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Bioconversion of rubber seeds to produce protein and oil-rich biomass using black soldier fly larva assisted by microbes

Muhammad Yusuf Abduh, Maryam Jamilah, Pramesti Istiandari, Syaripudin and Robert Manurung

Abstract
A co-conversion process using black soldier fly larvae (*Hermetia illucens*) and microbes was studied to convert rubber seeds into prepupal biomass from May to November 2016. De-oiled rubber seeds from West Bandung, Indonesia (cake to shell ratio of 2.5 wt%) was mixed with water (water to biomass ratio of 1.5-2.3 wt%) and pre-treated with two types of microbes; a liquid culture of *Aspergillus niger* (10^7 spore/ml) and a Biotaff solution containing consortium of microbes (10^7 cell/ml). Black soldier fly larvae were reared in rearing containers with a feeding rate of 68 mg/larvae/d and subjected with different shade rate (50-100%). The highest prepupal biomass productivity of 124.2 g/m^2.d on a dry basis with an assimilation efficiency of 28.7% and efficiency of conversion of digested feed of 25.9% was obtained when the larvae were cultivated using shade rate of 100% and fed with a substrate that had been pre-treated with a Biotaff solution containing 10^7 cell/ml. At this condition, the prepupal biomass has a protein content of 28.6 wt% and a fat content of 28.3 wt% which mainly consists of lauric acid (24.6%), oleic acid (24.5%), linoleic acid (19.3%) and palmitic acid (18.4%).

Keywords: *Hermetia illucens*, rubber seeds, *Aspergillus niger*, shade rate, biomass productivity

1. Introduction
Rubber trees (*Hevea brasiliensis*) are primarily cultivated for their latex as a source of natural rubber with Indonesia as the country with the largest rubber tree plantation in the world [1]. Apart from latex, rubber trees also produce rubber seeds (300-2060 kg/ha.y) but the valorisation of the seeds are still very limited [1-3]. Studies reported that the seeds consist of a shell (39 wt%, d.b) and a kernel (61 wt%, d.b.) with the kernels contain 40-50 wt%, d.b. oil and 17-20 wt%, d.b. protein [4].
Several studies on biorefinery of rubber seeds to produce other valuable bioproducts apart from latex have been carried out [5, 6]. The oil may be considered as a valuable source for producing biodiesel and biopolymers [7, 8] whereas the protein rich press cake may be valorised either as a valuable source for protein [3], biomaterials [9], biogas and thermochemical processes such as pyrolysis [10]. The remaining biomass after the isolation of oil from rubber seeds (shell and press cake) which is normally discarded as waste contain protein and lignocellulose. The shell contains 69 wt%, d.b. crude fibre, 3 wt%, d.b. protein and 1 wt%, d.b. oil [3]. When combined with the press cake which consists of protein, the mixture may be valorised as a substrate for the cultivation of *Hermetia illucens* which is commonly known as black soldier fly larvae (BSFL).
Black soldier fly is a non-pest fly that originates from America and has spread throughout the tropical and subtropical countries. The larva feeds on organic waste until it reaches a prepupal stage. The adult fly does not consume any food and does not pose and disease transmission risks [11]. Recent studies demonstrated that BSFL is an efficient converter for organic waste and contain relatively high amount of protein (40-45 wt%, d.b.) and fat (27-35 wt%, d.b.) which is suitable for an application as an animal feed [12, 13].
Bioconversion of remaining biomass from *Pandanus tectorius* fruit using BSFL for the production of protein rich biomass has been previously studied by Abduh et al (2017). At a feeding rate of 100 mg/larva.d, the efficiency of conversion is 27.4% and the produced prepupae has a protein content of 37.7 wt%, d.b [14]. Abduh et al (2017) also has carried out a systematic study to investigate factors such as cake to shell ratio, water to biomass ratio,

feeding rate and larval height that affect the bioconversion of *Reutealis trisperma* seed for the production of protein and oil rich biomass. At the optimum conditions, the estimated productivity of the prepupal biomass is 123.4 g/m^2.d with protein and fat content of 45 wt%, d.b. and 26.6 wt%, respectively [15].

