Study of fatty acid esters obtained from the biomass of *Hermetia illucens* larvae and the prospects of their use in the food industry

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Abstract. In this study we carried out a physicochemical analysis of fatty acid esters (FFA) obtained from the biomass of larvae, which can be used as ingredients in the food industry and for the manufacture of feed. The acute toxicity of fatty acid esters obtained from the biomass of *Hermetia illucens* larvae was determined. It was revealed that the acid and peroxide values of the fat fraction obtained from the biomass of *Hermetia illucens* larvae are within the limits established by the norm, which indicates the high quality of this product. The fatty acid composition is represented mainly by lauric, palmitic, oleic and myristic acids. It was shown that the differences in fatty acid composition of the samples, obtained from the biomass of *Hermetia illucens* larvae manually and using the Soxhlet extraction apparatus, were insignificant and within the acceptable values. In the study of presence of toxic elements in fat fractions, lead, cadmium, arsenic, mercury, iron were not detected or they did not exceed the established values.

1. Introduction

In the last decade there has been an increased interest in insects all over the world as a source of highly assimilable fodder protein, fat with unique properties, antioxidants, immunomodulators and raw materials for obtaining new drugs [1, 2]. An important factor is the possibility of cultivating some species in artificial conditions, which allows them to be used as new industrial producers in order to obtain physiological, biochemical and genetic characteristics of a particular crop, control the processes of bioconversion of substrates, on which insects develop, and also evaluate the qualitative and quantitative indicators of insect biomass products for subsequent use in feed, veterinary medicine, pharmacology [3, 4]. The black soldier fly (*Hermetia illucens*) is a large American fly from the family of Stratiomyidae, the natural distribution area of which is believed to be North and South America [5]. These insects are able to develop year-round in pure culture under artificial conditions, which allows them to be used for biotechnological purposes.

Modern trends in the food industry are associated with the search for innovative solutions and environmentally friendly technologies in the production of food products. As an alternative to animal and vegetable fats, new sources of fat obtained from insects are considered, which can be used as a
substitute for oils in bakery products [6] or as oil ingredients in the food industry [7, 8]. Biomass and proteins obtained from insects can be used as a source of animal protein and animal feed [9–14]. Studies [15] have revealed the potential of lipids obtained from the biomass of insects *Hermetia illucens* for use as an alternative to vegetable and animal lipids in products such as margarine or butter. Technological tests have shown successful replacement of solid starting fats with insect fats from *H. illucens* (with a replacement ratio of up to 60% of the total fat).

Consequently, the study of the physicochemical composition of the black soldier fly larvae is a relevant topic, the importance of which has increased significantly at the present time due to the need for import substitution of feed components and the search for new effective biological ingredients. First of all, this is due to the high nutritional value of the larvae, which are characterized by a high content of protein and fat, and in addition to the possibility of cultivation on organic waste, thereby helping to reduce the load on the environment.

2. Materials and methods

The fatty acid composition of the samples was carried out with the software complex for medical research based on the chromatograph “ChromatekKristall”.

The study of toxic elements was carried out using the fat fraction. The fat fraction from the larvae was obtained directly by 2 methods using the Soxhlet extraction apparatus and manually (mixing the crushed dried biomass of the milled larvae on the apparatus). Extraction took place in both cases with ethyl ether. The study was carried out for the presence of lead, cadmium, arsenic, mercury and iron in the samples. The experiment was carried out on an A-2 atomic absorption spectrometer with flame and electrothermal atomization. Sample preparation was carried out using an MS-6 microwave setup (dissolving the sample in nitric acid).

The acid and peroxide values of the fat fraction obtained by two methods were preliminarily investigated.

Tests for acute toxicity were performed according to the methodological instructions "Guidelines for conducting preclinical studies of drugs. Part One" (2012) and OECD GUIDELINE FOR TESTING OF CHEMICALS 402 (1987). Experiments on animals were carried out in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes ((ETS 123). Strasbourg, 1986).

