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Short communication

Analysis of Fatty Acid Composition and Phospholipid Content of Drone Pupae (*Apis mellifera* L.) Using Different Pre-treatment Methods

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Abstract Drone pupae are a natural resource suitable for the development of various food materials and formulations. In 2022, in Korea, drone pupae were approved as a food ingredient. However, research on quality standardization remains insufficient. Therefore, in this study, we aimed to establish quality control marker components and provide foundational data necessary for the utilization of drone pupae in food applications by analyzing their fatty acid composition and phospholipid content. Fatty acid analysis revealed eight types of saturated fatty acids and five types of unsaturated fatty acids, with palmitic and oleic acids being the most abundant saturated and unsaturated fatty acid types, respectively. The phospholipid content was 43.90 mg/g in drone pupae powder (DP-P) and 105.65 mg/g in drone pupae oil (DP-O). These findings suggest that the fatty acid composition and phospholipid content serve as quality control markers for drone pupae. In addition, this study provides foundational data for the future development of drone pupae-based food material.

Keywords Drone pupae, Saturated fatty acid, Unsaturated fatty acid, Phospholipid

INTRODUCTION

Lipids serve as a crucial source of energy in the body and play a major role in determining the physical characteristics of foods, such as flavor, texture, fluidity, and plasticity, all of which are essential in the production of various processed foods (Chung, 2007). Chemically, lipids are formed by the esterification of fatty acids with glycerol and are classified into saturated and unsaturated fatty acids based on their structural differences, specifically, the number of double bonds in their carbon chains. Saturated fatty acids are primarily found in animal fats, whereas unsaturated fatty acids are predominant in plant oils. Most naturally occurring unsaturated fatty acids exist in the cis configuration. However, trans fatty acids, although synthesized in the gastrointestinal tract of ruminants, are mainly produced during the hydrogenation of natural vegetable oils (Ascherio and Willet, 1997).

Biomembranes protect cells and tissues and play an essential role in various physiological functions, such as ion transport, cellular metabolism, and nutrient absorption (Shin and Bae, 2001). Phospholipids, essential components of cell membranes, include phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. Phosphatidylcholine is involved in nutrient absorption, basal metabolism regulation, and acetylcholine (neurotransmitter) production and reduces blood cholesterol levels, thereby preventing the development of cardiovascular diseases (Lee *et al*., 2011).

After evaluating their safety and nutritional properties, the Ministry of Food and Drug Safety (MFDS) recognized drone pupae as an edible insect in Korea. In 2022, drone pupae were formally approved as food ingredients by the regulatory authority (MFDS, 2020, 2022). Kim *et al*. (2018) demonstrated that drone pupae are rich in protein, carbohydrates, fats, various amino acids, minerals,

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and vitamins, serving as a highly valuable and nutritious food ingredient. Furthermore, drone pupae exhibit antioxidant, blood glucose-lowering (Kim *et al*., 2020a), anti-hair loss (Kim *et al*., 2020b), hydrolyzed protein-related (Kim *et al*., 2020c), and antidiabetic (Pyo *et al*., 2020) properties. Despite their potential to develop diverse food materials and formulations that could contribute to agricultural income and industrial growth, research on quality standardization remains insufficient.

Therefore, in this study, we aimed to establish quality control markers by analyzing the fatty acid composition and phospholipid content of drone pupae.

MATERIALS AND METHODS

1. Sample

The drone pupae (17 to 23 days post-oviposition) used in this experiment were collected from Western honey bees (*Apis mellifera* L.) reared at a beekeeping farm in Dangjin, Chungcheongnam-do, South Korea, in 2024. All processes, including collection, storage, and transportation, were conducted at −20°C.

