

Processing Strategies to Improve Animal Protein Digestibility for Age-Specific Customization

정 사 무 엘

Jung, Samooel

(충남대학교)

(Chungnam National University)

Curriculum Vitae

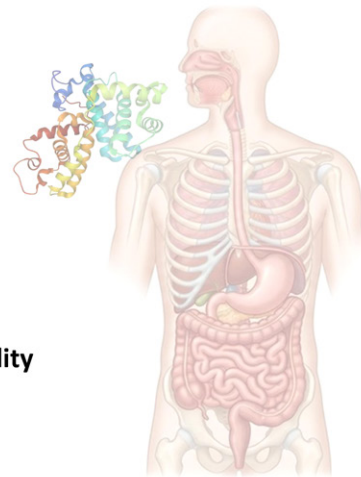
- ▶ 2014~현재 충남대학교 동물자원생명과학과 교수
- ▶ 2013~2014 충남대학교, 서울대학교 박사후 연구원
- ▶ 2010~2013 충남대학교 축산학과 농학박사
- ▶ 2008~2010 충남대학교 축산학과 농학석사
- ▶ 2001~2008 충남대학교 동물자원생명과학과 농학사

Processing Strategies to Improve Animal Protein Digestibility for Age-Specific Customization

SAMOOEL JUNG
CHUNGNAM NATIONAL UNIVERSITY

Contents

1. Quality of Animal Food as an Ideal Protein Source
2. Physiological Barriers in Protein Digestion
3. Molecular Mechanisms: Denaturation & Oxidation
4. Clinical Implications of Infants & the Elderly
5. Advanced Processing Strategies for Improving Digestibility
6. Conclusion & Future Outlook

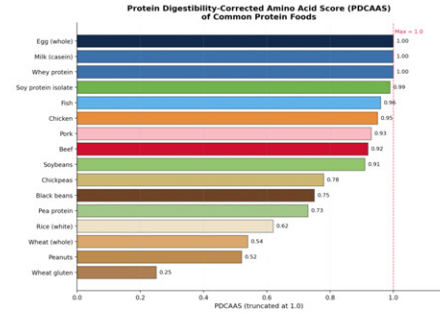


Nutritional Advantages of Meat & Eggs

- **High quality, complete protein**
 - All essential amino acid & high BCAA (leucine, isoleucine, valine) contents
 - High bioavailability with high digestibility

- **Highly bioavailable micronutrients**
 - Meat: heme iron, zinc, Vit. B12
 - Eggs: choline, Vit. D, Vit. A

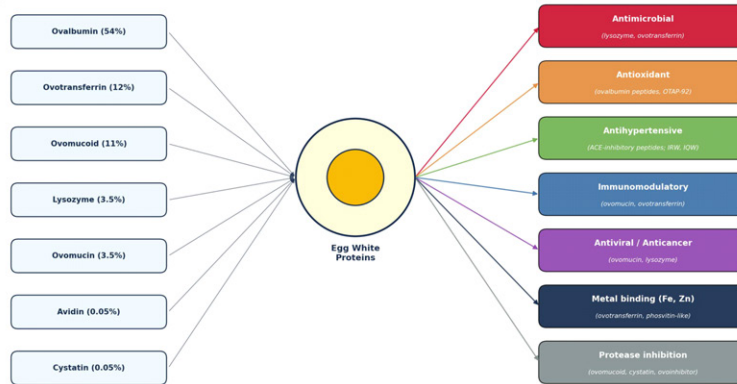
- **Bioactive peptides and non-protein nitrogen compounds**
 - Various peptides from protein digestion
 - Carnosine, anserine, creatine, taurine, and L-carnitine



Nutritional Advantages of Meat & Eggs

Bioactivity of egg white proteins

- Ovalbumin
- Ovotransferrin
- Ovomuroid
- Lysozyme
- Ovomucin
- Avidin
- Cystatin



Native proteins → GI digestion / enzymatic hydrolysis → Bioactive peptides → Systemic health effects