The application of remaining biomass after the isolation of oil from rubber seeds as a substrate for the cultivation of BSFL has not yet been studied. Hence, the present study aimed to investigate the bioconversion of rubber seeds using BSFL assisted by microbes to increase the protein and oil content of the prepupal biomass.

2. Materials and Methods

This study was conducted at Institut Teknologi Bandung from May to November 2016.

2.1 Materials

Seeds from rubber tree (*Hevea Brasiliensis*) were obtained from a rubber tree plantation owned by PT. Bajabang at West Bandung, Indonesia. Eggs of Black Soldier Fly were obtained from Depok, Indonesia. *Aspergillus niger* culture was obtained from Indonesian Culture Collection (Bogor, Indonesia). Biotaff was purchased from PT. Berlianplast (Jakarta, Indonesia). Hexane (99 vol%) was obtained from Bratachem (Bandung, Indonesia) whereas potato dextrose agar medium was obtained from Oxoid (Hampshire, England).

2.2 Hydraulic pressing of rubber seeds

Rubber seeds from PT. Bajabang were first dehulled to separate kernels from the shells. The kernels were grounded and dried in the oven at 60 ^{O}C overnight. Approximately 500 g of sample was placed in a pressing chamber of a laboratory scale hydraulic pressing machine and pressed at room temperature (27 ^{O}C) for overnight to isolate the oil from the kernel. The deoiled kernels or commonly known as pressed cake was used a substrate for the cultivation of BSFL [15].

2.3 Soxhlet extraction of black soldier fly oil

The oil within the prepupae of black soldier fly was extracted using a Soxhlet extraction procedure. The prepupae were dried at dried at 103 ^{O}C and ground using a coffee grinder. Approximately 7 g of sample was transferred into a Soxhlet thimble and extracted with n-hexane for at least 5 h. The solvent was evaporated using a rotary evaporator (atmospheric pressure, 69 ^{O}C) and the sample was subsequently dried at 103 ^{O}C until constant weight was achieved [15].

2.4 Preparation of microbial culture for pre-treatment of substrate

A.niger obtained from Indonesian Culture Collection was cultured using a potato dextrose agar medium at room temperature for 9 d until sporulation stage was attained. The spores were harvested and suspended in an aqueous solution containing tween 0.1% before filtered with a Whatman no. 1 filter paper to separate the spores and mycelium. The concentration of spores in the culture was 10^7 spore/ml as calculated using a haemocytometer. As for the preparation of Biotaff solution, every tablet of Biotaff which contain 10^9 bacterial cells was mixed sterile aquades until a concentration of 10^7 cell/ml was obtained.

2.5 Cultivation of BSFL with the remaining biomass from rubber seeds

Black soldier fly eggs obtained from Depok were initially hatched and later reared 1000 g with chicken meal. After 7 d, the larvae were placed inside rearing containers (6.8 x 6.8 cm) with a larval density of 5 larvae/cm^2. The larvae were fed with the remaining biomass obtained after the isolation of oil from the rubber seeds. The pressed cake was mixed with the shell (cake to shell ratio of 0.25 wt%, d.b) before ground and sieved (12 mesh). The mixed biomass was added with water (water to biomass ratio of 1.5 wt%) and used as a substrate for the cultivation of BSFL with a feeding rate of 68 mg/larva/d.

The substrate used for the cultivation of BSFL was subjected into different conditions; i) without pre-treatment (T1), ii) pre-treatment with a Biotaff solution containing 10^7 bacterial cell/ml (T2), iii) pre-treatment with a liquid culture of *A.niger* containing 10^7 spore/ml (T3). The BSFL was also subjected into different shade rates; i) shade rate of 50% (S1), ii) shade rate of 75% (S2), iii) shade rate of 100% (S3). During the treatment, the larvae and the residue which comprises of excretory products and unconsumed feed were weighed every 3 d.

The treatments were carried out until the most of the larvae (at least 50% population) had become prepupae (approximately 12 d). After most of the prepupae had become prepupae, the prepupae were inactivated by drying at 103 ^{O}C until constant weight was achieved. The final residue was also weight before and after drying at 103 ^{O}C.