The study design was based on the guidelines “Guidelines for experimental (preclinical) study of new pharmacological substances” (2005), “Guidelines for conducting preclinical studies of drugs. Part One” (2012) and OECD GUIDELINE FOR TESTING OF CHEMICALS 402 (1987).

The design and organization of the study are aimed at solving this goal and are based on the general principles of organizing studies to assess the acute toxicity of substances in laboratory animals (Table 1).

### Table 1. Design of Acute Toxicity Experiment

| Group | Species, sex of animals | Number of animals in a group | Drug (experiment variant) | Doses, number | Volume of solution for administration, ml / animal | Mode of administration |
|-------|--------------------------|-----------------------------|---------------------------|---------------|--------------------------------------------------|------------------------|
| 1     | Male mice weighing 20-25 g | 6                           | "Sample No. 1" (test sample)  | Several       | 0.1 – 0.5                                         | Intragastric, once     |
| 2     | Male mice weighing 20-25 g | 6                           | Sodium chloride solution 0.9% (control) | -             | 0.5                                              | Intragastric, once     |

Rodents are standard subjects for preclinical toxicity testing. Rats and mice are recommended in regulatory documents as the most adequate test systems for studying the general toxic properties of
potential pharmaceuticals.

The use of laboratory animals and the planning of the experiment were carried out in accordance with the rules adopted by the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. The personnel participating in the experiment are trained in the correct and humane handling of laboratory animals.

Characteristics of laboratory animals:
- White non-linear mice
- Sex: males.
- Age: 2-2.5 months.
- Weight: 20 - 25 g.

The animals were bred on purpose and had not previously participated in the experiments. Newly arrived animals were quarantined for 7 days in cages in a separate room.

During the quarantine period, the animals were monitored for clinical indicators of health.

The animals were kept in a vivarium in accordance with sanitary rules and on a standard diet in accordance with the Decree of the Chief State Sanitary Doctor of the Russian Federation of August 29, 2014, No. 51 "On the approval of SP 2.2.1.3218-14 Sanitary and epidemiological requirements for the device, equipment and maintenance of experimental biological clinics (vivariums)".

Acclimatization and care of animals was carried out in accordance with GOST R ISO 10993-2-2009.

The mice were kept in polycarbonate cages, respectively, 6 animals in each. Wood sawdust was used as a bedding.

The food was dry briquetted food.

The drinking water was tap water, which was given ad libitum from standard drinkers.

The animals were kept under controlled conditions:
- air temperature of 20-22 °C;
- relative humidity of 60-70%.

Air temperature and humidity were monitored in each room on a daily basis and the readings were documented. Lighting was natural, artificial (12 hours light / 12 hours dark).

The selection of animals into groups was carried out at random by the method of random numbers, using body weight as a criterion. Individual values of body weight did not deviate from the average value in the group by more than 10%. The animals were weighed on an MT 1.5 БИЗХА balance.

Each group of mice weighing 20-25 g consisted of 6 animals.

The weight of animals is indicated at the time of drug administration.

The preparation of mice for the experiment was carried out in accordance with the instructions of the General Pharmacopoeia Monograph "Abnormal toxicity" GF XII. Before the experiment, food and water were taken from the animals. After two hours, the animals were weighed and assigned to groups.

In assessing intragastric toxicity, the test sample was administered to white mice via a gavage directly into the stomach.

"Sample No. 1" (fatty acid esters obtained from the biomass of \textit{Hermetia illucens}) was administered in doses according to the dosage form indicated below.

The drug was administered intragastrically to male mice at doses of 6000, 8000, and 10000 mg / kg according to the dosage form.

Control mice were injected once intragastrically with a 0.9% sodium chloride solution in a volume of 0.2 ml.

Observation of the animals was carried out for 14 days, during the first day the animals were under continuous observation. When observing the animals, the following parameters were assessed and documented: intensity of physical activity, presence of seizures, coordination of movements, reaction to sound stimuli, condition of skin and coat, condition of mucous membranes, frequency of respiratory movements, type and consistency of fecal masses, food consumption.
3. Results and discussions
In the study of the acid and peroxide value in the fat fraction obtained from the biomass of *Hermetia illucens* larvae on the Soxhlet extraction apparatus and manually all indicators were within the limits established by the norm (Table 2).