Infant Gastrointestinal Track: Immature Physiology

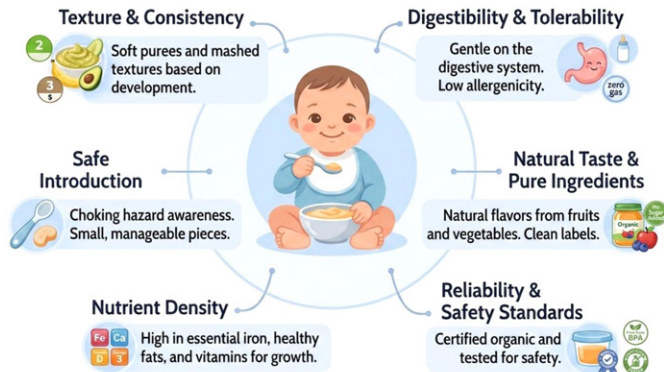
Digestive Physiology in the Infant

- Gastric Acid Secretion ↓**
Reduced initial protein denaturation
- Pepsin Activity ↓**
Slower cleavage of large proteins
Less complete protein pre-digestion
- Gastric Emptying ↑**
Faster movement to small intestine, reduced breakdown time
- Pancreatic Enzyme Secretion ↓**
Lower levels of trypsin, chymotrypsin, and other proteases
- Intestinal Brush Border Activity ↓**
Lower disaccharidase activity and other enzyme processes

Result:

- ⚠ Overall protein digestibility reduced in immature GI tract.
- ⚠ Requires tailored food design and optimized processing for absorption.

Infant-Friendly Food Requirements



Senescent Gastrointestinal Track: Age-related Decline

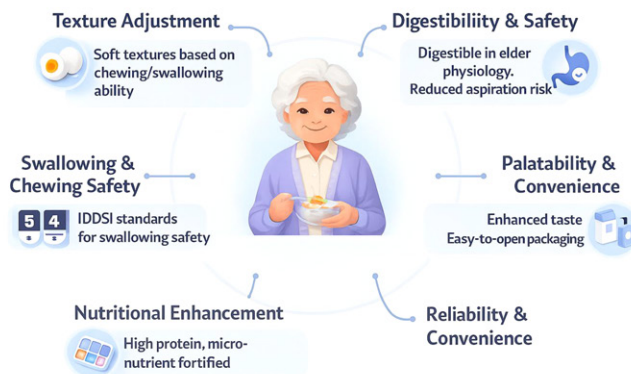
Digestive Physiology in the Elderly

- Gastric Acid Secretion ↓**
Reduced protein denaturation
- Pepsin Activity ↓**
Lower cleavage of large proteins
More undigested proteins entering intestine
- Gastric Emptying ↓**
Slower movement to small intestine
- Enzyme Secretion ↓**
(trypsin, chymotrypsin, pancreatic enzymes)
- Intestinal Absorption Efficiency ↓**
Lower amino acid uptake

Result:

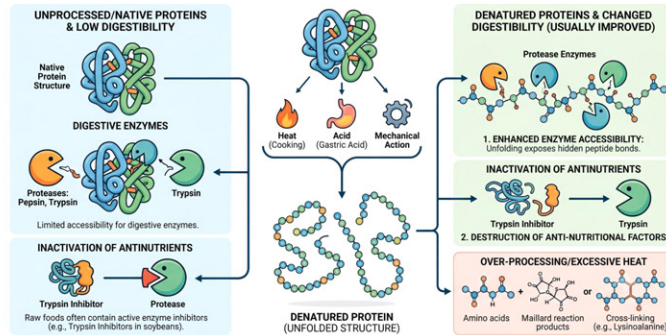
- ⚠ Overall protein digestibility significantly reduced
- ⚠ Risk of sarcopenia ↑, immune decline