2.6 Analytical methods

The lignocellulose content of the samples was analyzed by using a Gravimetric method [15] whereas the protein content of the sample was analyzed by Kjedahl method (SNI-01-2891-1992). The analysis was carried out at the Analytical Laboratory, University of Padjajaran, Jatinangor. The fatty acid composition of rubber seed oil and BSF oil was analyzed by gas chromatography-mass spectrometry (GC-MS) at the Chemical Laboratory, University of Education of Indonesia, Bandung.

2.7 Data analysis

The effect of co-conversion of rubber seeds into protein and oil-rich biomass using black soldier fly larvae and microbes was evaluated by calculating the assimilation efficiency (AE), efficiency of conversion of digested-feed (ECD) and waste reduction index (WRI) as suggested by previous studies [12, 16].

$$AE\ (\%) = (I-F)/I \times 100\% \quad (1)$$

$$ECD\ (\%) = B/(I-F)/I \times 100\% \quad (2)$$

$$WRI = AE/t \quad (3)$$

where I is the initial dry weight of feed (g) and F is dry weight of residue (g), B is the dry weight of prepupae (g), F is the dry weight of residue (g) and t is the period of treatment (d).

3. Results and Discussion

The substrate used in this study was highly rich with lignocellulosic materials with a rather small amount of protein and fat. The lignocellulose composition of the substrate consists of 13.1 wt% cellulose, 36.3 wt% hemicellulose and 35.1 wt% lignin. The substrate also contains 8.5 wt% protein and 7.9 wt% fat. A relatively low amount fat in the substrate was due to the prior isolation of oil from the kernels using a hydraulic pressing machine at room temperature [15].

In this study, the substrate which contains a high amount of lignocellulosic materials was pre-treated with two types of

microbes particularly a Biotaff solution containing 10^7 bacterial cell/ml and a liquid culture of *A.niger* containing 10^7 spore/ml to investigate the effect of co-conversion of rubber seeds into protein and oil-rich biomass using BSFL and microbes. In addition, the cultivation of BSFL was carried out in rearing containers equipped with a different shade rate (25-100%) to investigate the effect of light intensity on the growth rate of BSFL and the results are discussed in the following.

3.1 Effect of shade rate

Preliminary experiments were carried out to investigate the effect of light intensity caused by the use of different shade rate (50-100%) to the growth profile of BSFL. Fig. 1 shows the effect of shade rate on the weight profile of larvae fed with de-oiled cake and shell of rubber seeds that were not subjected to any pre-treatment with microbes. From the figure, it was observed that the BSFL reached a maximum weight of 7.3-9.0 g after 9 d before the weight slightly decreased to 7.2-8.8 g after 12 d of cultivation. Such profile may be rationalized by the behavior of BSFL that continuously feed until they have obtained the amount of energy required to become prepupae. The data shown in Fig. 1 suggests that after approximately 9 d, the larvae secreted prothoracicotropic hormone that trigged the larvae to stop feeding and developed into prepupae [12-14].

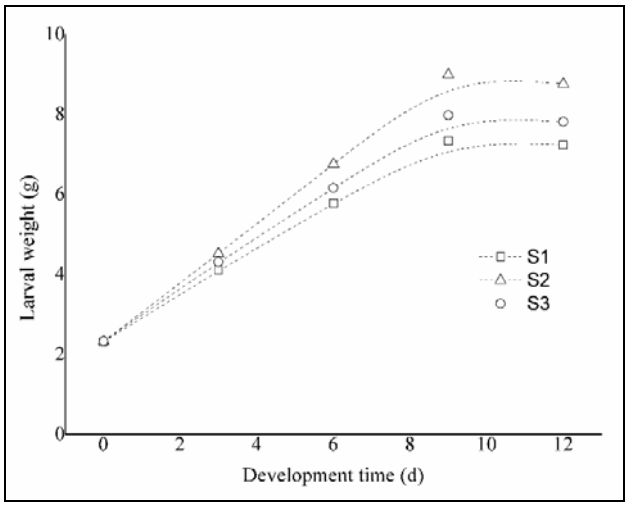

Fig 1: Effect of shade rate on the weight profile of larvae fed with de-oiled cake and shell of rubber seeds (cake to shell ratio: 0.25 wt%, d.b, water to biomass ratio: 1.5 wt%, w.b., feeding rate: 68 mg/larva/d, S1: shade rate of 50%, S2: shade rate of 75%, S3: shade rate of 100%).

