International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Optimization of depigmentation solvent dosage to remove melanin content in black soldier fly (*Hermetia illucens*)

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Abstract

Black soldier fly (BSF) cultivation has increasingly mushroomed in the world recently and BSF in certain phases tends to have an outer skeleton which is often called an exoskeleton which is generally black and contains melanin, chitin and chitosan. However, the melanin content in the BSF shell will be an obstacle in producing pure chitin and chitosan. So, removing the melanin content in the BSF shell needs to be done by depigmentation. This research aims to optimize the dose of depigmentation solvent to remove melanin content in black soldier flies (*Hermetia illucens*). This research uses various phases of BSF and BSF growing media. The BSF phases used were the prepupa and BSF exuviae phases and the BSF growing media used were fruit waste media from cultivator A and mixed waste media such as chicken feces, fish bones, vegetable waste and household waste from cultivator B. This study also used varying doses of depigmentation solvent, namely 3%, 6%, 9%, 12% and 15%. Parameters observed in this study namely BSF color morphology and BSF characterization in the form of color, yield, odor and texture. The research results showed that the best dose of depigmentation solvent to remove melanin content in BSF was 12% and the best BSF media was fruit waste media with PFWM extraction results having a white color, yield 17.89%, odorless and in powder form and EFWM had a white color, yield 19.01 %, odorless and in powder form. The 12% depigmentation solvent dosage is very cost efficient.

Keywords: Black Soldier Fly, Chitin, Depigmentation, Exoskeleton, Melanin.

Full length article *Corresponding Author*, e-mail: yunilas@usu.ac.id Doi # https://doi.org/10.62877/108-IJCBS-24-25-19-108

1. Introduction

Cultivation black soldier fly (*BSF*) has now been widely carried out by the public in various media and BSF research has also been carried out by many researchers in various phases throughout the world. BSF has several life phases, one of which is the prepupa and pupa phases. In the prepupa and pupa stages, BSF tends to have an external skeleton or exoskeleton [1]. BSF exoskeletons are generally black in color indicating the presence of melanin and BSF exoskeletons have great potential as a source of chitin and chitosan for insect-based futures which are useful in various industrial fields such as agriculture, fisheries, animal science, pharmacy and medicine which are not found in crustaceans [2-5]. Melanin is a black pigment with a high molecular weight that is formed through the oxidation and polymerization of phenolic compounds which consist of two types, namely dark pigmented eumelanin and light pigmented pheomelanin [6-7]. Melanin in BSF is found since entering the prepupal stage, because BSF actively produces eumelanin which is a brown to black pigment that can generally be found in the exoskeleton BSF [8-10].

Chitin and chitosan production can be carried out by extraction through several stages, namely deproteination, demineralization, depigmentation and deacetylation and by several methods, namely chemical and biological [11-13]. However, the presence of melanin in the BSF exoskeleton will be an obstacle in producing pure chitin and chitosan. Thus, removing the melanin content in the BSF exoskeleton needs to be done by depigmentation [14]. In addition, in optimal removal of melanin content in the BSF exoskeleton, you must pay attention to the efficiency of the dose of solvent used because it will affect costs. Based on this, the author conducted research related to optimizing the dose of depigmentation solvent to remove melanin content black soldier fly (*Hermetia illucens*).

2. Materials and methods

2.1. Materials

This research uses various phases of BSF and BSF growing media. The BSF phases used are the prepupa phase (18-25 days old) and BSF exuviae and the BSF growing media used are fruit waste media from cultivator A and mixed waste media such as chicken feces, fish bones, vegetable waste and household waste from cultivator B.

2.2. BSF Extraction

BSF extraction refers to [15]. BSF prepupa and BSF exuviae were sorted, then cleaned of foreign objects and washed with clean water. BSF prepupae were boiled with boiling water at 100^{O}C for 10-15 minutes and dried for 24 hours in the oven at 60^{O}C. Meanwhile, BSF exuviae were immediately dried for 24 hours in an oven at a temperature of 60^{O}C. Then the dried BSF prepupa and BSF exuviae are each put in an erlenmeyer then dissolved with 1N HCl and heated with *hotplate* for 20 minutes at 100^{O}C for demineralization process. The precipitate was washed with *Aquadest* to pH 6.8-7.0. Then soak the precipitate in 3.5% NaOH for 24 hours and heat with *hotplate* at a temperature of 80^{O}C for 1 hour for the deproteination process, then filtered with Whatman No. filter paper. 40. The resulting residue is washed with *Aquadest* until the pH is neutral. Then depigmentation was carried out using NaOCl with varying doses, namely 3%, 6%, 9%, 12% and 15% (soaked for 30 minutes), then washed with distilled water and filtered then dried in an oven at 60^{O}C for 24 hours. The resulting particles are called chitin.