Elderly-Friendly Food Requirements



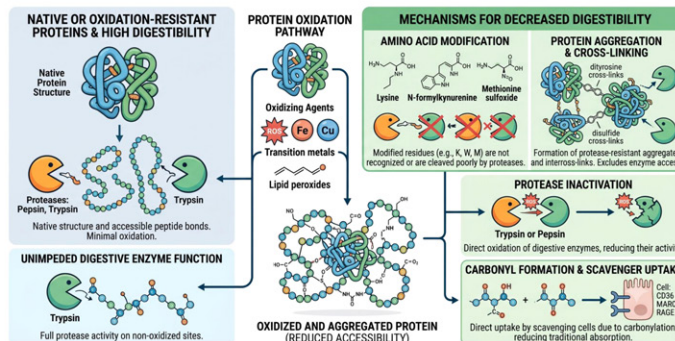
Double-Edged Sword of Protein Denaturation

- **Protein digestibility increased**
 - Increase in protease accessibility by exposing buried cleaving sites with unfolding
 - Inactivation of protease inhibitors
- **Protein digestibility decreased**
 - Decreased in protease accessibility by aggregations (physical or non-covalent clumping) of proteins
 - Unrecognition of protease by chemical side reactions (covalent cross-linking or Maillard reaction) of proteins

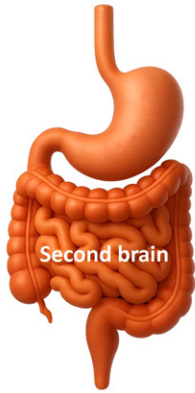


Detrimental Impact of Protein Oxidation

- **Structural collapse and aggregation**
 - Formation of protein-carbonyl compounds by chemical oxidation
- **Steric hindrance**
 - Oxidized proteins to re-collapse into highly compact, rigid polymers
 - Blocking protease access to binding pockets



Consequences of Poor Proteolysis in Infants



- **Growth faltering and nutritional deficiencies**
 - Amino acid deficiencies: Decrease in protein synthesis
 - Stunted growth: Delayed physical growth, poor weigh gain

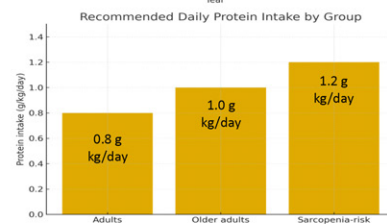
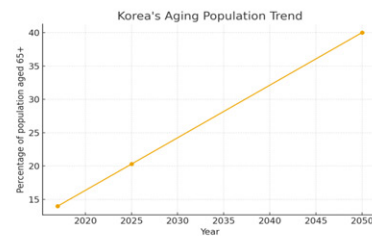
- **Gastrointestinal distress and discomfort**
 - Fermentation and gas: Fermentation of undigested protein in the large intestine (severe abdominal discomfort with the production of excess gas)
 - Altered stool patterns: Leading to diarrhea or constipation by causing osmotic imbalances

Consequences of Poor Proteolysis in the Elderly

- **Acceleration of sarcopenia and frailty**
 - Blunted muscle synthesis: Low absorption of essential amino acids
 - Increased frailty: Decrease in muscle mass

- **Impaired immune function and wound healing**
 - Delayed healing: Decrease in repairing skin barrier matrices
 - Higher infection risk: Low cellular immunity

- **Colonic fermentation and gut discomfort**
 - Toxic metabolites generated in the large intestine

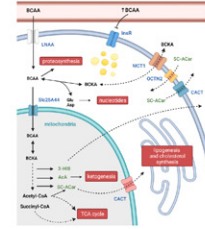


Advanced Processing Strategies for Improving Digestibility

Materials and Methods

Samples

- Beef for infant complementary foods
- Egg white for elderly-friendly foods



Static in-vitro digestion model

- INFOGEST protocol: young adult, infants, the elderly
- Digestibility: < 3 kDa via cut-off filter

