PAPER • **OPEN ACCESS**

Chemical composition, chitin and cell wall nitrogen content of Black Soldier Fly (*Hermetia illucens*) larvae after physical and biological treatment

To cite this article: Ainun Nafisah *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **546** 042028

IOP Conf. Series: Materials Science and Engineering **546** (2019) 042028 doi:10.1088/1757-899X/546/4/042028

Chemical composition, chitin and cell wall nitrogen content of Black Soldier Fly (*Hermetia illucens*) larvae after physical and biological treatment

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Abstract. The use of insects as feed may provide a potential solution to overcome the increasingly limited supply of feed, especially protein sources. Black soldier fly (BSF, *Hermetia illucens*) larvae is characterized by its easy production system, rapid growth, able to efficiently convert organic waste and contains high protein, i.e., around 40%. However, the BSF exoskeleton contains chitin which is a component of cell wall and may inhibit the process of nutrient utilization in the digestive tract of ruminants. This experiment aimed to reduce chitin in BSF through physical (separation of the exoskeleton part) and biological (fermentation with chitinolytic bacteria) treatments. There are four treatments tested in this study: BSF larvae control (T1), BSF larvae without exoskeleton (T2), fish meal (T3), fermented BSF larvae using *Bacillus subtilis* ATCC 19659 (T4).Physical and biological treatments were able to reduce crude fiber content from 20.27% (T1) to 4.29% (T2), 13.18% (T4), and there was a decrease in ADF content up to 21.78% (T2) and 19.69% (T4). *Bacillus subtilis* also had lipase enzyme activity, so it can reduce ether extract in BSF larvae by 25.83%. The value of deacetylation degree from chitin isolated without treatment (T1) had a higher value of 66.11. This shows that the fermentation process using chitinolytic bacteria is able to reduce the quality of chitin and change the characteristics of chitin. It can be concluded that physical and biological treatment of BSF prepupa larvae can reduce chitin content, which is indicated by a decrease in crude fiber and fiber fraction (NDF and ADF). Fermentation using chitinolytic bacteria can change chitin characteristics and affect chitin quality.

1. Introduction

Land conversion is one of the factors that threatens the availability of feed for both cereals and forages. In addition to the availability that is not guaranteed throughout the year, especially in the dry season, the quality of forages in the tropics has significant fluctuation due to environmental factors and the diversity of soil quality. Forages, especially grasses, generally have low protein content and some nutrients such as minerals. Leguminosa has a relatively higher protein content than grass, which also experiences limited production due to the narrowing of agricultural land for growing feed crops. This is what causes farmers to depend on feed ingredients for animal protein sources such as fish meal which is more expensive than legumes. The high price of fish meal makes farmers unable to buy or give it in small amounts. In addition, fish meal is also expected to have contamination in the form of heavy metals and pathogenic microbes such as Salmonella. Both of these factors lead to the need for alternative feed

ingredients that are cheap, good quality animal protein sources, do not require extensive land and can be easily supplied.

Insects are animals that can be fed and have high protein content [1] with a good and balanced amino acid profile [2]. The use of insects as feed may provide a potential solution to overcome the increasingly limited supply of feed, especially protein sources. The advantages of insects compared to other food sources from plants and animals are their ability to convert organic waste into a body with a very high level of efficiency with low water requirements [3]. One species of insect that has the potential to be used as feed is black soldier fly (BSF, *Hermetia illucens*) larvae is characterized by its easy production system, rapid growth, able to efficiently convert organic waste and contains high protein, i.e., around 40% [4]. However, the BSF exoskeleton contains chitin which is a component of cell wall. The compound can inhibit the process of nutrient utilization in the digestive tract of ruminants. Therefore, it is necessary to perform an effective treatment on BSF in order to reduce the negative effect of chitin and to simultaneously increase the protein content of BSF larvae. Decreasing chitin in BSF is performed physically (separation of the exoskeleton part) and biologically (fermentation with chitinolytic bacteria).