2.3. BSF Morphology

BSF morphology refers to [16]. Samples were taken of seven BSF prepupae and seven BSF exuviae, and the BSF prepupa and BSF exuviae were arranged on rough, smooth paper, then the differences in color of the BSF prepupa and BSF exuviae were observed.

2.4. Characterization of BSF Chitin

Characterization consists of color, texture, odor and yield. Color, texture and smell are observed visually. Whereas The chitin yield obtained from the extraction results can be calculated using the formula below:

$$\text{Chitin yield (\%)} = \frac{chitin\ mass}{dry\ biomass\ mass} \times 100\%$$

3. Results and Discussions

3.1. BSF Morphology

Observing BSF morphology is observing the shape of the outside of an organism's body, by observing the physical shape of the BSF prepupa and BSF exuviae, namely the color, to determine the effect of using different growing media on the BSF produced. The color morphology of BSF in this study produced various colors, namely BSF prepupa fruit waste media (PFWM) was brown, BSF prepupa mixed waste media (PMWM) was blackish brown, BSF exuviae fruit waste media (EFWM) was black and BSF exuviae mixed waste media (EMWM) is very black. Changes in the color morphology of BSF prepupae and BSF exuviae showed varying results due to the melanin pigment content attached to the BSF exoskeleton. The different BSF color morphology occurs due to the influence of the media used and the color of the animal's body is related to the pigment cells in the animal's body. This can occur because it is influenced by the presence of pigments (natural dyes) in the food, which are then synthesized by the body. animals that consume it. This is in accordance with the statement by Tu et al., (2022) that the color of BSF can be influenced by the media used when cultivating it [17]. The melanin content in the BSF exoskeleton also provides good resistance. Jana and Mukherjee (2014); Cordero and Casadevall (2017) stated that melanin provides resistance to insects because of its toxicity [18-19]. Ushakova et al., (2019) added that melanin plays an important role in the animal body to protect against the damaging effects of extreme environmental factors [20]. Apart from that, the color morphology of BSF, namely brown to black, functions as a means of protecting against ultraviolet (UV) radiation. Burraco and Orizaola (2022) stated that the black color of BSF is a means of protecting against ultraviolet (UV) radiation and as a form of camouflage [21]. The color morphology of BSF is shown in Table 1.

Table 1: BSF morphology.

Sample	Color
PMLB	Chocolate
PMLC	Blackish Brown
EMLB	Black
EMLC	Very Black

3.2. Characterization of BSF Chitin

The characteristics of BSF chitin including color, odor, texture and yield are presented in Table 2. This research produced various colors, namely PFWM 3% (very brown), 6% (brown), 9% (slightly brown), 12% (white) and 15% (very white). PMWM color 3% (very blackish brown), 6% (blackish brown), 9% (slightly blackish brown), 12% (brown) and 15% (white), EFWM color 3% (black), 6% (slightly black), 9% (brownish white), 12% (white) and 15% (very white) and colored EMWM 3% (very black), 6% (black), 9% (slightly black), 12% (brownish white) and 15% (white). NaOCl solution at a dose of 12% is the best dose because the color, smell and texture produced meet the chitin quality standards according to [22] is brownish white to white in color, odorless and in powder form. The yield of chitin produced in a 12% NaOCl solution varied, namely PFWM 17.89%, PMWM 18.17%, EFWM 19.01% and EMWM 19.27%.

Table 2: Characterization of BSF chitin.