The growth rate of the larvae increases when the shade rate was increased from 50% to 75% but then decreases when the shade rate was further increased to 100%. When the larvae were subjected to a shade rate of 75%, the maximum weight of the larvae was 9.0 g which was higher in comparison to the corresponding weight when the larvae were subjected to a shade rate of 50% and 100% (7.2 and 8.0 g, respectively).

A possible explanation may be due to the fact that most immature insects avoid direct exposure to light which may increases water losses [17-19]. Increasing activities along with water losses from transpiration may reduce the biomass of BSFL as observed in this study when the shade rate was decreased from 75% to 50%. However when the shade rate was increased to 100% (the larvae were subjected to almost completely dark condition), the maximum weight slightly decreased from 9.0 to 8.0 g. As such suggests that there is an optimum light intensity for the cultivation of BSFL. Hence, a shade rate of 75% was used for the proceeding experiments to investigate the effect of pre-treatment with microbes as discussed in the following section.

3.2 Effect of substrate pre-treatment with microbes

Fig. 2 shows the effect of pre-treatment with microbes on the weight profile of larvae fed with de-oiled cake and shell of rubber seeds that were subjected 75% shade rate. It was observed that the growth rate of BSFL fed with non-pre-treated substrate resembles the profile when BSFL was fed with a substrate that had been pre-treated with a Biotaff solution containing 10^7 bacterial cell/ml. Both treatments obtained a maximum weight of approximately 8.7 g after 9 d. A higher maximum weight of 9.6 g was obtained when the substrate was pre-treated with a liquid culture of *A.niger* containing 10^7 spore/ml.

In this study, the substrate used as a feed for the cultivation of BSFL were pre-treated with bacteria and fungi because they are capable of degrading lignocellulosic materials [20] which can facilitate further conversion by BSFL. A Biotaff solution that was used in this study contain bacterial consortium that are able to decompose organic waste and has been widely used for septic tank application. Similar growth profile between T1 (substrate without pre-treatment) and T2 (substrate pre-treated with a Biotaff solution containing 10^7 bacterial cell/m) as illustrated in Figure 2 indicates that when subjected to 75% shade rate, pre-treatment of the substrate with bacteria had no significant effect on the growth of BSFL.

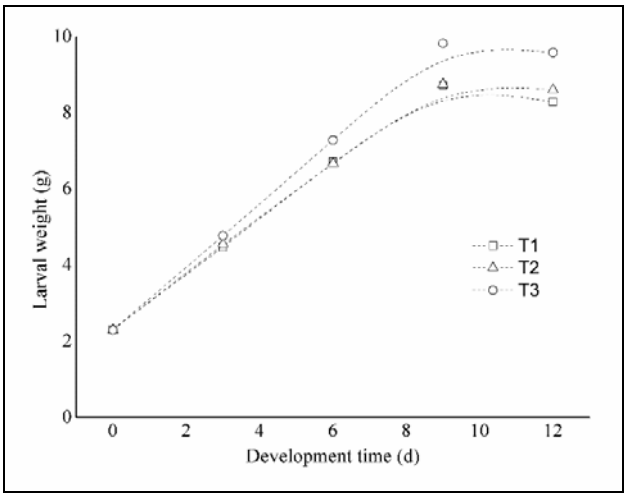

Fig 2: Effect of pre-treatment with microbes on the weight profile of larvae fed with de-oiled cake and shell of rubber seeds (cake to shell ratio: 0.25 wt%, d.b, water to biomass ratio: 1.5 wt%, w.b., feeding rate: 68 mg/larva/d, T1: no pre-treatment, T2: pre-treatment with Biotaff solution containing 10^7 bacterial cell/ml, T3: pre-treatment with *A. niger* containing 10^7 spore/ml.