2. Pre-treatment methods

The preparation of freeze-dried drone pupae powder and fat extraction were performed as previously described by Kim *et al*. (2019, 2023). Briefly, The drone pupae powder samples were sterilized by heating at 121°C for 10 min using a high-pressure sterilizer and then dried for 72 hours at −40°C to −45°C using a freeze dryer (Kim *et al*., 2019). The drone pupae oil samples were prepared by adding distilled water to the raw drone pupae, grinding the mixture, and filtering it through a 300 μm mesh. The extract was then subjected to primary centrifugation (15,000 rpm, 30 min) at 4°C, and the lipid layer was collected. The collected lipid layer was subjected to secondary centrifugation (15,000 rpm, 30 min, 40°C), and the resulting final lipid extract was used as the sample (Kim *et al*., 2023).

3. Fatty acid composition in drone pupae

The saturated and unsaturated fatty acid content in drone pupae was analyzed using the fatty acid test method outlined in the Korean Food Code (MFDS, 2024a). Fat was extracted from the homogenized sample using 2−3 mL of chloroform and 2−3 mL of diethyl ether. The extracted fat was dissolved and concentrated under nitrogen in a 40°C water bath. The sample was then mixed with 2 mL of 7% trifluoroborane methanol solution and 1 mL of toluene, heated in a 100°C oven for 45 min, and then cooled to room temperature. Next, 5 mL of distilled water, 1 mL of hexane, and 1 g of anhydrous sodium sulfate were added to the sample, followed by shaking and settling. For dehydration, the upper layer was transferred into a vial containing approximately 1 g of anhydrous sodium sulfate, and the solution was used for analysis. Quantitative analysis was conducted using a gas chromatograph (GC, Agilent 7890A) equipped with a flame ionization detector and a capillary column (Sigma Aldrich SPTM-2560, MO, USA), under the following conditions: detector temperature 285°C, flow rate 0.75 mL/min, injection volume 1 μL, oven temperature program 100°C (4 min), 208°C (3°C/min, 5 min), 244°C (2°C/min, 15 min).

4. Analysis of phospholipid content

Phospholipid (measured as acetone-insoluble matter) analysis in drone pupae was conducted following the phospholipid method from the Health Functional Food Code (MFDS, 2024b) and calculated using the following formula:

$$\text{Phospholipids (mg/g)} = \frac{\text{A (mg)} - \text{B (mg)}}{\text{C (g)}}$$

A: Weight of the sample (mg)
B: Weight of acetone-soluble matter (mg)
C: Sample weight (g)

5. Statistical analysis

The results obtained from this experiment were expressed as means ± standard deviation (SD).

RESULTS AND DISCUSSION

1. Fatty acid composition

Fatty acids are classified as saturated and unsaturated fatty acids based on the presence or absence of carbon-carbon double bonds in their hydrocarbon chains. Upon excess consumption, saturated fatty acids cause cardiovascular diseases, whereas unsaturated fatty acids are an important indicator for the improvement of cardiovascular health (Jeong *et al*., 2014). Analysis of the fatty acid composition in pre-treated drone pupae revealed the presence of eight and five saturated and unsaturated fatty acid types, respectively (Tables 1 and 2). Among the detected saturated fatty acids, palmitic acid was predominant, with 7.76 g/100 g in DP-P and 37.22 g/100 g in DP-O, followed by stearic acid and myristic acid. The total saturated fatty acid content was 10.79 g/100 g in DP-P and 51.62 g/100 g in DP-O. Additionally, oleic acid was the most abundant unsaturated fatty acid, with 7.92 g/100 g in DP-P and 38.23 g/100 g in DP-O. The total unsaturated fatty acid content was 8.53 g/100 g in DP-P and 40.07 g/100 g in DP-O. According to Kim *et al*. (2018), raw drone pupae contain 3.14 g/100 g saturated fatty acids and 2.31 g/100 g unsaturated fatty acids, highlighting observed content differences based on differences in sample pre-treatment. These findings serve as foundational data for the development of food formulations using drone pupae and establishing quality control indicators. Furthermore, drone pupae have been recognized as a food ingredient through temporary registration as a food material and subsequent transition to a general food ingredient. Consequently, drone pupae produced by domestic beekeeping farms are now officially acknowledged as a food resource. In the future, to ensure systematic quality control of domestically produced drone pupae, we plan to conduct studies on quality characteristics based on different regions.

