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ПРЕДВАРИТЕЛЬНОЕ ИССЛЕДОВАНИЕ СУХИХ ЛИЧИНОК ЗЕЛЁНОЙ МУХИ ПАДАЛЬНИЦЫ LUCILIA CAESAR «РЕСУРСОСБЕРЖЕНИЕ / ДИВЕРСИФИКАЦИЯ»

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Аннотация. *Зеленая муха падальница (Lucilia caesar) относится к семейству отряда двухкрылых Calliphoridae. Было проведено исследование с целью изучения приблизительного состава и возможностей использования личинок зеленой мухи падальницы путем обезжиривания растворителем. На основании предварительных анализов, содержание сырого белка в массе из личинок зеленой мухи падальницы значительно увеличилось с 45,20 до 64,95% после обезжиривания. Аналогичным образом содержание золы и влаги в образцах увеличилось до 7,10 и 4,95%, соответственно. Кроме того, выход масла из зеленой мухи падальницы составил 31,03%. Было зарегистрировано увеличение содержания углеводов, включая клетчатку, с 16,03 до 23,01% после обезжиривания, что указывает на присутствие в личинках хитина. Полученные данные показывают возможность использования личинок зеленой мухи падальницы в качестве источника белка путем его концентрирования в кормах для животных, маслах для использования в биодизельном топливе, а также волокнах/хитине для производства продукта промышленного использования. Таким образом, дальнейшее детальное исследование личинок зеленой мухи падальницы (Lucilia caesar) является актуальным.*

Ключевые слова: зелёная муха падальница, личинки, Lucilia caesar, содержание белка, обезжиривание, хитин

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Original article

PRELIMINARY STUDY ON DRIED GREEN BOTTLE FLY LUCILIA CAESAR "RESOURCE SAVING/DIVERSIFICATION"

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Abstract.*The green bottle fly (GBF) (Lucilia caesar) belongs to family Calliphoridae in order Diptera. A simple study was conducted to investigate the proximate composition and examine the green bottle fly larvae utilization possibilities by solvent defatting. Based on the proximate analyses, the crude protein content of ground green bottle fly larvae paste had significantly increased from 45.20 to 64.95 % after defatting. In similar manner, the ash and moisture content of the samples also increased to 7.10 and 4.95 % respectively. Additionally, the yield of extracted of green bottle fly was 31.03 %. The carbohydrate including fiber content have been recorded to increase from 16.03 to 23.01 % after defatting that those values signify the presence of chitin in the larvae. The findings indicate the possibilities of utilizing green bottle fly larvae in producing diversified products such as source of protein by concentrating for animal feed ingredients; as oil source for use in biodiesel trails; and sources of fiber/chitin to produce chitin derived product for industrial use. Thus, the study recommends further detailed research on the larvae.*

Keywords: *green bottle fly, larvae, Lucilia caesar, proximate, protein content, defatting, chitin*

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Introduction

Insects are among the diverse and abundant class of the phylum arthropods. The diversity and abundance of insects, is beyond their eco-logical and environmental role extended for industrial applications. Nowadays insects are becoming one of the promising resources viable and sustainable protein sources and are also source of lipid and chitin components. Insects have been reported to contain considerable amount of essential amino acids, fatty acids, vitamins, and mineral for their application in food and animal feed [1, 2]. The protein content of insects ranges from 10,3 % in raw samples to 82,6 % in protein concentrates [1, 3, 4]. The protein content vary with species, developmental stages and source environment. For instance, protein content of Eulepidamashona (beetle) 13,85–16,54 % and Henicuswhellani (cricket) 14,66–20,96 % [5], five insect species 53,2 to 58,3 % [6], tree locust flour protein content of 6,24–67,75 % [7], Tenebrio molitor (mealworm) meals range 51,8–59 % and Hermetiaillucens meals 49,9–58,8 % [8], raw dried BSF flour (Hermetiaillucens) 42 % [9], T. molitor (mealworm) before defatting 52 % and after defatting 76,5 % [10], and freeze dried migratory locust (Locustamigratoria) 65,87 % and 82,26 % for protein concentrates [4]. The results of those findings indicates the protein content of insect species varies with the application of processing methods

such as size reduction, drying, filtration, defatting etc. therefore, the results are comparable with the plant and other animal origin food products and signify their potential in replacing some previous meals.

