GC-MS Analysis of Fatty Acid Components of Black Soldier Fly Larvae Fed on Kitchen Waste at Different Ages

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Abstract. Black soldier fly larvae, as a biological converter of organic waste, can efficiently and safely degrade kitchen waste, convert it into their own nutrients, and realize the resource utilization of kitchen waste. Since the content of crude oil in black soldier fly larvae accounts for more than 35% of the dry weight of insects, the oil of larvae in the fifth instar and prepupal stage is used as the test raw material, with reference to the national standard GB5009.168-2016 fatty acids were extracted and methylated with methanol solution, Gas chromatography-mass spectrometry was used. The results showed that the main chemical components of the esterification products of the fifth instar insect oil were oleic acid, palmitic acid and lauric acid, and the three fatty acids accounted for 66.27%, 17.23% and 11.39% of the total respectively. The main chemical components were methyl laurate, methyl oleate, methyl linoleate and methyl palmitate. The four fatty acids accounted for 46.62%, 17.88%, 17.42% and 13.23% respectively.

1 Introduction

Kitchen waste is a kind of food residue formed in people's daily diet, and it is the main component of Municipal solid waste, including rice, meat, fruit, vegetables, and edible oil. Kitchen waste has complex components, and its chemical composition is cellulose, fat, carbohydrates, fatty acids, inorganic salts, and moisture. Incineration and landfill are the existing traditional treatment methods, which will cause secondary pollution to the atmospheric environment and groundwater [1-5]. Kitchen waste has great potential [6]. With the enhancement of people's awareness of ecological protection, the resource utilization and harmless treatment of kitchen waste have received extensive attention. Therefore, it is urgent to find a green and efficient treatment method to realize the recycling of kitchen waste [7].

Insects account for about 60% of all animal species, and are a sustainable biological resource. Insect treatment technology is a new treatment technology in recent years. The black soldier fly(Hermetia illucens L.), as a resource insect, has the advantages of short growth cycle, fast production speed and convenient feeding[8-10]. Its protein content exceeds 40% of the dry weight, and the crude fat content exceeds 35% of the dry weight. It has a wide range of food sources, such as animal feces, kitchen waste and other wastes [11-13]. As an organic waste converter, black soldier fly can efficiently and safely degrade kitchen waste and convert it into its own nutrients, which can realize resource recycling without adverse effects on humans [14].

The larvae of black soldier flies have a strong ability to withstand hunger. Even after a long period of starvation and then resuming feeding, their prepupal rate will not be affected, but the weight of the prepupae will increase significantly. This characteristic makes the larvae of black soldier flies flexible in actual production and enables them to adapt to different environmental conditions to a certain extent. In addition, due to their powerful degradation ability, the larvae of black soldier flies can quickly decompose and degrade organic substances such as paper, food waste, and fertilizer residues. They themselves are also high-protein and high-fat insects with high nutritional value and have wide applications in medicine. The application prospects of black soldier fly larvae in the treatment of organic waste are very broad. They can effectively convert organic waste such as kitchen waste and livestock and poultry manure into high-quality insect protein and organic fertilizer. This conversion process not only reduces the volume and pollution of waste but also realizes the recycling of resources[15-17]. The characteristics of black soldier fly larvae make them an ideal choice for the resource utilization of urban kitchen solid waste.

At present, the application of protein as feed has received extensive attention, and the application of insect oil is still in the blank stage [18]. Black soldier fly larva is rich in oil and has a reasonable composition of fatty acids, including long-chain saturated fatty acids such as lauric acid and palmitic acid, which can be used as a sustainable oil source. In order to fully develop the application value of black soldier fly as a resource insect, this study extracted crude oil from black soldier fly larvae at two

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different instars fed on kitchen waste, analyzed and compared the fatty acid composition of the oil, and further promote the high-value application of resources, thereby promoting the greening process of resources in China.

