

OP-231 [Beekeeping Technology and Quality] Hydrolysis for Drone pupa (*Apis mellifera* L.) Utilization

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The drone pupa (*Apis mellifera* L.) was approved as an edible insect and food ingredient in South Korea in 2020, allowing it to be used in a variety of foods. Drone pupae are an edible insect resource rich in protein (approximately 42% dry matter) and unsaturated fatty acids. However, drone pupae have not been studied or manufactured into food compared to other edible insects. Hydrolysis is one of the effective methods to enhance the bioactivity of proteins. The present study was conducted to prepare drone pupa hydrolysate and analyze its antioxidant activity and effects on muscle cells. The drone pupae were freeze-dried and powdered, and the enzymes were hydrolyzed by mixing flavorzyme (FZ) and nutrase (NT) in proportion. The enzyme ratios were CON (no hydrolysis), T1 (FZ, 100), T2 (NT, 100), T3 (FZ:NT, 30:70), T4 (FZ:NT, 50:50), and T5 (FZ:NT, 30:70). The experiments were performed by SDS-page, hydrolysis, ABTS and DPPH radical scavenging activity, and added to muscle cells (C2C12) to determine the effects on differentiation and gene expression. The hydrolysis treatment increased the hydrolysis degree, as measured by the formation of more small molecular peptides on SDS-page. Both ABTS and DPPH radical scavenging activity indicated lower antioxidation in CON. leucine was significantly higher in the hydrolyzed treatment compared to CON, with the highest values in T3 and T4. The fusion index and myotube area, which are indicators of muscle maturity, were significantly higher in the drone pupa extract and hydrolysate. Also, gene expression levels showed a similar trend. Based on the above results, drone pupae promote myotube formation, and hydrolyzing drone pupae enhances their antioxidant activity. Drone pupa is being discarded as a byproduct of the beekeeping industry. Therefore, the results of this study are expected to be used as basic data to create a new source of income for the beekeeping industry.

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농촌진흥청
Rural Development
Administration