nutrac protocols **PROTOCOL**

INFOGEST static in vitro simulation of gastrointestinal food digestion

Andri Brodbeck¹, Lutz Egger², Marie Abminger³, Paula Aho⁴, Ricardo Assunção⁵, Sonja Ballmann⁶, Tapani Böhler⁷, Claire Bruchler-Lacand⁸, Rachel Brunsen⁹, Frédéric Carreau¹⁰, Alfonso Clemente¹¹, Milena Corredig¹², Didier Dupont¹³, Claire Dufour¹⁴, Catherine Edwards¹⁵, Matt Goding¹⁶, Sajeel Karkhanavala¹⁷, Boris Kricheldorf¹⁸, Steven La Feunteun¹⁹, Ulf Lorenzen²⁰, Adam Macfarlane²¹, Alan H. Mackie²², Carla Martinez²³, Sebastian Merra²⁴, David Julian McClements²⁵, Christa Muehle²⁶, Maria Mäkelä²⁷, Risto Partanen²⁸, Claudio N. Sant'Ana^{29,30}, Isabelle Souchoir³¹, B. Paul Singh³², Gergely Varga³³, Martin S. J. Wickham³⁴, Werner Watzusch³⁵ and Isabella Beecher³⁶

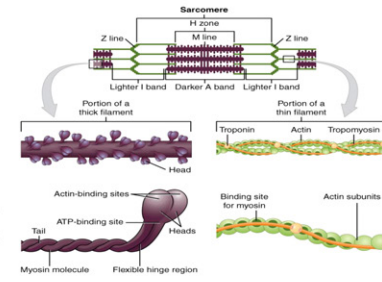
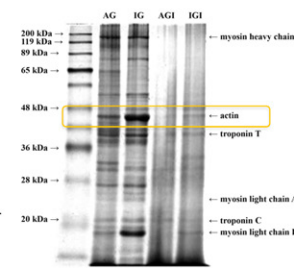
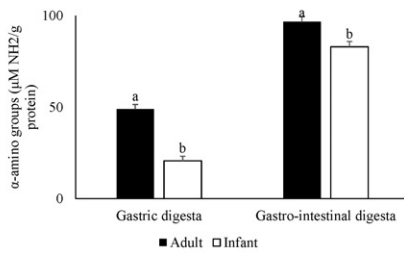
Developing a mechanistic understanding of the impact of food structure and composition on human health has increasingly become a challenge. In the upper gastrointestinal tract, these components have to be broken down into different conditions that often have very little physiological relevance, and this involves the meaningful combination of results. The standardized protocol presented here is based on an international consensus developed by the INFOGEST network. The method is designed to be used with standard laboratory equipment and requires limited resources to reproduce a wide range of researchers to adopt it. It is a static digestion method that uses constant rates and time to digest foods and is consistent for each step of digestion. This makes the method simple to use but not suitable for assessing digestion kinetics. Using this method, food samples are subjected to sequential and sequential digestion with parameters such as substrate, enzymes, time, substrate pH and time of digestion are based on available physiological data. This standardised and detailed digestion method (INFOGEST 2.0) is available for download with the original method, such as the inclusion of the oral phase and the use of gastric juice. The method can be used to assess the indicators resulting from digestion of foods by analyzing the digestion products (e.g., peptides/amino acids, fatty acids, simple sugars) and evaluating the release of micronutrients from the food matrix. The whole protocol can be completed in 7-8, including 5 if required for the determination of enzyme activities.

Step	Protocol
1	Perform enzyme activity and bile assays
2	Prepare SSF and SSF stock solutions
4	Perform pH test adjustment experiment
7-12	Mix Food with SSF (1:1, wetwt)
13	Include CaCl ₂ (1.5 mM in SSF)
14	Add salivary amylase, if necessary (75 U/mL)
15, 16	Inoculate whole mung (2 min, 37 °C, pH 7)
17, 18	Mix oral bolus with SSF (1:1 (wetwt))
19	Include CaCl ₂ (0.15 mM in SSF)
20, 21	Add pepsin, gastric lipase (2,000, 60 U/mL)
22-24	Inoculate whole mung (2 h, 37 °C, pH 3.0)
25, 26	Mix gastric chyme with SSF (1:1 (wetwt))
27	Include bile (10 mM bile salts)
28	Include CaCl ₂ (0.6 mM in SSF)
29	Add pancreatin (trypan activity 100 U/mL)
30-32	Inoculate whole mung (2 h, 37 °C, pH 7.0)

Sampling procedure and sample treatment (Table 1)