Although the fatty acid composition of black soldier fly oil has been studied to some extent, there are still some gaps in the research related to kitchen waste. For example, although it is known that the feeding substrate has an effect on the fatty acid composition of black soldier fly, the specific mechanism and degree are still unclear. Whether the effects of different types of kitchen waste (such as vegetable residues, meat residues, etc.) on the fatty acid composition of black soldier fly are different, and whether this effect will change the quality and feeding value of black soldier fly oil, these issues have not been fully studied. In addition, many studies at present mainly focus on the bioconversion technology of black soldier fly as kitchen waste treatment, but there are few studies on the application potential of black soldier fly oil itself in the treatment of kitchen waste, especially the influence of its fatty acid composition on the treatment effect[19-20]. Whether the specific fatty acids in black soldier fly oil can help to improve the treatment efficiency of kitchen waste, or whether it can be used as an accelerator in the treatment process, further research is needed to confirm. These are also the main background of this article.

2 Experiment

2.1. Instruments and Materials

The fifth instar and prepupa stage crude oil of black soldier fly used in the experiment were obtained from the Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. Silica gel, petroleum ether, ethyl acetate, sodium hydroxide, ethanol and methanol were purchased from Shanghai Titan Scientific Co. Ltd. Main equipment: Trace 1310+ISQ Gas chromatography-mass spectrometry (GC-MS) gas-mass spectrometer (Thermo Fisher Scientific, USA).

In the experiment, the fifth instar and prepupal stages of black soldier fly were selected to carry out the experiment of kitchen waste recycling, because the feeding conditions of this type of larvae were carried out under the condition of artificial feed with 70 % water content. Compared with the larvae under the artificial feeding condition with 50 % water content, the average weight of the larvae was significantly larger and heavier, and the breeding cycle was about 10 days less, which had a strong growth advantage and breeding advantage.

2.2 Feeding black soldier fly larvae and extracting body oil

The newly hatched larvae of the first instar were fed with waste homogenate mixed with half wheat bran (w/v=1:2). After 48 hours, they entered the second instar. After that, they were fed with kitchen waste pre-fermented with lactic acid bacteria, and food was ensured daily. The fifth instar and prepupa stage larvae were taken for subsequent treatment. After inactivation with boiling water, rinse with clean water to remove surface water to obtain fresh weight, then dry with hot air at 60°C for 50 hours until the mass change is less than 0.3% to obtain dry weight; after crushing, ultrasonically extract with n-hexane, and stir and extract at room temperature, the organic layer was rotary evaporated to obtain the crude extract.

The specific drying time and temperature of drying at 60 degrees Celsius for 50 hours until the mass change is less than 0.3 % is the result of comprehensive consideration of many factors such as health and safety, nutrient retention, water control, economic benefits and energy efficiency. First of all, 60 °C is a common high temperature treatment temperature, which can effectively kill pathogens and pests in garbage and ensure the safety of products. Secondly, when dealing with kitchen waste, the 50-hour dry black soldier fly is to ensure that the pathogen is killed while minimizing the nutritional loss of black soldier fly larvae or other useful components. Finally, the standard of mass change less than 0.3 % is to ensure that the dried product has an appropriate moisture content. This is very important for subsequent storage and transportation, and can prevent product mildew or corruption. Taken together, this approach aims to ensure that the black soldier fly process of kitchen waste is efficient and safe, while maximizing the nutritional and economic value of the product.

The relevant data are shown in Table 1.

Table 1. Extraction of crude insect oil from black soldier fly

instar	Fifth	pre-pupa
fresh weight /g	1346.63	876.43
dry weight /g	448.01	366.28
crude extract quality /g	159.94	116.18
dry weight ratio	35.70%	31.72%

2.3 Crude oil refining and fatty acid preparation

The crude insect oil is purified by column chromatography, the filler used is silica gel powder, namely silicon dioxide, and the eluent of the column chromatography is a mixed solvent of petroleum ether and ethyl acetate. The mesh number of the silica gel powder in the column chromatography is 200 meshes, and the volume ratio of petroleum ether and ethyl acetate is 20:1. The obtained crude insect oil was added to a 200-mesh silica gel column, eluted with petroleum ether/ethyl acetate (volume ratio of 10:1), the eluate was collected and the solvent was spin-dried to obtain the refined insect oil.

With reference to the national standard GB5009.168-2016, 4g of the above-mentioned fifth instar and prepupa stage oil were placed in a 250ml round-bottomed flask, 20mL of 1mol/L sodium hydroxide aqueous solution was added and mixed, 10ml of ethanol was added to improve solubility, and the oil bath was refluxed. Stir at 95 °C for 4h, after cooling to room temperature, add 50 ml of distilled water, add an appropriate amount of 1 mol/L dilute hydrochloric acid to adjust pH=7, extract three times with 50 ml of ethyl acetate, combine the organic phases and wash three times with saturated sodium chloride solution, Use anhydrous sodium sulfate to dry and dehydrate, filter, recover the filtrate, and then distill off ethyl acetate under reduced pressure to obtain insect oil fatty acid.