However, when the substrate pre-treated with *A.niger* were fed to BSFL and subjected 75% shade rate the growth rate increased and reached a maximum larval weight of 9.6 g after 9 d. *A. niger* is known to have cellulase enzymes that can cleave the β-1,4 D glucan bond in cellulose to produce glucose, cellobiose, and cello-oligosachharides [20, 21]. *A. niger* also able to hydrolyse polysaccharide such as starch and pectin as well as hemicellulose like xylan due to the presence of a xylanase enzyme [22]. As such facilitates the degradation of the substrate that contains a high amount of lignocellulose for the BSFL to better digest the substrate for their growth.

3.3 Effect of shade rate and pre-treatment of substrate with microbes

Fig. 3 shows the effect of both shade rate and pre-treatment with microbes on the weight profile of larvae fed with de-oiled cake and shell of rubber seeds. It was observed that the growth profile is similar for all conditions. The BSFL reached a maximum weight after 9 d before the weight slightly decreased after 12 d of cultivation.

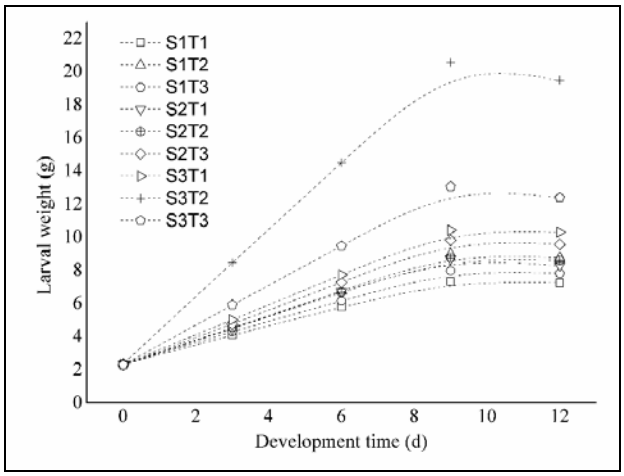

Fig 3: Weight profile of larvae fed with de-oiled cake and shell of rubber seeds pre-treated with microbes in rearing containers subjected to different shade rate (S1: shade rate of 50%, S2: shade rate of 75%, S3: shade rate of 100%, T1: substrate with no pre-treatment, T2: substrate pre-treated with a Biotaff solution containing 10^7 cell/ml, T3: substrate pre-treated with a liquid culture of *A.niger* containing 10^7 spore/ml).

The highest growth rate was observed when the substrate was pre-treated with a Biotaff solution containing 10^7 cell/ml and the cultivation of BSFL was subjected to a shade rate of 100% (S3T2) with a maximum weight of 20.6 g. Second highest growth rate was observed when the substrate was pre-treated with a liquid culture of *A.niger* containing 10^7 spore/ml and the cultivation of BSFL was subjected to a shade rate of 100% (S3T3) with a maximum weight of 13.1 g. These values are almost 2-3 times higher in comparison to the maximum average weight of 7.3 g attained by the prepupae when the substrate was not pre-treated and the larvae were cultivated under 50% shade rate (SIT1).

These results confirm that pre-treatment of the substrate with microbes increased the growth rate of the BSFL. The substrate used in this study was rich with lignocellulosic materials (13.1 wt% cellulose, 36.3 wt% hemicellulose and 35.1 wt% lignin). Hence pre-treatment of the substrate with a Biotaff solution (10^7 cell/ml) and a liquid culture of *A.niger* (10^7 spore/ml) increased the growth rate of BSFL because the microbes are capable of degrading lignocellulosic materials [20] which facilitates further bioconversion by BSFL. The results also suggest that the larvae preferred dark conditions during cultivation possibly because direct exposure to light increases water losses from transpiration which may results in biomass reduction of the larvae [17-19].

Fig. 4 shows the relative proportion of substrate that was converted into prepupal biomass, remains as residual matter and used for metabolism of the larvae. Around 2.9-7.5% of the substrate was transformed into prepupal biomass. Almost 71.3-77% of the substrate remains as residual matter which includes faeces excreted by the larvae or substrate that had not been consumed by the larvae. Taking into account the proportion of substrate that was converted into prepupal biomass and that remains as residual matter, the proportion of substrate that was used by the larvae for their metabolic needs lies in the range of 20.1-21.6%.