The demand for energy are one of the push-ing factors to discover of fuel sources, among which the green energy in biodiesel/fuel production is to reduce environmental concerns [11, 12]. Compared to petroleum sources, bio-diesel is ecofriendly, renewable, reduces coun-tries dependence on imported petroleum prod-uct. For those reason, insects have been target-ed for oil sources due to their fat content of 10 to 60 % [8, 13], good bio-converters of wastes for clean production, short life cycle, and high reproduction rate [14, 15]. The larval developmental stage has higher fat than adults that is present in different forms such as triacylglycerols, cholesterol, and others. The fat content of insect species varies with the application of processing methods. This is mainly, the processing by defatting either chemically or mechanically, filtration to obtain oil that may be used in biodiesel production. Some of the reports of insects as potential source of oil, fat content of raw dried BSF flour (Hermetiaillucens) 36,2 % [9], T. molitor (mealworm) 30,8 % [10], Tenebrio molitor (mealworm) meals range 16,6–29,8 % and Hermetiaillucens meals 11,3–29,0 % [8], termite (Macrotermes sub-hylanus) 19,8–42,3 % and grasshoppers (Ruspoliadifferens) 12,8–43,1 % [16], five insect species 11,9 to 34,5 % [6], freeze dried migratory locust (Locustamigratoria) 23,81 % [4]. The findings of the researcher is interesting and leads to the selection of potent species and search of new potential species.

The rising interest in insects as protein and oil sources is reflected to increasing scientific literatures, but regarding the green bottle fly lar-vae (GBFL) (Luciliacaesar) is almost no infor-mation.

The genus Lucilia are well known as scavengerinsects, belongs to family Calliphoridae in order Diptera. Regarding this species, very few previous preliminary researches in Russian federation have demonstrated that protein-lipid preparation from dried and crushed fly larvae of the species Luciliacaesar as promising feed alternative resource containing 44,2 % of crude protein, and crude fat of 11,6 % [17]. The finding suggests further research on this species to ex-plore its potential use.

Therefore, in this paper, we explored the protein and oil potential of dried green bottle fly larvae (GBFL) by chemical defatting. At the same time, its ash and moisture composition of the GBFL and predicting the presence of chitin.

Materials and methods

Samples, chemicals/reagents Allchemicals and reagents were of analytical grade. Water used in the experiments was deionized water.

Sample of whey protein isolate powder (WPI, Lithuania) used in the present study was acquired from a commercial retailer specialized on nutritional supplements that was used as control.

Samples of Green bottle fly larvae Sam-ples of Green bottle fly larvae (L. caesar) were obtained from the enterprise ‘New Biotechnolo-gies’ LLC (Lipetsk, Russian Federation), that were dried to a range of 60–70 ºC temperature until the moisture content of the biomass reach-es less than 4 % [17]. This dried protein-lipid preparation commercially named as ‘Zooprotein’ were brought packed in sterile plastic containers to the laboratory of Faculty of Biotechnology of ITMO University.

Dried insects paste and flour preparation The dried GBF larvae were unpacked and ground in Grind-mix grinder (type GM200, Retsch GmbH, Germany) for 10 minutes at 6000 RPM and repeated five times. It was manually sieved to pass through 1mm mesh size and di-vided into two parts. One part (10 g) was defatted with n-hexane in ratio (1 : 5) at room temperature [15] with some modifications. After 48 h, the liquid part with n-hexane was transferred to another beaker that Evaporation of n-hexane and water droplets was done at 105 ºC in a water bath for 3–4 h. Then, the remaining liquid was centrifuged at 13000 RPM for 15 minutes to separate the fat and residues. The solid matrix (not soluble in n-hexane was dried in at 37ºC for 2 h and then homogenized. Here, two components were obtained, one dried fat extracted flour and a second one, crude fat. These samples were weighed to determine yield and fat content of samples. And the other (second) part was only dried sample without defatting. Then, both of them, ground non-defatted paste and defatted powder GBFL were kept properly and analyzed within 2 weeks.