2. Materials and Methods

The BSF larvae used in this study was the prepupa phase, aged 20-23 days. The treatments given in this study were BSF larvae control (T1), BSF larvae without exoskeleton (T2), fish meal (T3), fermented BSF larvae using *Bacillus subtilis* ATCC 19659 (T4). BSF larvae without exoskeleton were obtained by manually and physically separating between the exoskeleton and the inside of BSF larvae. The fermentation process with *Bacillus subtilis* was carried out by adding *B. subtilis* inoculums as much as 3.8×10^7 cfu/mL (6% dry matter substrate) incubated for 3 days at 40ºC.

Samples were subjected to proximate analysis, i.e. determinations of dry matter (DM), ash, crude protein (CP), ether extract (EE) and crude fiber (CF) by following AOAC procedures [5]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to [6]. Residues obtained in NDF and ADF analyses were continued with CP determination to obtain neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) contents as described by [7]. The results obtained show the percentage of proteins that are on the cell wall of feed ingredients. NDICP and ADICP contribute to the percentage of low-quality protein in feed ingredients, especially ADICP. The higher the level of ADICP, the lower the protein that can be used by rumen microbes and the animals themselves [8].

BSF larvae control (T1) and fermented BSF larvae using *Bacillus subtilis* (T4) were delipidated using hexane for 6 hours. Chitin was isolated according to [9]. Chitin characterized by using FTIR (Fourier-transform infrared spectroscopy). FTIR spectrum analysis for chitin was carried out in the functional group and fingerprint area with a frequency of 4000 cm^{-1}-400 cm^{-1}. The degree of deacetylation (DD) was determined by the formula:

$$DD\ (\%) = 100 - \left[\left(\frac{A_{1650}}{A_{3450}}\right) \times \left(\frac{100}{1{,}33}\right)\right]$$

A1655 shows absorption in the amide band, A3450 shows absorption in the hydroxyl band, and factor 1.33 shows the ratio value of A1655 / A3450 to fully deacetylation degree.

3. Result and Discussion

BSF larvae (T1) had protein content of 42.99% (Table 1). Protein content of BSF larvae is around 40% [4]. The difference in nutrient components in BSF larvae is apparently due to age and substrate multiplication [10]. The second highest component of T1wasether extract, i.e., 36.13%. Crude fiber in BSF larvae is sourced from the exoskeleton, wherein there is also chitin content. Chitin has a broad spectrum distribution such as crustacean skin, insect exoskeleton (25-58%), and fungal cell walls of about 22-40% [11,12]. Chitin contributes to the skeleton composition of insects [13]. One insect that has chitin content is BSF. Chitin composition in BSF is about 5.4% of dry matter [14]. This is also

evident in the T2 treatment which experienced a decrease in crude fiber content of 15.98% from T1. In addition, the separation of the exoskeleton from BSF larvae will increase the percentage component of ether extract.

Fermentation using chitinolytic bacteria (*Bacillus subtilis*) succeeded in reducing crude fiber by 7.09% from the control (T1). In addition to having chitinase enzyme activity [15], *Bacillus subtilis* also produces lipase enzymes at an incubation temperature of 10ºC and continues to increase activity to a temperature of 60ºC [16] and protease enzymes [17]. So that there was also a decrease in crude protein and crude fat content in BSF larvae.

Table 1. Chemical composition (% dry matter) of BSF larvae control (T1), BSF larvae without exoskeleton (T2), fish meal (T3), fermented BSF larvae using *Bacillus subtilis* (T4).

Component	Treatment			
	T1	T2	T3	T4
Ash	20.58	11.04	25.63	12.32
CP	42.99	30.82	64.53	42.05
EE	36.13	56.21	12.16	10.30
CF	20.27	4.29	0.80	13.18
NDF	35.53	38.23	83.30	14.78
ADF	29.27	7.49	1.55	9.58
NDICP (% CP)	27.81	36.70	11.30	10.93
ADICP (% CP)	32.04	23.34	1.01	7.97

ADF, acid detergent fiber; ADICP, acid detergent insoluble crude protein; CF, crude fiber; CP, crude protein;EE, ether extract; NDF, neutral detergent fiber; NDICP, neutral detergent insoluble crude protein.