The highest conversion percentage of feed into biomass (7.5%) was observed when the substrate was pre-treated with a Biotaff solution containing 10^7 cell/ml and the cultivation of BSFL was subjected to a shade rate of 100% (S3T2). The lowest conversion percentage (2.9%) was recorded when the substrate was not pre-treated and the larvae were cultivated under 50% shade rate (SIT1). As such indicates that pre-treatment of the substrate with microbes as well as light intensity during the cultivation of BSFL play a crucial role in the bioconversion of the substrate into prepupal biomass. The amount of substrate that was converted into prepupal biomass obtained in this study is slightly higher that the results reported by Abduh et al (2017) which lies in the range of 2.8-5.4% when the BSFL was fed trisperma seed that was not pre-treated with microbes [15]. Hence, pre-treatment of the substrate with microbes is necessary to degrade the lignocellulosic materials into glucose which can be readily uptake by the larvae as their biomass.

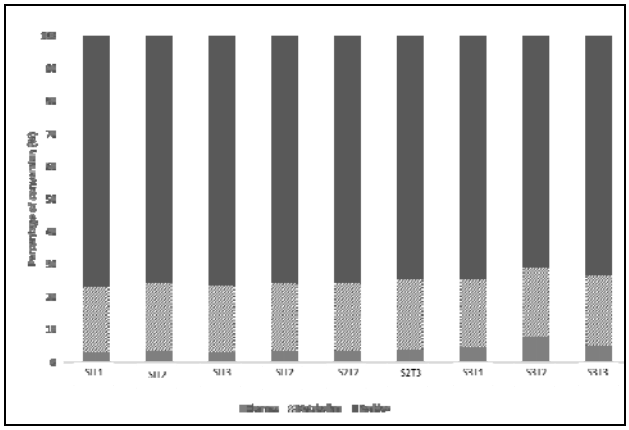

Fig 4: Relative proportion of substrate converted into biomass (prepupal weight), used for metabolisms and remains as residuals

The performance of co-conversion of rubber seeds into protein and oil-rich biomass using BSFL and microbes may be assessed by calculating the assimilation efficiency (AE), efficiency of conversion of digested-feed (ECD) and waste reduction index (WRI) and the results are presented in Table 1. Based on the results in Table 1, it can be seen that the assimilation efficiency varies from 23-28.7% with a higher shade rate generally results in higher value AE. These values resemble the assimilation efficiency of 26.1% reported by the previous study when BSFL was reared in a dark condition and fed trisperma seed [15]. These results again emphasize the importance of reducing light intensity during the cultivation of BSFL to increase their efficiency in converting the substrate they ingested for growth.

The efficiency of conversion of digested feed varied from 12.5 to 25.9%.These values indicate how well the digested substrate was converted to prepupal biomass. These values were slightly higher than the values reported by the previous study which lie in the range of 11.to 20.3% when the larvae were fed with trisperma seeds that had not been pre-treated with microbes [15]. As such indicates that pre-treatment of the substrate with microbes may increase the efficiency of BSFL to digest the substrate and converted the substrate to their biomass.

Table 1: Assimilation efficiency, efficiency of conversion, waste reduction index and estimated productivity of prepupal biomass (dry based)

Cultivation condition	AE (%)	ECD (%)	WRI (-)	Productivity of prepupae ($g_{prepupae}/m^2_{container}.d$)
S1T1	23.0	12.5	2.1	36.9
S1T2	24.3	14.3	2.2	48.3
S1T3	23.5	13.1	2.1	40.9
S2T1	24.0	13.7	2.2	44.8
S2T2	24.1	14.1	2.2	47.3
S2T3	25.1	15.0	2.3	54.2
S3T1	25.5	16.8	2.3	62.8
S3T2	28.7	25.9	2.6	124.2
S3T3	26. 7	18.6	2.4	77.5

Another parameter particularly the waste reduction index was calculated by dividing the assimilation efficiency with 12 days; the period required by the larvae to transform into prepupae. In this study, the values of WRI vary from 2.1 to 2.6 which lie in the range of 1.3 to 2.9 as reported by the previous study when the BSFL was fed trisperma seed as a substrate [15]. Based on the results in Table 1, it was clearly seen that the cultivation condition with the highest values for AE (28.7%), ECD (25.9%) and WRI (2.6) is S3T2; the substrate was pre-treated with a Biotaff solution containing 10^7 cell/ml and the cultivation of BSFL was subjected to a shade rate of 100%.