Contents



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- ✓ **Conclusion**



Bee products



Honey



Royal jelly



Bee venom



Drone pupa



Propolis



Bee pollen



Figure 1. Global drone pupa and larva products available in various countries

Nutrition

- Protein: 52%
- Fat: 26%
- Carbohydrate: 16%
- Mineral & Vitamin

Antioxidant

- Polyphenol
- Flavonoid

Safety

- Heavy metal
- Pathogenic microorganism

Enzymatic hydrolysis

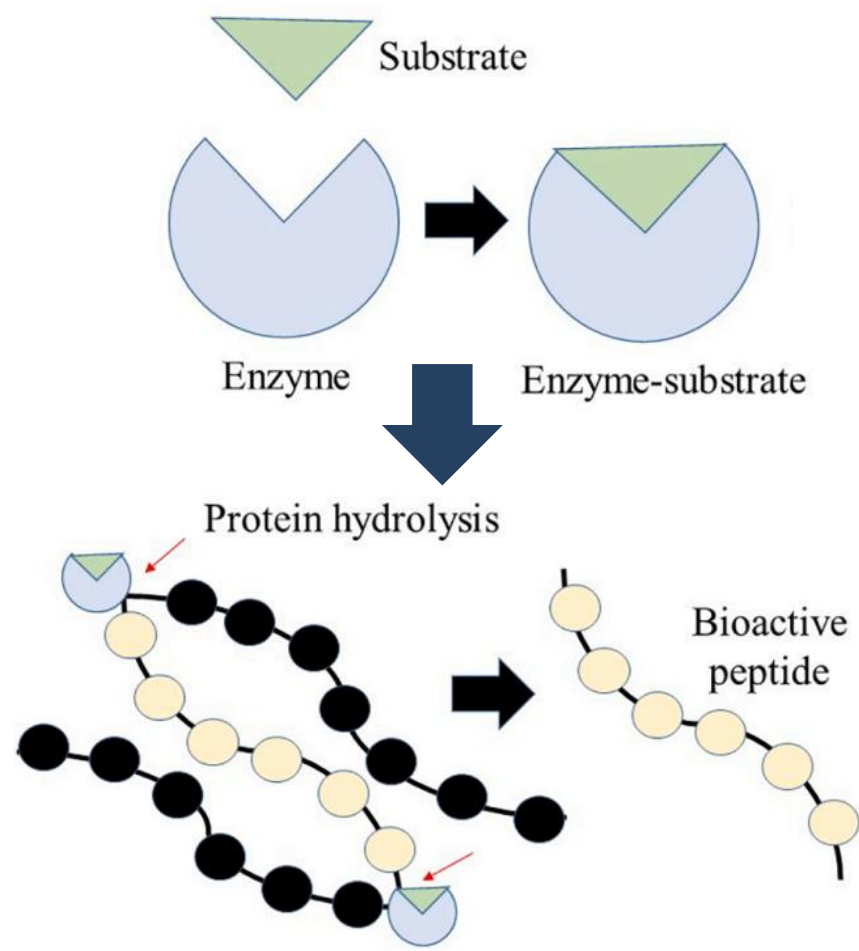


Figure 2. Graphic representation of the enzymatic hydrolysis process in the release of bioactive peptides (Cruz-Casas et al., 2021).

Table 1. The Bioactive peptides from various insects and their potential health benefits (Hasnan et al., 2023)

Insect Source	Enzyme Used	Bioactivity
Silkworm/domestic silk moth (<i>Bombyx mori</i>)	Pepsin, trypsin, R-chymotrypsin	ACE inhibitory effects, with 100% activity in hydrolysates
Cotton leafworm (<i>Spodoptera littoralis</i>)	Pepsin, trypsin and α -chymotrypsin	In vitro ACE inhibitory activity (IC50: 2123 μ M)
Mealworm larva (<i>Tenebrio molitor</i>)	Alcalase	ACE inhibitory activity (IC50: 0.017 mg/mL)
Silkworm pupae (<i>Bombyx mori</i>)	Alcalase, Prolyve, Flavourzyme, Brewers Clarex	Antioxidant activity (ROS reduction, superoxide dismutase (SOD) expression and glutathione (GSH) production activity)
Tropical house crickets (<i>Gryllodes sigillatus</i>)	Alcalase-generated protein hydrolysates	Anti-hypertensive, anti-glycemic, anti-inflammatory activities
Black soldier fly (<i>Hermetia Illucens</i>)	-----	Antimicrobial activity against various pathogens
Silkworm pupae (<i>Bombyx mori</i>)	Acidic protease, Neutral protease	Antioxidant activity, ABTS radical scavenging
Crickets (<i>Gryllus bimaculatus</i>)	Alcalase	Antioxidant activity
White-spotted flower chafer larva (<i>Protaetia brevitarsis</i>)	Flavourzyme	ACE inhibitory activity, NO production in cells
Brown seaweed (<i>Laminaria digitate</i>)	Hydrolysates, fermentation generated peptides or generated from high-pressure processing	ACE-1 inhibitory activity, Potential DPP-IV inhibition

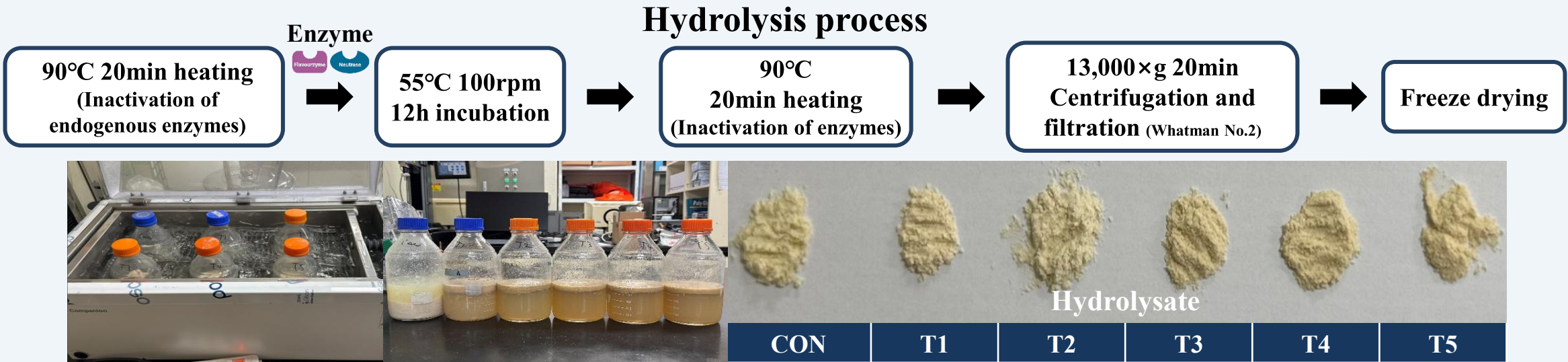
- Objective -

**In this study, we analyzed the antioxidant activity,
amino acid content, and functionality
of enzymatically hydrolyzed drone pupae.**