Table 1. Saturated fatty acids content and saturated fatty acids composition in different sample (powder and oil) of drone pupae

Compounds (g/100 g)	DP-P[1)]	DP-O
Saturated fatty acid	10.79±0.47[2)]	51.62±0.39
Myristic acid (C14 : 0)	0.52±0.36	2.88±0.27
Pentadecanoic acid (C15 : 0)	0.03±0.48	0.16±0.36
Palmitic acid (C16 : 0)	7.76±0.28	37.22±0.24
Stearic acid (C18 : 0)	2.14±0.17	10.60±0.21
Arachidic acid (C20 : 0)	0.10±0.36	0.36±0.17
Behenic acid (C22 : 0)	0.05±0.22	0.05±0.29
Tricosanoic acid (C23 : 0)	ND[3)]	0.04±0.05
Lignoceric acid (C24 : 0)	0.10±0.17	ND

1)DP-P: Drone pupae powder, DP-O: Drone pupae oil.
2)Values are mean±SD in triplicates.
3)ND: Not detected.

Table 2. Unsaturated fatty acids content and unsaturated fatty acids composition in different sample (powder and oil) of drone pupae

Compounds (g/100 g)	DP-P	DP-O
Unsaturated fatty acid	8.53±0.27[1)]	40.07±0.19
Palmitoleic acid (C16 : 1)	0.11±0.31	0.43±0.25
Oleic acid (C18 : 1)	7.92±0.35	38.23±0.28
Linoleic acid (C18 : 2)	0.16±0.23	0.70±0.17
Alpha-linolenic acid (C18 : 3)	0.33±0.04	0.62±0.36
Cis-11-eicosenoic acid (C20 : 1)	0.02±0.12	0.08±0.04

1)Values are mean±SD in triplicates.

2. Phospholipid content

Phospholipids are polar lipids composed of glycerol, a phosphate group, and two non-polar fatty acids. Phospholipids possess emulsifying functions and play essential nutritional roles in neonatal growth, brain development, and the formation of nervous tissues (Sanders and Zeisel, 2007). Phospholipid content (measured as acetone-insoluble matter) analysis in the pre-treated drone pupae samples revealed 43.90 mg/g in DP-P and 105.65 mg/g in DP-O (Table 3). Similar to the fatty acid composition and content results, differences in phospholipid content were observed with differences in drone pupae pre-treatment techniques. Phospholipids, which are a type of lipid (acetone-insoluble) or phosphatidylcholine, are registered as functional indicators in healthy functional foods; phosphatidylcholine improves blood cholesterol levels and is included in lecithin-based processed foods (MFDS, 2024c). Additionally, krill oil, a general food product, must contain more than 30% phospholipid content (MFDS, 2024d). According to a market report on lecithin and phospholipids, the market size was valued at USD 5.1 billion in 2021 and is projected to reach USD 9.0 billion by 2028 (Polaris Market Research, 2022).

Table 3. The content of phospholipids in different sample (powder and oil) of drone pupae

Compounds (mg/g)	DP-P	DP-O
Phospholipids	43.90±0.43[1)]	105.65±0.27

1)Values are mean±SD in triplicates.

Thus, drone pupae, a natural source of phospholipids, could contribute to the development of bio-healthcare technologies.

CONCLUSION

This study analyzed the fatty acid composition and phospholipid content of drone pupae to establish quality control markers. Eight saturated fatty acid types and five unsaturated fatty acid types were observed in DP-P and DP-O, with palmitic and oleic acids as the most abundant saturated and unsaturated fatty acids, respectively. The phospholipid content (measured as acetone-insoluble matter) was found to be 43.90 mg/g in DP-P and 105.65 mg/g in DP-O. Overall, the findings of this study suggest that the fatty acid composition and phospholipid content in drone pupae serve as potential quality control indicators. Furthermore, this study serves as a foundational study for the development of drone pupae-based food materials.

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