Advanced Processing Strategies for Improving Digestibility

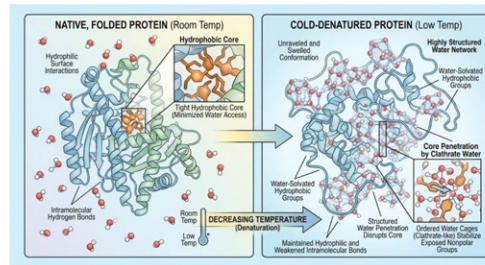
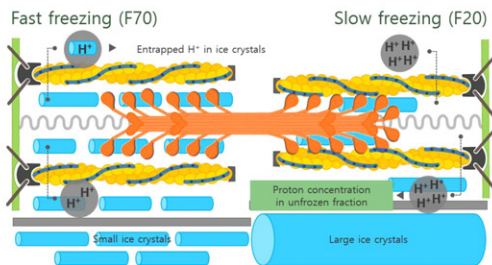
Beef Protein Digestion: Adult vs. Infant



Results

- Beef protein digestibility was lower in both the gastric and gastrointestinal phases of the infant simulated digestion model than in the adult model.
- Actin is the main protein with **digestive resistance** because the trypsin or chymotrypsin can not cleave **F-actin** since the cleaving sites are buried by the actin-actin interaction (Hozumi, The Journal of Biochemistry 104, 285).

Beef Protein Digestion: Freezing-then-aging

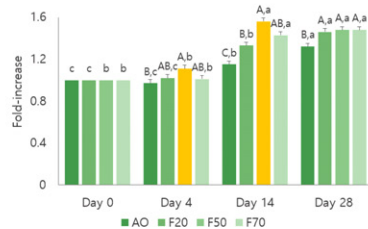


Hypothesis

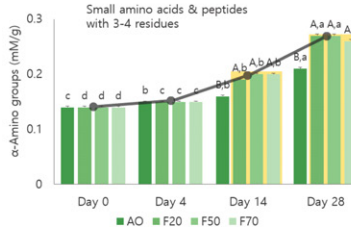
- During the freezing process, the ice crystal formation results in the physical damage of the lysosome and release of cathepsin. Cold denaturation of muscle protein may change the behavior of protein digestibility.
- During the aging of beef, the degradation of muscle protein may improve the digestibility.

Beef Protein Digestion: Freezing-then-aging

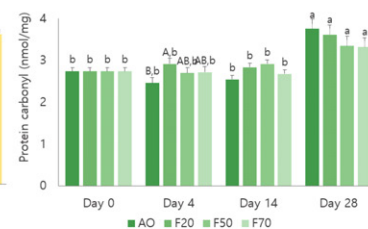
Cathepsin B activity



α-Amino group content



Protein oxidation

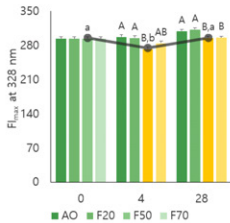
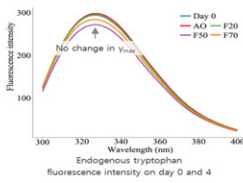


Results

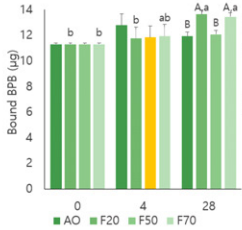
- Cathepsin B activity was increased in the frozen-thawed beef, especially in F50 at day 14.
- Degradation of muscle proteins was increased in the frozen-thawed beef.
- Protein oxidation was increased after the 28-days aging process. However, no significant effect of freezing on the protein oxidation was found.