Chitin isolated from T1 and T2 had the characteristics as presented in Figures 1 and 2. Chitin quality is indicated by the value of deacetylation degree. The higher the value of deacetylation, the better the quality of chitin. DD T1 value is 63.25, and at T4 is 44.11. The decrease in chitin quality was caused by the presence of chitinase activity by *Bacillus subtilis*.

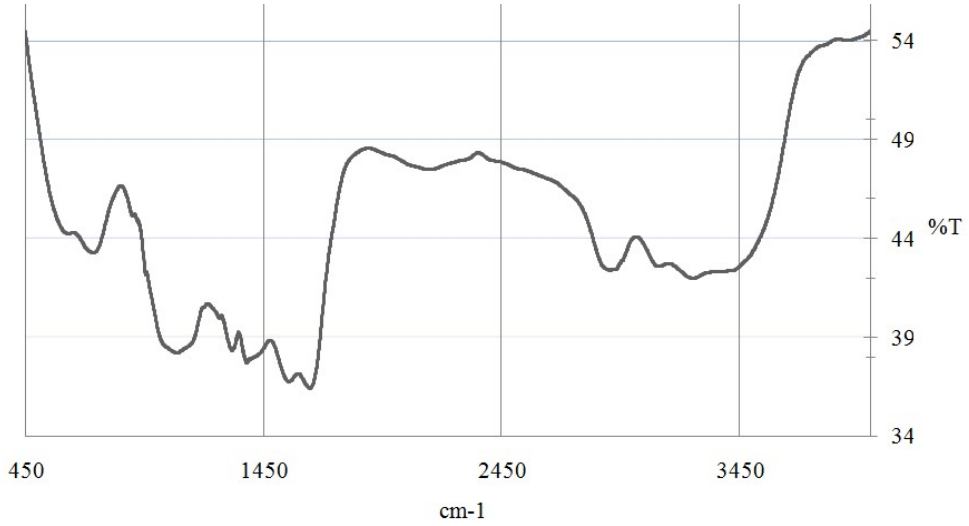

Figure 1. FTIR spectra of chitin larva BSF (T1).

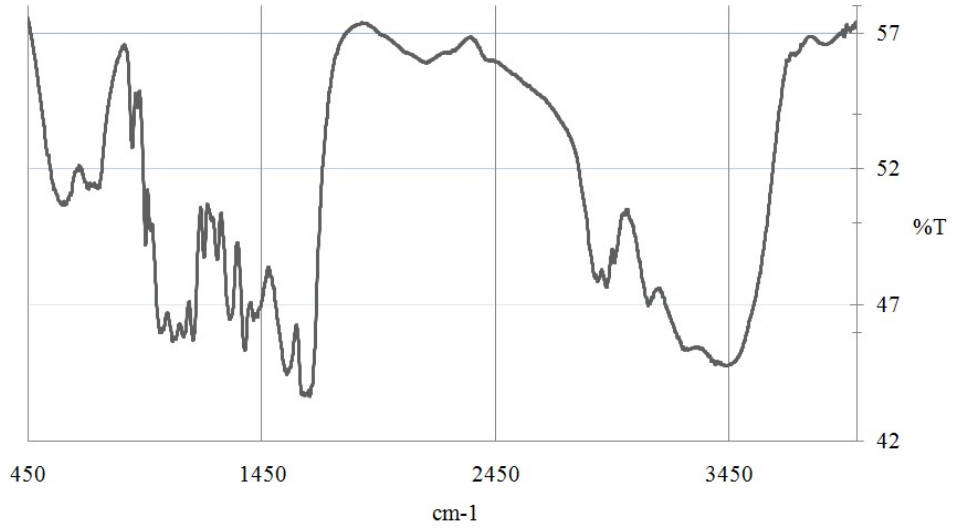

Figure 2. FTIR spectra of chitin fermented BSF larvae using *Bacillus subtilis* (T4).

4. Conclusion

Physical and bilogical treatment of BSF prepupa larvae can reduce chitin content, which is indicated by a decrease in crude fiber and fiber fraction (NDF and ADF). Fermentation using chitinolytic bacteria can change chitin characteristics and affect chitin quality.

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