2.4 Fatty acid methylation

Weigh 2g of each of the above products into a 250 mL round-bottomed flask, add an appropriate amount of anhydrous methanol, mix well, slowly add 1 ml of concentrated sulfuric acid dropwise, and reflux in an oil bath for 4 h at 115 ° C. After the reaction is completed, let it cool to room temperature, add 50 ml of distilled water, extracted three times with 50 ml of ethyl acetate, combined the organic phases, washed with saturated sodium chloride until neutral, added anhydrous sodium sulfate to the organic phase, dehydrated and dried, filtered. Ethyl acetate was removed under reduced pressure on a rotary evaporator to give the methyl esterified fatty acid. The product was dissolved in 1 ml of dichloromethane and transferred to a 1.5 ml gas chromatography bottle for GC-MS analysis.

2.5 Test Conditions

GC conditions: Column Agilent DB-1HT (30 m×250 μm×0.1 μm), carrier gas is helium, column initial temperature 40 °C, heated to 300 °C at a rate of 15 °C/min, maintained for 1 min, equilibration time 10 min, the injection volume was 1 μL, the column flow rate was 1.5 mL/min, and the split ratio was 1:20.

MS conditions: EI ion source, electron energy 80 eV, electron multiplier voltage 1 kV, mass scanning range 45-800 m/z, ion source temperature 280 ℃, GC-MS interface temperature 280 ℃.

2.6 Identification and Analysis

The GC-MS spectrum was analyzed by Thermo Xcalibur Qual Browser. According to the GC-MS measurement results, the data in the database were matched to determine the chemical structure of each component and related data.

3 Results and discussion

GC-MS was used to analyze and detect the oil components of the fifth instar and prepupa stage. The total ion chromatogram of fatty acid methyl esters was shown in Figure 1. The NIST 11 database was used, and the analysis results were obtained after searching, as shown in Table 2 and table 3. The analysis showed that the fatty acids of the fifth instar and prepupa stage are mainly lauric acid, oleic acid, palmitic acid, myristic acid, linoleic acid and stearic acid.

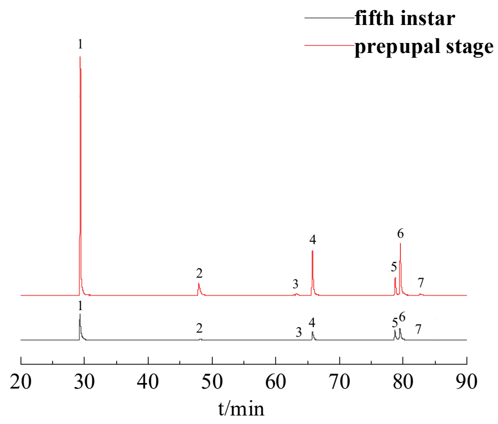

Figure 1. Total ion current chromatogram of fatty acid methyl ester from fifth instar and prepupal stage black soldier fly oil

3.1 Analysis of Fatty Acid Methyl Esters in Insect Oil at Different Periods

The obtained mass spectrum was matched with the instrument database to determine the chemical composition of the methyl esterification products of the fifth instar insect oil. The detailed results are shown in Table 2 and Table 3, and each component of the insect oil was quantitatively analyzed.

According to Fig. 1, the GC-MS detection results in Table 2 and Table 3 show that the fifth instar and the prepupal stage insect oil each detected 7 peaks, after matching with the database, 7 compounds were identified, each accounting for the total amount of fatty acid methyl esters. 100.00% of the amount. The analysis and testing results showed that the main components of the oil of black soldier fly at the fifth instar were lauric acid, oleic acid, palmitic acid and linoleic acid. The main components of black soldier fly oil in the prepupa stage were lauric acid, oleic acid and palmitic acid.