Beef Protein Digestion: Freezing-then-aging

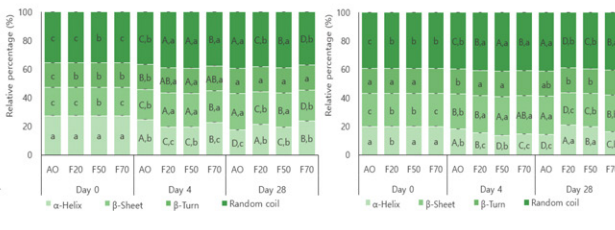
Fluorescence intensity



Surface hydrophobicity



Secondary structure of myosin and actin fractions

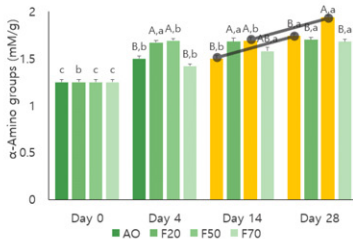


Results

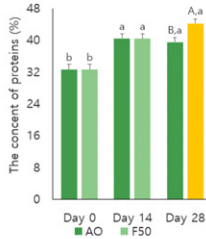
- Fluorescence intensity of F50 was low at day 14 and 28. However, the surface hydrophobicity showed no consistent results.
- In myosin and actin, denaturation and renaturation properties were observed on day 14 and 28, respectively, in frozen-thawed beef except for F50 myosin.

Beef Protein Digestion: Freezing-then-aging

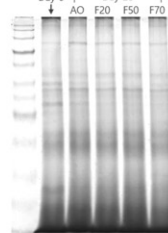
α-Amino groups of digesta



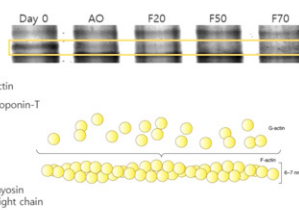
Proteins <3 kDa



SDS-PAGE



Actin band

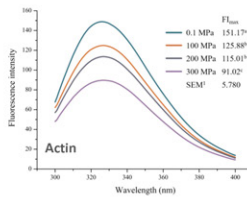
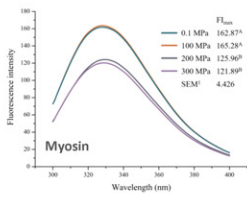


Results

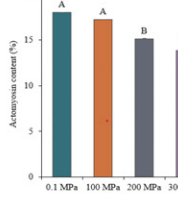
- After aging, the **actin** band disappeared in the freezing-then-aging beef.
- Aging and the pre-freezing process resulted in an increase in beef protein digestibility.
- F50 (frozen at -50°C) with 28 days of aging showed the highest protein digestibility.

Beef Protein Digestion: High Hydrostatic Pressure

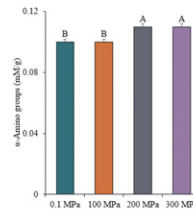
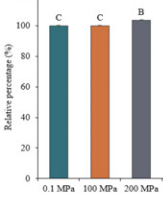
Fluorescence intensity



Beef Properties



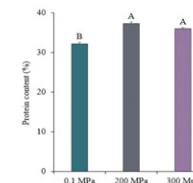
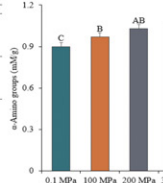
Beef Properties



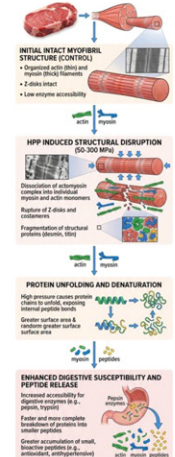
Secondary structure

Treatment	α-Helix	β-Sheet	β-Turn	Random coil
Myosin	0.130 ^a	14.08 ^a	17.72 ^a	64.07 ^a
100 MPa	14.08 ^a	17.72 ^a	15.91 ^a	52.82 ^a
200 MPa	12.36 ^a	24.21 ^a	15.90 ^a	47.53 ^a
300 MPa	12.36 ^a	39.03 ^a	15.80 ^a	32.19 ^b
SEM ^c	0.211	0.377	0.080	0.176
Actin	0.130 ^a	12.50 ^a	40.17 ^a	47.19 ^a
100 MPa	11.24 ^a	40.90 ^a	15.59	31.91 ^a
200 MPa	10.53 ^a	41.43 ^a	15.57	31.24 ^a
300 MPa	10.20 ^a	41.85 ^a	15.59	30.36 ^a
SEM ^c	0.209	0.421	0.083	0.141