**Table 2.** The results of the study of the acid and peroxide number of the fat fraction obtained by two methods

| Indicator                              | Results | ND for test methods (measurements)* |
|----------------------------------------|---------|------------------------------------|
|                                        | Soxhlet extraction | Manual |                               |
| Acid number, mg KOH / g                | 65.8±0.4 | 65.4±0.4 | GOST 13496.18-85              |
| Peroxide number, mmol 1 / 2O2 / kg     | 20.12±1.81 | 20.87±1.88 | GOST 31485-2012              |

The fatty acid composition of fatty fractions from the biomass of *Hermetia illucens* larvae obtained on the Soxhlet extraction apparatus and manually was within the acceptable values (Tables 3, 4).

**Table 3.** Fatty acid composition of fatty fractions from the biomass of *Hermetia illucens* larvae obtained on the Soxhlet extraction apparatus

| Component              | Area  | Height | Concentration |
|------------------------|-------|--------|---------------|
| C10:0 Capric acid      | 43.747| 6.400  | 1.362         |
| C12:0 Lauric           | 1577.598 | 162.169 | 49.100        |
| C14:0 Myristic         | 357.664 | 32.761 | 11.132        |
| C14:1 Myristoleic      | 18.887 | 2.003  | 0.588         |
| C15:0 Pentadecanoic    | 8.743 | 0.674  | 0.272         |
| C16:0 Palmitic         | 441.984 | 28.227  | 13.756        |
| C16:1 Palmiteoleic     | 102.338 | 8.704  | 3.185         |
| C17:1 Margarinooleic   | 10.629 | 0.404  | 0.331         |
| C18:0 Stearic          | 86.906 | 3.774  | 2.705         |
| C18:1n9c Oleic         | 295.557 | 15.394  | 9.199         |
| C18:2n6c Linoleic      | 180.420 | 12.897  | 5.615         |
| C18:3n6 Gamma Linoleic | 3.397 | 0.324  | 0.106         |
| C18:3n3 Linolenic      | 36.983 | 1.642  | 1.151         |
| C20:0 Arachic          | 3.558 | 0.269  | 0.111         |
| C20:1 Eicosenic        | 13.763 | 1.169  | 0.428         |
| C22: 1n9 Erucic        | 0.978 | 0.276  | 0.030         |
| C20:5n3cis5,8,11,14,17 Eicosapentaenoic | 1.180 | 0.214  | 0.037         |
Table 4. Fatty acid composition of fatty fractions from the biomass of *Hermetia illucens* larvae obtained manually

| Component     | Area  | Height | Concentration |
|---------------|-------|--------|---------------|
| C10: 0 Capric acid | 12.874 | 1.883  | 0.893         |
| C12: 0 Lauric  | 557.720 | 63.111 | 38.678        |
| C14: 0 Myristic | 132.653 | 12.950 | 9.200         |
| C14: 1 Myristoleic | 5.520  | 0.712  | 0.383         |
| C15: 0 Pentadecanoic | 2.728  | 0.233  | 0.189         |
| C16: 0 Palmitic | 217.837 | 14.110 | 15.107        |
| C16: 1 Palmitoleic | 53.897 | 4.701  | 3.738         |
| C17: 1 Margarinoleic | 3.619  | 0.209  | 0.251         |
| C18: 0 Stearin  | 66.458  | 2.858  | 4.609         |
| C18: 1n9e Oleic | 204.475 | 11.538 | 14.180        |
| C18: 2n6e Linoleic | 141.070 | 10.535 | 9.783         |
| C18: 3n3 Linolenic | 19.145 | 1.197  | 1.328         |
| C20: 0 Arachidic | 2.673   | 0.246  | 0.185         |
| C20: 1 Eicosenic | 9.314   | 0.831  | 0.646         |
| C20: 5n3cis5,8,11,14,17 Eicosapentaenoic | 1.253 | 0.264  | 0.087         |

Results of the fatty acid composition determination using the ChromatekCrystal chromatograph in the biomass of *Hermetia illucens* larvae obtained by the Soxhlet extraction apparatus and manually show that the differences in the indicators were insignificant and were also within the acceptable values.

