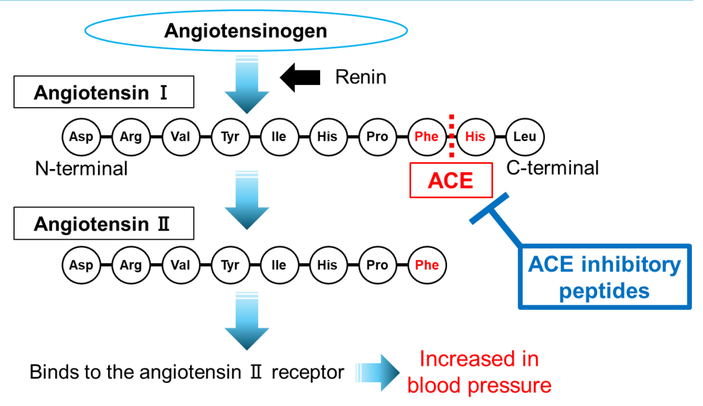
In recent years, the expansion of aquaculture production has led to the generation of large amounts of fish processing by-products, such as fish skin and scales. Although these underutilized resources are rich in collagen and gelatin therefore represent valuable biomaterials, most of them are discarded. Effective utilization of these resources is expected to contribute to sustainable aquaculture and the development of high-value functional materials.

Marine gelatin has attracted attention as an alternative to mammalian gelatin because it involves fewer religious restrictions and possesses potential physiological functions. In particular, **peptides** with Angiotensin I-converting enzyme (ACE) inhibitory activity are expected to be useful as functional food ingredients due to their antihypertensive effects.

▼ Methods for Extracting Marine Gelatin



▼ Renin-Angiotensin System(RAS)



In this study, **the physicochemical properties of gelatin** extracted from discarded fish skin and scales of cultured Malabar grouper (*Epinephelus malabaricus*) produced in Okinawa, Japan, were evaluated.

In addition, **the ACE inhibitory activity of its enzymatic hydrolysates** was investigated.