Parameter	NaOCl Dosage (%)				
	3	6	9	12	15
PFWM					
Color	Very Chocolate	Chocolate	A little chocolate	White	Very White
Yield (%)	-	-	-	17.89	-
Odor	-	-	-	Odorless	-
Texture	-	-	-	Powder	-
PMWM					
Color	Very dark brown	Blackish Brown	A little dark brown	Brownish white	White
Yield (%)	-	-	-	18.17	-
Odor	-	-	-	Odorless	-
Texture	-	-	-	Powder	-
EFWM					
Color	Black	A little black	Brownish white	White	Very White
Yield (%)	-	-	-	19.01	-
Odor	-	-	-	Odorless	-
Texture	-	-	-	Powder	-
EMWM					
Color	Very Black	Black	Little Black	Brownish white	White
Yield (%)	-	-	-	19.27	-
Odor	-	-	-	Odorless	-
Texture	-	-	-	Powder	-

The yield resulting from this research (PFWM 17.89% and PMWM 18.17%) is the same as research by Nasution et al., (2020), namely 18.05%, because it uses 0.315% NaOCl [23]. Likewise, research by Mirwandhono et al., (2022) was 18.05%, because it used 0.315% NaOCl [15]. However, the yield produced in this research was greater than the research by Yunilas et al., (2023), namely 11.60-14.60%, because it used 0.315% NaOCl [24]. Meanwhile, the yield resulting from this research (EFWM 19.01% and EMWM 19.27%) is greater than research by Lin et al., (2021) namely 12.4%, because it uses H_2O_2 30% [25]. Likewise, research by Zlotko et al., (2021) was 5.7-8.0%, because it used 1% H_2O_2, $MKnO_4$, $C_2H_2O_4$ [26].

4. Conclusions

Based on the research results, it shows that the best dose of depigmentation solvent to remove melanin content in BSF is 12% and the best BSF media is fruit waste media with PFWM extraction results having a white color, yield 17.89%, odorless and in powder form and EFWM has a white color, yield 19.01%, odorless and in powder form. The 12% depigmentation solvent dosage is very cost efficient.

References

[1] E. Mirwandhono, Yunilas, N. Ginting, G. A. W. Siregar, M. I. A. Nasution, S. Wahyuni, Siswanto (2024). Isolation and characterization of chitosan from black soldier fly exuviae. IOP Conference Series Earth and Environmental Science. 1362 012013

[2] C. S. Wu, S. S. Wang. (2018). Bio-based electrospun nanofiber of polyhydroxyalkanoate modified with black soldier fly's pupa shell with antibacterial and cytocompatibility properties. ACS Applied Materials & Interfaces. 10: 42127-42135.

[3] L. Bastiaens, L. Soetemans, E. D'Hondt, K. Elst. (2019). Sources of chitin and chitosan and their isolation. In Chitin and Chitosan: Properties and Applications. 1-34.

[4] A. Khayrova, S. Lopatin, V. Varlamov. (2021). Obtaining chitin, chitosan, and their melanin complexes from insects. International Journal of Biological Macromolecules. 167: 1319-1328.

[5] K. Y. Barragan-Fonseca, L. O. Greenberg, G. Gort, M. Dicke, J. J. A. V. Loon. (2023). Amending soil with insect exuviae improves herbivore tolerance, pollinator attraction and seed yield of Brassica nigra plants. Agriculture, Ecosystems and Environment. 342: 1-9.

[6] F. Solano. (2014). Melanins: skin pigments and much more-types, structural models, biological functions, and formation routes. New Journal of Science. 2014: 1-28.

[7] A. Y. Glagoleva, O. Y. Shoeva, E. Khlestkina. (2020). Melanin pigment in plants: current knowledge and future perspectives. Frontiers in Plant Science. 11: 770.

[8] M. Sugumaran, H. Barek. (2016). Critical analysis of the melanogenic pathway in insects and higher animals. International Journal of Molecular Sciences. 17: 1753.

[9] N. A. Ushakova, A. E. Dontsov, A. I. Bastrakov, N. Y. Garmash, D. S. Pavlov. (2017). Paramagnetics melanin and Mn2+ in black soldier fly *Hermetia illucens*. Doklady Biochemistry Biophysics. 473: 102-105.