- Materials & Methods -

Table 2. Enzyme ratio for hydrolysis of drone pupae

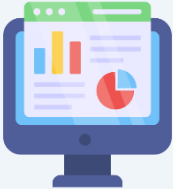
Enzyme (%)	CON	T1	T2	T3	T4	T5
Flavourzyme	-	-	100	30	50	70
Neutrase	-	100	-	70	50	30





Experiments

- SDS-PAGE
- DPPH/ABTS radical scavenging activity
- Free amino acid content
- C2C12 cell differentiation (IF, RT-qPCR)
- Degree of hydrolysis



Statistical analysis

- IBM SPSS Statics (28.0)
 - One-way ANOVA
 - Tukey’s test
- MetaboAnalyst 6.0
 - Partial least squares discriminant analysis (PLS-DA)

- Degree of hydrolysis & Antioxidant activity

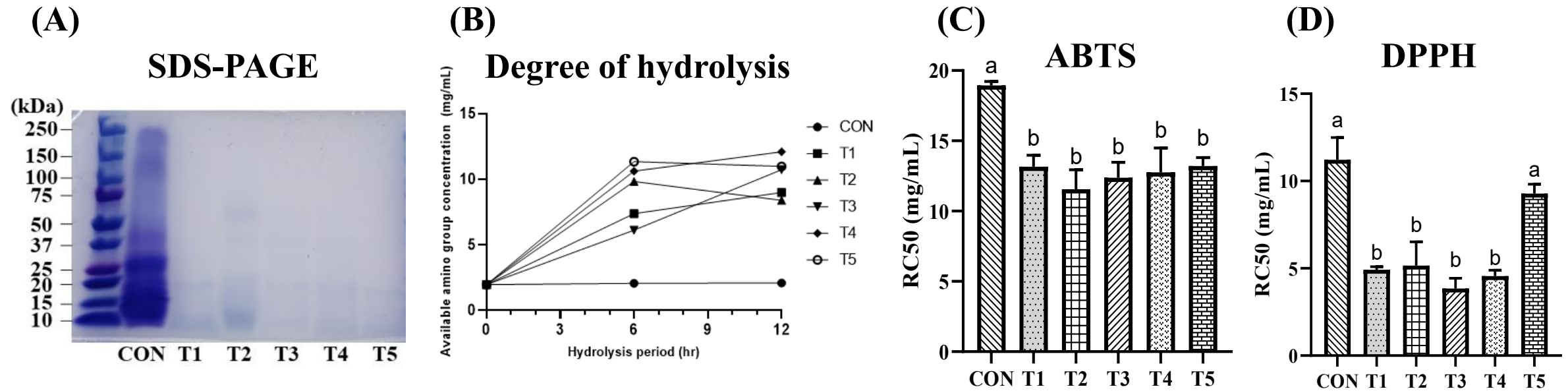


Figure 3. (A) SDS-PAGE profile, (B) degree of hydrolysis, and antioxidant activities ((C) ABTS and (D) DPPH radical-scavenging assays) of drone-pupa hydrolysates. a-b Different superscripts indicate statistically significant differences ($p < 0.05$).

- ✓ The combined use of the two enzymes **improves the hydrolysis**
- ✓ Enzymatic hydrolysis of the drone pupa **increases its antioxidant activity**.