Proximate / Chemical Analysis The crude protein content of samples was determined by the Kjeldahl method according AOAC method 981,10 for green bottle fly larvae and AOAC method 991,20 for whey protein isolate. A 0,5–2 g samples (both non-defatted ground paste GBFL and defatted powder GBFL) were weighed and transferred to digestion tubes/flask and samples were in triplicate. Sample digestion, distillation and titration was carried out in Kjeld-hal set up (VELP-Scientifica-UDK 159 instru-ment). The percentage of nitrogen was convert-ed to crude protein by multiplying with Nitrogen factor 6,25 for green

bottle fly larvae and with Nitrogen factor 6,38 for whey protein isolate.

The moisture content was conducted in automatic moisture/balance analyzer (type-MOC 120H, Shimadzu Corp., Japan). Around 1–2 g of sample from both non-defatted ground paste GBFL and defatted powder GBFL in triplicate were weighed in analytical balance. Then the samples were placed in the heating pan of au-tomatic analyzer and heated at 100 ºC for 3–8 minutes. The moisture content in percentage was automatically displayed and recorded for each sample.

Ash content was determined gravimetrically on incineration according to the AOAC standard methods 942.05 [18] as described in Zhang et al. [19]. The non-defatted ground paste GBFL and defatted powder GBFL samples were weighed in 1–2 g in triplicate. The samples used for moisture analysis were used to determine ash content, that were burned in hot plate at 150–170 ºC in fume hood until the smoking com-pletely stops. After the preheating combustion is completed, it will be transferred to a muffle furnace (model LOIP LF 9/13 G2, LOIR, Russia) for at least 4 h at 550–600 ºC or until constant weight. The crucible were allowed to cool in des-iccator for 30 min and weighed to the nearest digits. The ash content was calculated from the percentage of ash resi-due compared to the initial weight of the moisture-free sample:

$$\%Ash = M_1 \times 100 / M_2 \ , \qquad (1)$$

where M1 – weight of residue, g; M2 – sample weigh, g.

The method of extraction is described in previous sections [15].

The extracted crude oil percentage of samplesis detailed here, that the final fat obtained was calculated by difference of the final weight of the sample with flask minus the weight of initial sample with flask divided by initial weight of sample without flask. It was ex-pressed in percentage.

Carbohydrate was obtained by difference (100 – sum of moisture, protein, fat, crude fibre/chitin and ash) [18]. Carbohydrate content was calculated by the following equation:

Carbohydrate (%) = (100 – (moisture + ash + crude fat + crude protein + fiber/chitin)) x 100 %

(Carbohydrate +crude fiber/chitin) = 100 – (moisture + ash + crude fat + crude protein).

Statistical analysis. Results were analyzed and expressed as means ± standard deviation of the replicates. Analysis of variance (ANOVA) was performed to determine significance differences between treatments. Post hoc analysis (Tukey – Duncan) were performed to determine significantly different treatments at 5 % level of significance ($p < 0,05$). Analysis was done using SPSS software version 20.