[10] U. D'Amora, A. Soriente, A. Ronca, S. Scialla, M. Perrella, P. Manini, J. W. Phua, C. Ottenheim, R. D. Girolamo, A. Pezzella, M. G. Raucci, L. Ambrosio. (2022). Eumelanin from the black soldier fly as

sustainable biomaterial: characterisation and functional benefits in tissue-engineered composite scaffolds. Biomedicines. 10: 2945.
[11] H. Ding, L. Lv, Z. Wang, L. Liu. (2020). Study on the “glutamic acid-enzymolysis” process for extracting chitin from crab shell waste and its by-product recovery. Applied Biochemistry Biotechnology. 190: 1074-1091.
[12] K. Mohan, A. R. Ganesan, T. Muralisankar, R. Jayakumar, P. Sathishkumar, V. Uthayakumar, R. Chandirasekar, N. Revathi. (2020). Recent insights into the extraction, characterization, and bioactivities of chitin and chitosan from insects. Trends in Food Science & Technology. 105: 17-42.
[13] T. Behiye, O. Hakan, A. Ahmet, Q. Tugba, B. M. Ozkan, T. Mesut. (2021). Preparation of chitosan from waste shrimp shells fermented with *Paenibacillus jamilae* BAT1. International Journal of Biological Macromolecules. 183: 1191-1199.
[14] A. Khayrova, S. Lopatin. (2022). The potential of Hermetia illucens as a source of chitin, chitosan and their melanin complexes. Polymer Science: Peer Review Journal. 3: 1-7.
[15] E. Mirwandhono, M. I. A. Nasution, Yunilas. (2022). Extraction of chitin and chitosan black soldier fly (*Hermetia illucens*) prepupa phase on characterization and yield. IOP Conference Series Earth and Environmental Science. 1114 012019.
[16] R. H. K. Yusuf, F. Fathul, R. Sutrisna, L. Liman. (2023). Pengaruh berbagai media terhadap morfologi (warna, panjang, lebar), produksi per ekor, segar dan bahan kering maggot black soldier fly. Jurnal Riset dan Inovasi Peternakan. 7: 287-297.
[17] N. P. C. Tu, N. N. Ha, N. T. T. Linh, N. N. Tri. (2022). Effect of astaxanthin and spirulina levels in black soldier fly larvae meal-based diets on growth performance and skin pigmentation in discus fish, Symphysodon sp. Aquaculture. 553: 738048.
[18] B. K. Jana, S. K. Mukherjee. (2014). Notes on the distributio of phytomelanin layer in higher plants a short communication. Journal of Pharmaceutical Biology. 4: 131-132.
[19] R. J. Cordero, A. Casadevall. (2017). Functions of fungal melanin beyond virulence. Fungal Biology Reviews. 31: 99-112.
[20] N. Ushakova, A. Dontsov, N. Sakina, A. Bastrakov, M. Ostrovsky. (2019). Antioxidative properties of melanins and ommochromes from black soldier fly *Hermetia illucens*. Biomolecules. 9: 408.
[21] P. Burraco, G. Orizaola. (2022). Ionizing radiation and melanism in chornobyl tree frogs. Evolutionary Applications. 15: 1469-1479.
[22] Badan Standarisasi Nasional. (2013). Kitosan-syarat mutu dan pengelolaan SNI 7949-2013 Jakarta (ID): BSN.
[23] M. I. A. Nasution, Yunilas, E. Mirwandhono. (2020). Black soldier fly (*Hermetia illucens*) prepupa phase fermentation by organic acids to decrease chitin content. Jurnal Peternakan Integratif. 8: 159-165.
[24] Yunilas, M. I. A. Nasution, E. Mirwandhono, A. F. Qohar. (2023). Effect of fermentation time and organic acid level on organoleptic quality and chemical components of black soldier fly prepupae silage. Advances in Animal and Veterinary Sciences. 11: 1651-1658.
[25] Y. S. Lin, S. H. Liang, W. L. Lai, J. X. Lee, Y. P. Wang. (2021). Sustainable extraction of chitin from spent pupal shell of black soldier fly. Processes. 9: 976.
[26] K. Zlotko, A. Wasko, D. M. Kaminski, I. Budziak-Wieczorek, P. Bulak, A. Bieganowski. (2021). Isolation of chitin from black soldier fly (*Hermetia illucens*) and its usage to metal sorption. Polymers. 131: 1-6.