Results and Discussion

1-1. Structural and Functional Properties of MG

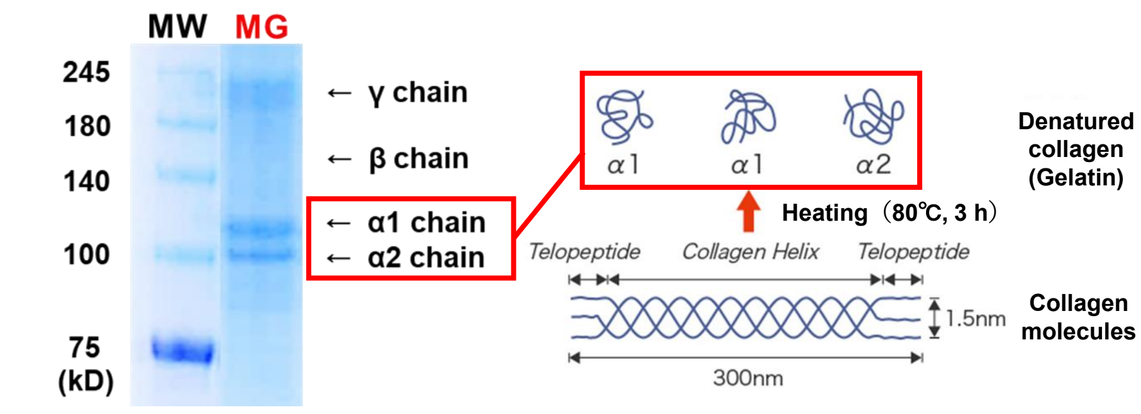


Fig 1. SDS-PAGE Profile of MG

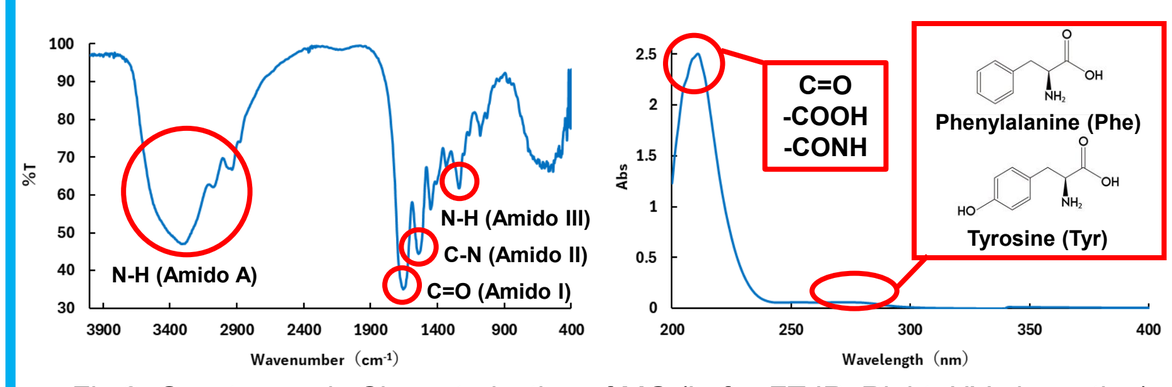


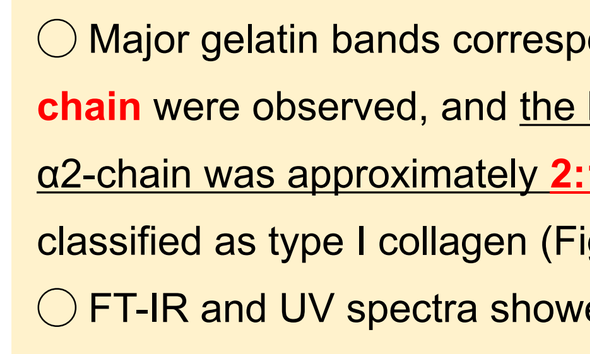
Fig 2. Spectroscopic Characterization of MG (Left：FT-IR, Right: UV absorption)

○ Major gelatin bands corresponding to the **α-chain and β-chain** were observed, and the band ratio of the α1-chain to α2-chain was approximately **2:1**, indicating that MG was classified as type I collagen (Fig. 1).

○ FT-IR and UV spectra showed characteristic peptide bonds-derived structures and aromatic amino acid (Phe and Tyr) absorption, respectively (Fig. 2).

MG retained the characteristic structures derived from collagen.

1-2. Sensory Evaluation Milk Jelly Made with MG



Conditions

- **Panelists**: n = 20
- **Evaluation method**: 7-point relative scale
- **Control milk jelly**: Commercial pork gelatin used

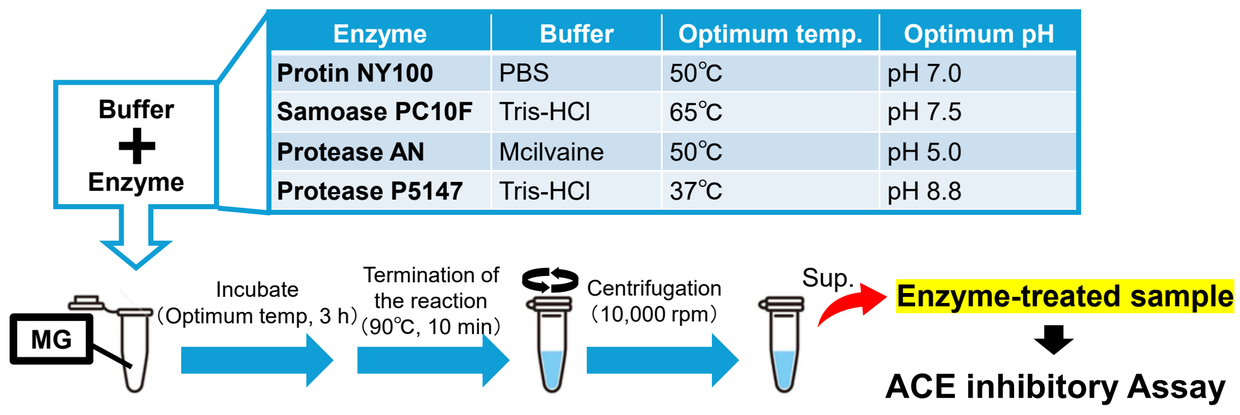
Fig 3. Sensory Characteristics of Milk Jelly

○ Milk Jelly exhibited a characteristic melting behavior specific to fish-derived gelatin and melted near oral temperature, resulting in smooth mouthfeel and strong milk flavor and sweetness（Fig. 3）.

MG may serve as a functional food material with properties distinct from mammalian gelatin.