Results and discussion

Physical observation and Defatting of GBFL

Based on the physical observation of dried GBFL, it was initially with distinct smell after unpacking from its package. The dried GBFL pieces had color ranges of light yellow, light grey, light brown, black against a white background surface to the naked eye of the observer (Fig. 1, *a*). They had elongated shape and firmer to touch. Up on grinding, the dried GBFL was ob-served to the leaching of oily substance to the surface of the grinder. When the ground GBFL mass passed through a less than 1 mm mesh sieve, it was placed in paper surface and was examined to stain the paper with oily substance and had to look like dark yellow colored paste item (Fig. 2, *b*.). The ground GBFL oil was ex-tracted using n-hexane. The extracted crude oil was observed to light yellow coloration up on placing 2 ml microfuge (Fig. 2, *c*.). The solid resi-due of GBFL was changed to a powder up on evaporation of n-hexane. Defatted GBFL powder had found to have creamy color with little smell of the initial dried GBFL (Fig. 2, *d*.). There are several methods of extraction of crude oil such as mechanical, filtration, solvents or combination. Solvent extraction is simple for defatting at laboratory basis and there are different types of differing in degree of polarity properties and they can give different yield of larval crude oil [20]. Among which, in this study n-hexane was selected due to its availability As indicated in Table 1, the yield of dried GBFL derived oil was 31,03 % that is in similar raw dried BSF flour (Hermetiaillucens, 36,2 %) [9], T. molitor (mealworm; 30,8 %) [10], but it seems low as compared to the previous of research. In one study, ethanol, acetone and petroleum ether extraction from black soldier fly larva have reported 50 to 60 % of crude oil extract, in which ethanol had been recorded to have highest value than petroleum ether and lowest acetone [20]. In black soldier fly, it was reported to have nearly 40 % of oil content in black soldier fly larvae biomass by mechanical pressing, and combination of pressing and solvent extraction removed about 90 % of total oil content [14]. The lower crude oil yield in this study could be the method extraction, type of solvent used and substrate/species. This could help to suggest for further study and improvement of research depth.

Table 1 – Chemical composition of research samples

Таблица 1 – Химический состав исследуемых образцов

Analysis	Non-defatted ground paste GBFL	Defattedpowder GBFL	Wheyproteinisolate (control)
	Mean ± SD	Mean ± SD	Mean ± SD
Crudeprotein (%)	45,20 ± 1,01[a]	64,95 ± 0,7[b]	89,40 ± 0,78[c]
CrudeAsh (%)	5,30 ± 0,17[a]	7,10 ± 0,13[b]	3,02 ± 0,1[c]
Moisture (%)	2,44 ± 0,04[a]	4,95 ± 0,06[b]	5,77 ± 0,11[c]
Yieldof Oilextracted	31,03 ± 0,65	notdetectablelevel	notdetectablelevel
Carbohydrate + crudefiber / chitin	16,03 ± 1,54[a]	23,01 ± 0,72[b]	1,80 ± 0,73[c]
Note: Values in the same row and subtable not sharing the same subscript are significantly different at p < 0,05 in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances. Tests are adjusted for all pairwise comparisons within a row of each innermost subtable using the Bonferroni correction.			

Proximate Composition of GBFL

The protein content of ground non-defatted GBFL paste, defatted GBFL powder, and whey protein isolate are summarized in Table 1. There was significant (p < 0,05) difference in the crude protein content among the ground non-defatted GBFL paste, defatted GBFL powder, and whey protein isolate. The crude protein content was higher defatted GBFL powder than the ground non-defatted GBFL paste, but was highest in the whey protein isolate (control). The protein content of defatted GBFL powder and ground non-defatted GBFL paste obtained in this study was within the range of reported studies [1, 4, 6]. The ground non-defatted GBFL paste was 45,2 % which is comparable with results of dried green bottle fly larvae (44,2 %) reported by [17] and raw dried BSF flour (Hermetiaillucens) (42 %) reported by Huang et al., [9]. Defatting was found to increase the level of protein content to 64,95 % in the present study.

(a) (b)

(c) (d)

(a) dried GBFL (4 % moisture);
(b) oily ground non-defatted GBFL paste;
(c) creamy defatted defatted GBFL powder;
(d) crude oil extract by n-hexane in microfuge

Figure 1 – Research samples

Similarly, defat-ting increased the crude protein of T. molitor (mealworm) to 76,5 % [10], of migratory locust (Locustamigrato-ria) protein concentrates to 82,26 % [4] and black soldier fly prepupae meal to 63,9 % [14].

The results indicate that the crude protein content of the ground non-defatted GBFL paste, defatted GBFL powder are comparable with the most common protein source in animal feeds such as soybean meal and fishmeal; and other insects. Moreover, the removal of crude fat from BSF prepupae not only improved the crude protein content of GBFL but also the removal of crude fat from high fat containing feedstuffs is important in mixing/processing of animal feeds ingredients and also to improve the storability in reducing oxidation level. Further, fat removal may increase of protein digestibility of the insect derived feed or concentrates.