Table. 2. The analysis of fifth instar stage insect oil fatty acid component

No.	Retention time	Name	Molecular formula	Molecular weight
1	29.27	methyl laurate	C13H26O2	214
2	48.17	methyl myristate	C15H30O2	242
3	63.44	9methyl hexadecenoate	C17H32O2	268
4	65.78	methyl palmitate	C17H34O2	270
5	78.71	methyl linoleate	C19H34O2	294
6	79.47	methyl oleate	C19H36O2	296
7	82.75	methyl stearate	C19H38O2	298

Table. 3. The analysis of prepupal stage insect oil fatty acid component

No.	Retention time	Name	Molecular formula	Molecular weight
1	29.36	methyl laurate	C13H26O2	214
2	47.91	methyl myristate	C15H30O2	242

3	63.27	9methyl hexadecenoate	C17H32O2	268
4	65.76	methyl palmitate	C17H34O2	270
5	78.75	methyl linoleate	C19H34O2	294
6	79.55	methyl oleate	C19H36O2	296
7	82.64	methyl stearate	C19H38O2	298

3.2 Fatty acid components of insect oil at fifth instar and prepupa stage

The types of fatty acids contained in the oil of the fifth instar and prepupa stage were the same, mainly lauric acid, followed by oleic acid. The content of lauric acid in insect oil in the two periods was 37.93% and 54.11%, and the average was 46.02%. The stearic acid content was the lowest, 3.52% and 1.10%, respectively, with an average of 2.31%. The contents of lauric acid and myristic acid in the fifth instar insect oil were lower than those in the prepupa stage insect oil, which were 37.93% and 5.05%, respectively. The contents of other components were higher than those in the prepupa stage insect oil. The contents of lauric acid and myristic acid in the prepupa stage insect oil were 54.11% and 6.60%, respectively. The other components were lower than the fifth instar insect oil. As show in table 4.

Table. 4. Proportion of fatty acids component in insect oil at different stages (%)

No.	Name	fifth instar stage	prepupal stage
1	methyl laurate	37.93	54.11
2	methyl myristate	5.05	6.60
3	9methyl hexadecenoate	3.35	1.71
4	methyl palmitate	16.30	14.57
5	methyl linoleate	14.35	5.61
6	methyl oleate	19.50	16.3
7	methyl stearate	3.52	1.10

Table 5 is the analysis of the fatty acid saturation of the black soldier fly in different periods. It can be seen from the table that the difference in fatty acid saturation in different stages of insects is obvious, and the total saturated fatty acid content of insect oil in the prepupa stage is higher, which is 76.38%.

Table. 5. Fatty acid saturation proportion in insect oil at different stages (%)

Fatty acid	fifth instar stage	prepupal stage
Total saturated acids	62.8	76.38
Total unsaturated acids	37.2	23.62

4 Conclusion

The test and analysis results showed that a total of 7 peaks were detected in the oil methyl esterification products of fifth instar and prepupa stage. After matching with the database, 7 compounds were identified, accounting for 100.00% of the total fatty acid methyl ester. The results showed that the black soldier fly is rich in lipids and had reasonable fatty acid composition, including long-chain saturated fatty acids such as lauric acid and palmitic acid, and unsaturated fatty acids such as oleic acid. Can be used as a sustainable oil source. Black soldier fly has a high development value, which provides a theoretical basis for further realizing the recycling of kitchen waste and the application of high-value oil.

The research results have an important impact on the resource recovery industry and waste management field, and have strong practical significance. First of all, the quality and applicability of these resource-based by-products can be grasped by analyzing and measuring the fatty acids of the oil after the treatment of black soldier fly. The composition of fatty acids in oil is crucial to determine its potential in biofuels or other industrial applications. Accurate analysis can help to optimize the feeding conditions and treatment processes of black soldier fly, thereby improving the quality and yield of oil. In addition, understanding the composition of fatty acids in oils and fats will also help to develop new resource markets and applications, such as the use in the cosmetics or pharmaceutical industries, bringing more resource-based economic benefits. In addition, the analysis and determination of fatty acids in black soldier fly oil is helpful for waste managers to understand the efficiency of treatment process and the market value of products. This can not only enhance the attractiveness of waste management services, but also provide data support for policymakers to develop more effective waste management and resource recovery strategies. In addition, with the in-depth study and optimization of the black soldier fly treatment technology, this technology is expected to become an important part of the waste management field and promote the innovation and development of the entire industry.

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