Protein digestibility

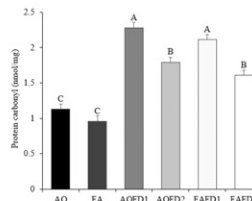


PHYSIOLOGICAL FRAGMENTATION OF MYOFIBRILS IN RAW MEAT BY HIGH-PRESSURE PROCESSING (50-300 MPa)

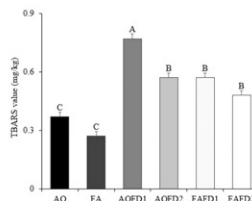


Beef Protein Digestion: Freeze-drying with room or low temperature

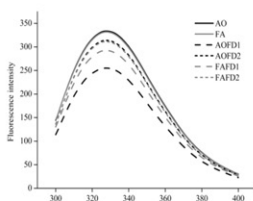
Protein oxidation



Lipid oxidation



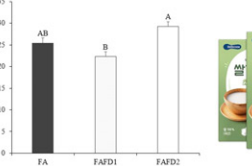
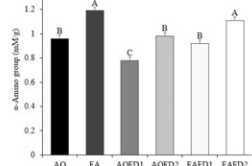
Fluorescence intensity



Secondary structure

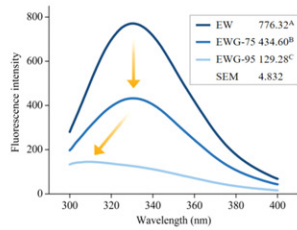
Treatment	α-Helix	β-Sheet	β-Turn	Random coil
Myosin	AO 17.15 ^c	24.67 ^a	17.54	40.60 ^a
FA	21.83 ^b	22.21 ^b	17.40	38.55 ^b
AOFD1	17.67 ^c	24.74 ^a	17.77	39.82 ^a
AOFD2	21.09 ^b	22.71 ^b	17.45	38.76 ^b
FAFD1	22.38 ^b	22.29 ^b	17.44	37.89 ^b
FAFD2	25.55 ^a	20.66 ^c	17.28	36.51 ^c
SEM ^c	0.335	0.205	0.110	0.197
Actin	AO 13.40 ^b	27.33 ^b	17.41 ^{ab}	41.86 ^a
FA	15.04 ^a	26.34 ^b	17.54 ^{ab}	41.08 ^{ab}
AOFD1	12.23 ^b	28.60 ^a	17.47 ^{ab}	41.74 ^a
AOFD2	13.60 ^b	27.57 ^b	17.52 ^{ab}	41.30 ^{ab}
FAFD1	12.88 ^c	27.76 ^c	17.79 ^a	41.57 ^{ab}
FAFD2	15.50 ^a	26.36 ^b	17.56 ^{ab}	40.58 ^b
SEM ^c	0.101	0.169	0.064	0.235

Protein digestibility

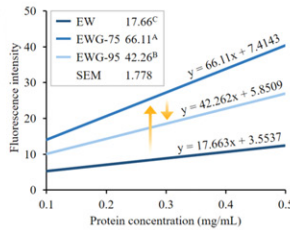


Egg White Protein Digestion: Different heating temperature

Fluorescence intensity



Surface hydrophobicity



Secondary structural components

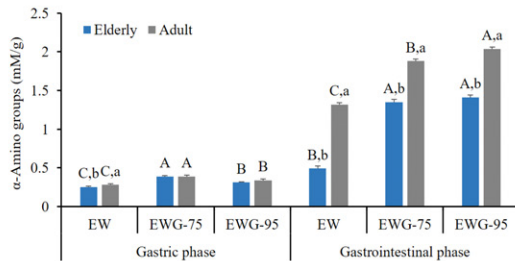
Treatment	α-Helix	β-Sheet	β-Turn	Random coil
EW	41.81 ^A	14.11 ^B	17.60 ^B	26.49 ^A
EWG-75	42.15 ^A	13.13 ^B	16.93 ^C	27.80 ^A
EWG-95	30.57 ^B	25.85 ^A	19.53 ^A	24.05 ^B
SEM	0.870	0.681	0.127	0.633