The moisture of ground non-defatted GBFL paste, defatted GBFL powder, and whey protein isolate are given in Table 1. The moisture content of ground non-defatted GBFL paste, defatted GBFL powder, and whey protein isolate are 2,44 %, 4,95 % and 5,77 %, respectively. Moisture content of ground non-defatted GBFL paste and defatted GBFL powder are comparable to the values reported in literature. Moisture content obtained in cricket powder (6,79 %) [21], and another cricket 6,40 % in Paiko et al. [22] and Zooprotein of L.ceasar 12 % [17]. The level of moisture content lower 10 % is good for the storage of high protein products to avoid any chemical changes and microbial activity. This could signify for products of defatted GBFL for further us-age as animal feed ingredient.

From nutritional point of view, ash con-tent level is important for source essential minerals such calcium, magnesium, iron, potassium etc for the functioning of cell of an organism. The ash content of ground non-defatted GBFL paste, defatted GBFL powder, and whey protein isolate are presented in Table 1, in which there was indicated significant ($p < 0,05$) difference in the ash content among the ground non-defatted GBFL paste, defatted GBFL powder, and whey protein isolate. The higher ash content in defat-ted GBFL than ground non-defatted GBFL paste may be the removal of fat cause to change in proximate constituent's proportion. The results of ash of this study were 5,3 % for ground non-defatted GBFL paste and 7,1 % for defatted GBFL. Other studies have documented on ash content of 4,65 % in T. molitor larvae flour [23], and of 4,9 % in T. molitor again [24]. The slight difference observed in comparison to the results reported by previous researches may be due to differences in rearing conditions, type of species, feed compositions, different sample preparation and analysis techniques.

As indicated in Table 1, the carbohydrate including fiber had significant difference ($p < 0,05$) among the three samples examined. It can be suggested that the defatting of GBFL samples caused to increase from 16,03 % to 23,01 %. The results of non-defatted GBFL carbohydrate content plus fiber are comparable with findings of other insects species studied. In one research paper, the fiber content (carbohydrate) had reported in range of 4,03 % to 11,06 % in larvae of Alomyrinadichotoma; Protaetiabrevitarsis; and T. molitor; and 9,53 % to 10,37 % in adults Telogryllusemma, and Gryllusbimaculatus [6]. Bednářová, [13] have also reported the fiber content of seven insect species to range 8–27 %, in which the highest value was documented in African migratory locust (Locustamigratoria). In another study of L. caesar larvae, the crude fiber was reported to be 8,1 % [17]. The slight variation of carbohydrate/ crude fiber among research reports may be due to the developmental stages, methods of analysis; and species. However, the results of this study carbohydrate including fiber for both non-defatted and defatted GBFL are good indicators of for the presence of quantifiable amount chitin that is the most common insoluble form of fiber in the body of insects contained mainly in their exoskeleton. This finding leads to further study for the extraction of chitin derived products from GBFL and the removal of chitin-antinutritional factor furthermore may improve extracted insect protein digestibility.

Conclusion

The present study has found that GBFL has appreciable quantity of proximate constituents for its use in product diversification. In this, the protein content of non-defatted GBFL signifies for its use in animal nutrition. The solvent defat-ting of GBFL samples also further improved the protein content. The lower moisture content also seems to be essential for increased shelflife of higher protein content derived products by re-ducing hydrolytic effect and microbial activity. GBFL derived oil has a good results, which could further be diverted in producing insect biodiesel to be use as commercial ingredient. Moreover, the amount of carbohydrate/fiber in GBFL gave clues to the presence of chitin, as this insoluble component is the main fiber component of in-sect's exoskeleton and is important for industrial use. Overall, GBFL can be utilized in producing of diversified products such as protein concentrates, oil sources and chitin derived products by successfully implementing efficient technology for generating the products

with simultaneous organic waste conversion. Thus, further research study on the species is highly recommended.

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