Enzyme	CON	T1	T2	T3	T4	T5
Flavourzyme	-	-	100	30	50	70
Neutrase	-	100	-	70	50	30

- Free amino acid composition

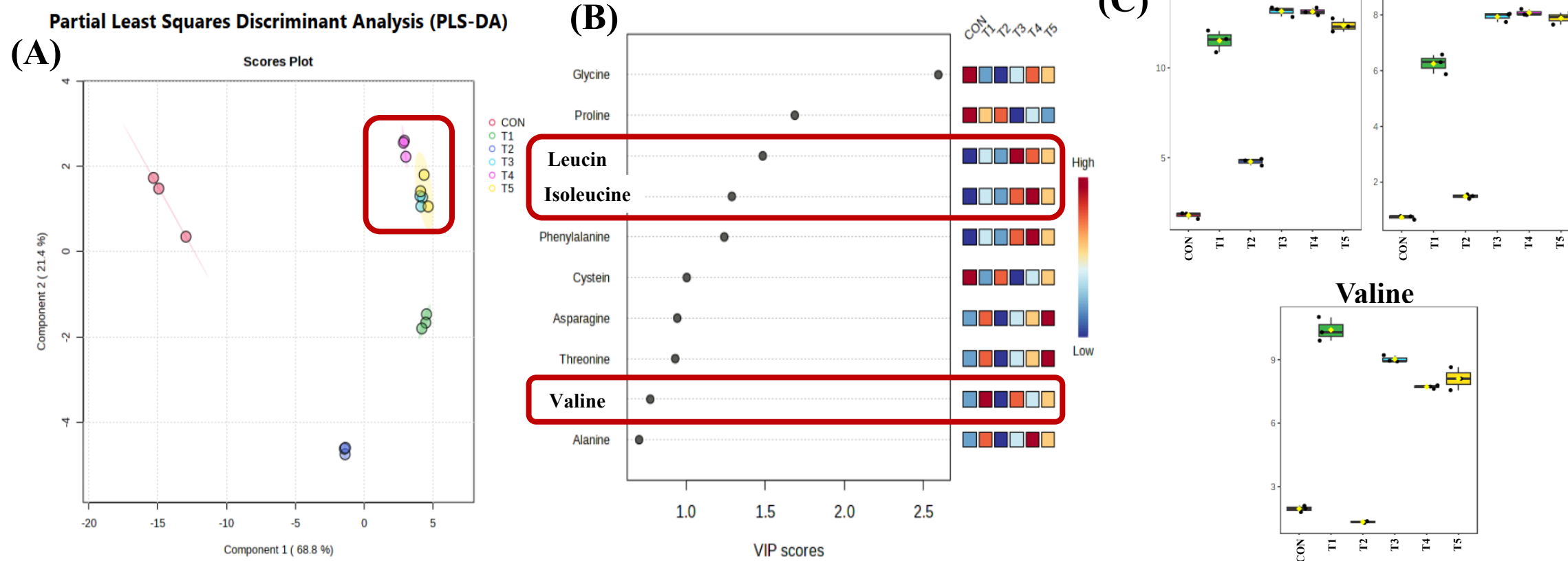


Figure 4. Partial least squares discriminant analysis (PLS-DA) and variable importance in projection (VIP) score using amino acid content data of hydrolyzed drone pupa.

✓ The combined use of the two enzymes **increases the content of branched chain amino acids (Leucine, Isoleucine, Valine)**.