Results

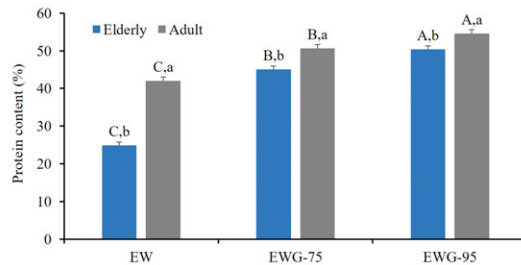
- EWG-95 showed the lowest fluorescence intensity with a blue shift.
- The surface hydrophobicity was increased in EWG-75 and 95 compared to EW. However, EWG-95 showed lower surface hydrophobicity than EWG-75.
- EWG-95 consisted of lower α-helix and higher β-sheet content compared to EW and EWG-75.

Egg White Protein Digestion: Different heating temperature

α-Amino groups of digesta



Contents of protein < 3 kDa

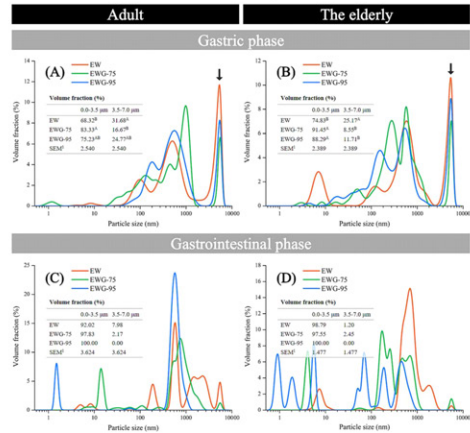


Results

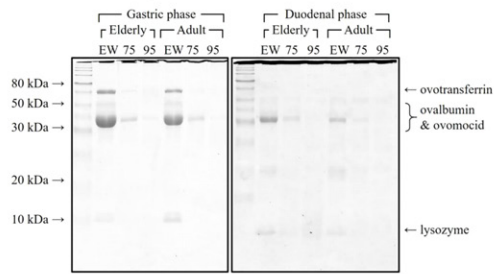
- Amino group contents in the gastrointestinal phase were higher in the adult model than in the elderly model.
- Protein digestibility increased in EWG compared to EW.
- EWG-95 had higher protein digestibility than EWG-75.

Egg White Protein Digestion: Different heating temperature

Particle size distribution of digesta



SDS-PAGE of digesta



Results

- Disruption of native EWPs including protease inhibitor, may play a more significant role in enhancing protein digestibility despite severe aggregation.

Conclusion & Future Outlook

Conclusion

- Animal food proteins (meat and eggs) possess excellent initial nutritional profiles, but their dense, native macromolecular networks limit actual bioaccessibility in underdeveloped or declining gastrointestinal tracts.
- Optimizing structural processing conditions can significantly enhance *in vitro* protein digestibility and kinetics.
- Animal foods provide maximum nutritional value when custom-tailored to the physiological needs of specific target consumers.

Future Outlook

- *In vivo* translation and clinical dietary trials
- Comprehensive peptidomic mapping and bioactivity tracking
- Industrial scale-up and development of age-specific medical foods



Thank you

Appendix

List of Published Core Research (Our Publications)

- Low protein digestibility of beef puree in infant in vitro digestion model. 2019. Food Sci. Ani. Resour. 39:1000-1007.
- Improvement of meat protein digestibility in infants and the elderly. 2021. Food Chem. 356, 129707.
- Freezing-then-aging treatment improved the protein digestibility of beef in an in vitro infant digestion model. 2021. Food Chem. 350, 129224.
- Changes in beef protein digestibility in an in vitro infant digestion model with prefreezing temperatures and aging periods. 2023. Heliyon, 9, e15611.
- High-pressure processing of beef increased the in vitro protein digestibility in an infant digestion model. 2023. Meat Sci. 205, 109318.
- Characterization of peptides released from frozen-then-aged beef after digestion in an in vitro infant gastrointestinal model. 2024. Meat Sci. 212, 109468.
- Heat-induced gelation of egg white proteins depending on heating temperature: Insights into protein structure and digestive behaviors in the elderly in vitro digestion model. 2024. Int. J. Bio. Macromol. 262, 130053.