When studying fat fractions obtained from the biomass of *Hermetia illucens* larvae, toxic elements such as lead, cadmium, arsenic, mercury, iron were not detected in the sample, or they did not exceed the established indicators (Table 5).

Table 5. Results of the fat fractions study for the presence of toxic elements

| Indicator              | Results          | Error of test results | ND for test methods (measurements) * |
|------------------------|------------------|-----------------------|-------------------------------------|
| Toxic elements, mg / kg | Less than 0.1    | Not detected          | GOST 30692-2000                     |
| Lead                   | Not detected     | -                     |                                     |
| Cadmium                | Less than 0.1    | Not detected          | GOST 30692-2000                     |
| Arsenic                | Less than 0.01   | Not detected          | GOST R 51766-2001                   |
| Mercury                | Less than 0.002  | Not detected          | GOST R 53183-2008                   |
| Iron                   | Less than 250 g/t | Not detected          | GOST 26573.2-2014                   |
| Zinc                   | Less than 1.0    | Not detected          | GOST 30692-2000                     |

The results of determining acute toxicity by administering the test sample to white nonlinear male mice are shown in Table 6. As follows from the data in the table, the administration of the test sample...
at doses of 6000, 8000 and 10000 mg/kg in the dosage form did not lead to the death of the animals. In animals that were injected intragastrically with "Sample No. 1" at doses of 6000 and 8000 mg/kg of body weight according to the dosage form, symptoms of intoxication were not observed. At the same time, in white nonlinear mice after administration of the drug at a dose of 10,000 mg/kg, depression was noted, they were hypodynamic. These symptoms disappeared within 1-2 hours after administration. Subsequently, the experimental mice did not differ from the control animals.

In the control group of animals, which were injected with the control substance in the maximum allowable volumes, mortality and signs of intoxication were not observed.

Table 6. Results of acute toxicity studies after a single intragastric administration of FESA (sample No. 1) to white non-linear male mice

| Sample dose (mg/kg) | Number of mice in the experiment | The number of mice killed after a single injection of the drug in various doses every other day | Result |
|--------------------|----------------------------------|-------------------------------------------------|--------|
| 6000               | 6                                | 0 0 0 0 0 0 0 0                                | 0/6    |
| 8000               | 6                                | 0 0 0 0 0 0 0 0                                | 0/6    |
| 10000              | 6                                | 0 0 0 0 0 0 0 0                                | 0/6    |
| Control            | 6                                | 0 0 0 0 0 0 0 0                                | 0/6    |

After a single intragastric administration of fatty acid esters (sample 1), the death of animals in all groups was absent throughout the entire observation period, LD100 could not be established, LD50 is more than 10000 mg/kg. Consequently, esters of fatty acids obtained from the biomass of *Hermetia illucens* larvae according to the degree of impact on the body according to GOST 12.1.007 belong to the 4th hazard class - low-hazard substances.

4. Conclusion

Thus, it was revealed that the acid and peroxide values of the fat fraction obtained from the biomass of *Hermetia illucens* larvae are within the limits established by the norm, which indicates the high quality of this product. The fatty acid composition is represented mainly by lauric, palmitic, oleic and myristic acids. It was shown that the differences in fatty acid composition of the samples, obtained from the biomass of *Hermetia illucens* larvae using the Soxhlet extraction apparatus and manually, were insignificant and within the acceptable values. In the study of presence of toxic elements in fat fractions, lead, cadmium, arsenic, mercury, iron were not detected or they did not exceed the established values. When studying the acute toxicity of fatty acid esters, it was proved that in terms of the degree of impact on the body, they belong to the 4th hazard class - low-hazard substances according to GOST 12.1.007. Consequently, the substances of fatty acid esters from the biomass of *Hermetia illucens* larvae have a high potential for further use in various fields of industry, including production of food and feed.

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