2-1. Preparation of MGP

Enzyme	Buffer	Optimum temp.	Optimum pH
Protin NY100	PBS	50℃	pH 7.0
Samoase PC10F	Tris-HCl	65℃	pH 7.5
Protease AN	Mcilvaine	50℃	pH 5.0
Protease P5147	Tris-HCl	37℃	pH 8.8



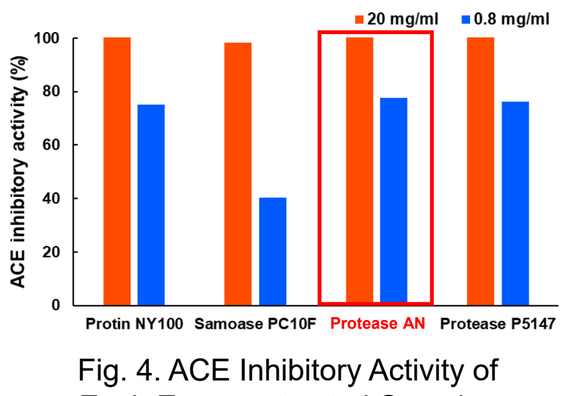


Fig. 4. ACE Inhibitory Activity of Each Enzyme-treated Samples

○ High ACE inhibitory activity was observed for all enzymes except Samoase PC10F.

○ **Protease AN** was considered suitable for generating short-chain peptides favorable for ACE inhibition.

Differences in substrate specificity among four types of enzymes likely affected peptide structures and ACE inhibitory activity.

2-2. Fractionation of MGP

Enzyme-treated sample



Econo-Column® (**DIAION HP20**)
1.5 × 20 cm
（Mitsubishi Chemical Co., Ltd.）

※HP20：Synthetic adsorbent resin

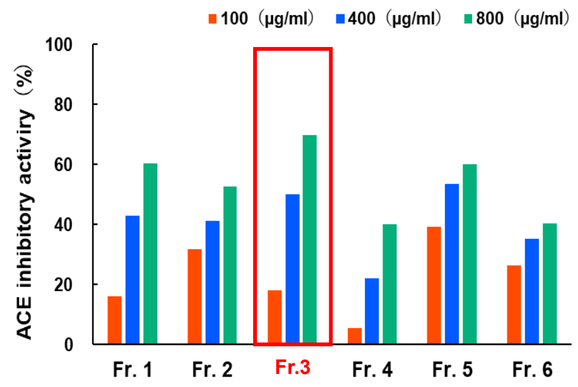
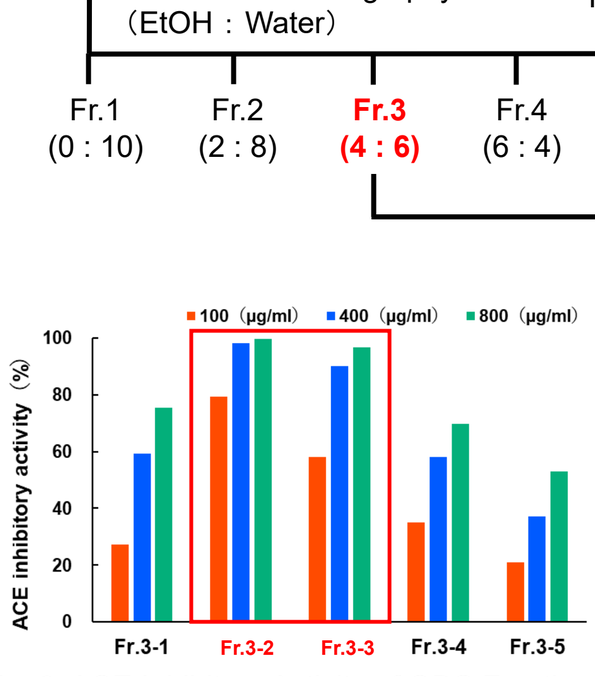


Fig. 5. ACE Inhibitory Activity of HP20 Fractions

Column chromatography
（EtOH：Water）

Fr.1	Fr.2	Fr.3	Fr.4	Fr.5	Fr.6
(0：10)	(2：8)	**(4：6)**	(6：4)	(8：2)	(10：0)





UNIVERSAL™ (**ODS**)
3.0 × 16.5 cm
（Yamazen Co., Ltd.）

Column chromatography
（EtOH：Water）

Fr.3-1	Fr.3-2	Fr.3-3	Fr.3-4	Fr.3-5
(0：10)	**(2：8)**	**(4：6)**	(6：4)	(10：0)

Fig. 6. ACE Inhibitory Activity of ODS Fractions

○ Further ODS fractionation of HP20 Fr.3 increased ACE inhibitory activity by approximately 2-3 fold (Fr.3-2 and Fr.3-3).

Further purification and identification of ACE inhibitory peptides will be performed using HPLC and Orbitrap LC-MS/MS.