Enzyme	CON	T1	T2	T3	T4	T5
Flavourzyme	-	-	100	30	50	70
Neutrase	-	100	-	70	50	30

- C2C12 cell differentiation

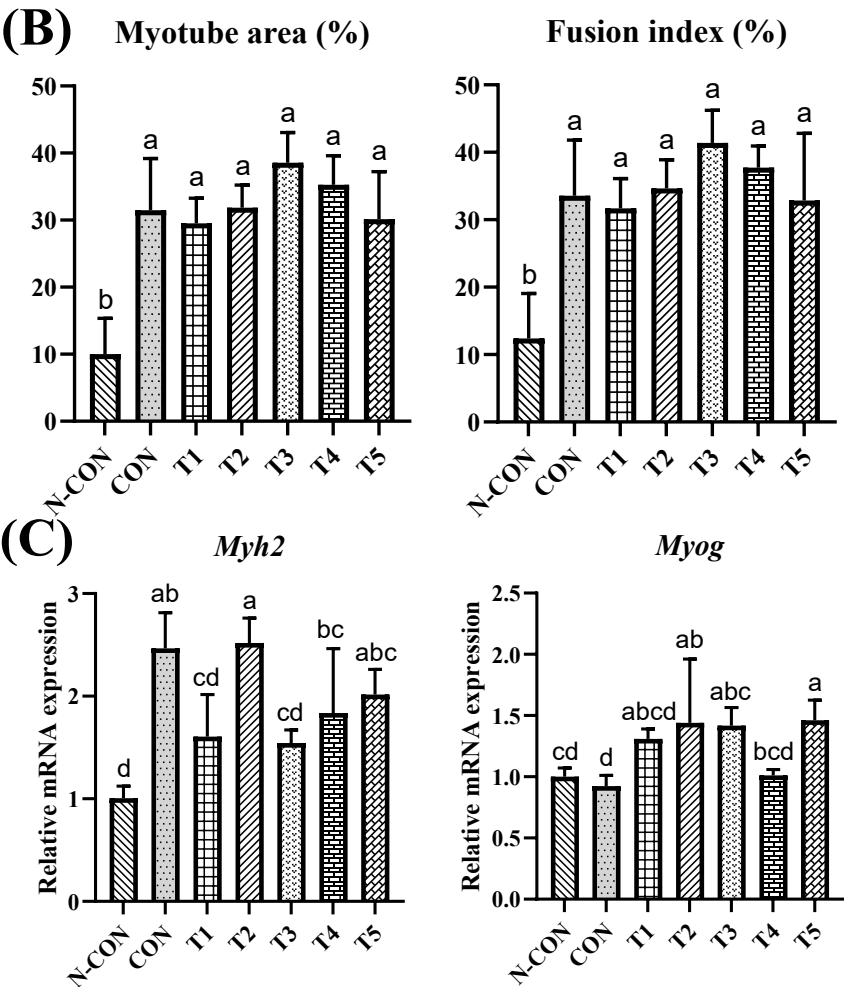
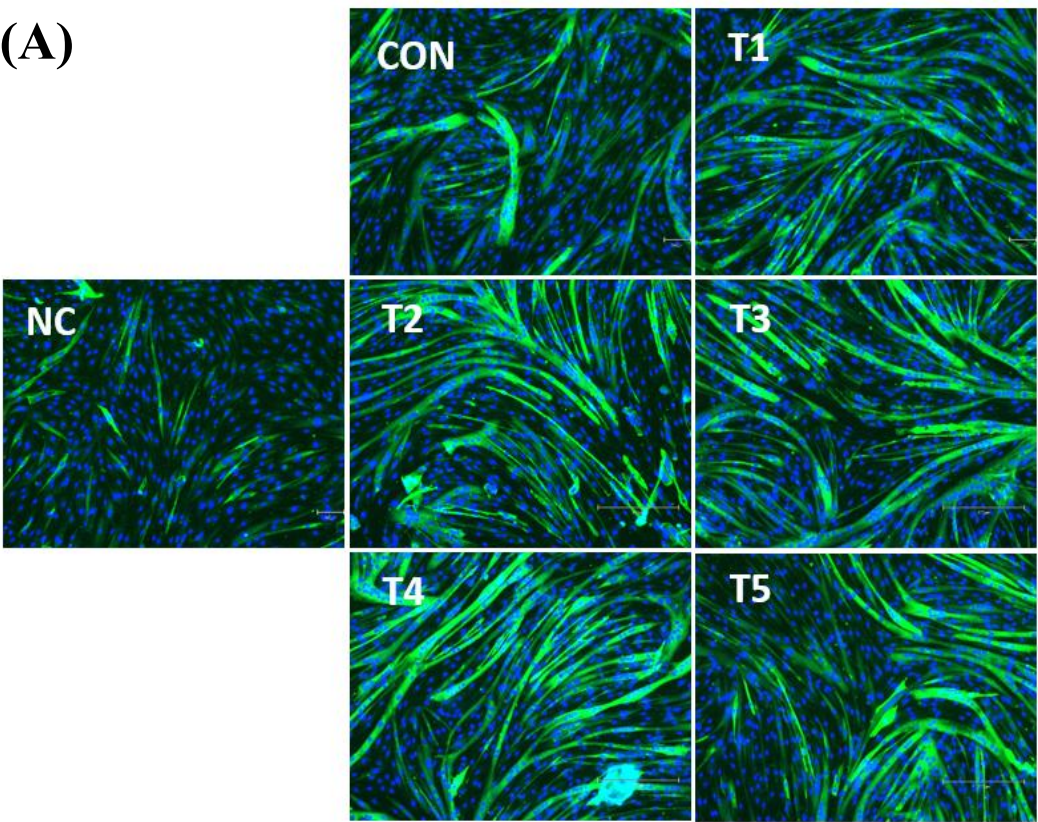
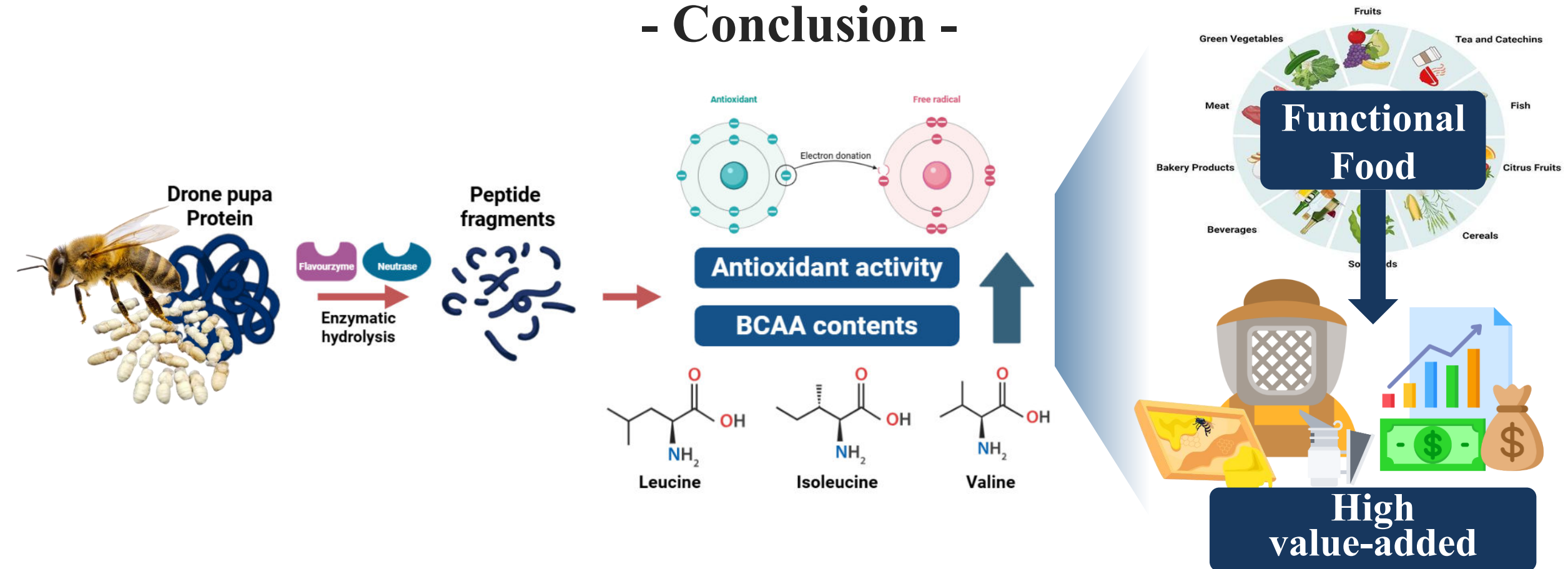


Figure 5. All experiments were performed using day 3 differentiated. (A) Immunofluorescence staining images. (B) Myotube area and fusion index measured using ImageJ. (C) Relative mRNA expression levels of *Myh2* and *Myog* genes (described as fold change) determined by RT-qPCR.

✓ The hydrolysate and extract of the drone pupa **enhanced differentiation** in C2C12 cells.

Enzyme	CON	T1	T2	T3	T4	T5
Flavourzyme	-	-	100	30	50	70
Neutrase	-	100	-	70	50	30

- Conclusion -



Enzymatic hydrolysis of drone pupae improves their functional properties, suggesting their **potential application in functional foods** and contribution to **higher value-added utilization**



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Thank you

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- Supplementary information (1)



16d



17d



19d



23d

Figure S1. Morphological Changes of Drone (*Apis mellifera*) by Developmental Age
(Apiculture Ecology Division, National Institute of Agricultural Sciences, Rural Development Administration, Hyoyoung Kim)

- Supplementary information (2)



Figure S2. Procedure for Collecting Drone Pupae (*Apis mellifera*)