The productivity of the prepupal biomass ($g/m^2.d$) on a dry basis at different cultivation conditions was estimated from the dry weight (the larvae have an average water content of 59%) of the prepupal biomass divided by the area of the rearing container (0.0046 m^2) and period of cultivation (12 d). The estimated productivity of the prepupae which lies in the range of 36.9 to 124.2 $g/m^2.d$ are shown in Table 1. The lowest productivity was recorded when the substrate was not pre-treated and the larvae were cultivated under 50% shade rate (SIT1). Increasing the shade rate and pre-treating the substrate with microbes increased the productivity of the prepupal biomass up to more than three times (124.2 $g/m^2.d$) when the substrate was pre-treated with a Biotaff solution containing 10^7 cell/ml and the cultivation of BSFL was subjected to a shade rate of 100% (dark condition). This productivity lies within the range of 123 to 145$g/m^2.d$ reported in other studies [12, 15].

3.4 Composition of protein and oil in the prepupal biomass

The prepupal biomass obtained after the cultivation of BSFL fed with the remaining biomass of rubber seeds after the isolation of oil using a hydraulic pressing machine was analyzed to determine the protein and fat content and the results are shown in Table 2. The residue remains in the rearing containers was also analyzed to determine its fat and protein content in comparison to the values prior to the cultivation.

Initially, the substrate had a fat and protein content of 7.9 wt% and 8.5 wt%, respectively. After 12 d of treatment, the fat and protein content of the residue in the rearing containers decreased to 0.6 wt% and 7.0 wt%, respectively. The prepupal biomass had a fat content in the range of 18.9 to 28.3 wt%; 139-258% higher than the initial value in the substrate. The protein content in the prepupae lies in the range of 28.6 to 55.2 wt%; 236-549% higher than the initial value in the substrate.

Table 2: Protein and fat content of prepupal biomass harvested from different cultivation conditions

Cultivation condition	Protein content (wt%)	Fat content (wt%)
S1T1	41.9	18.9
S2T1	55.2	22.0
S3T1	47.7	20.5
S3T2	28.6	28.3
S3T3	50.9	20.5

According to the previous studies, the fat content in the prepupae lies in the range of 26-34 wt% whereas the protein content varies from 37-45 wt% [12, 15]. The results obtained in this study are slightly out of range in comparison to the reported values in the literature. Most probably the difference is due to the ability of BSFL to digest different types of organic matter that depends on the composition of material as well as microbial symbionts and digestive enzymes [23].

The highest protein content in the prepupae (55.2 wt%) was recorded when the larvae were cultivated using a shade rate of 75% and fed with a substrate that had not been pre-treated with microbes (S2T1). At this condition, the fat content of the prepupae was 22 wt%. The lowest protein content in the prepupae (28.6 wt%) was obtained when the larvae was cultivated using a shade rate of 100% and fed with a substrate that had been pre-treated with a Biotaff solution containing 10^7 cell/ml (S3T2). At this condition, the fat content in the prepupal biomass was the highest as compared to the values at other conditions. When the larvae were subjected to a shade rate of 100% and fed with a substrate that had been pre-treated with a liquid culture of *A.niger* containing 10^7 spore/ml (S3T3), the protein content was almost double (50.9 wt%) but with a lower fat content (20.5 wt%) in comparison when the substrate was pre-treated a Biotaff solution.

The amount of fat content the prepupae was very much correlated with the estimated productivity of the prepupal biomass as shown in Table 1. When the larvae were subjected to a shade rate of 50% and fed with a substrate that had not been pre-treated with microbes, the estimated productivity of the prepupal biomass was the lowest (36.9 $g_{prepupae}/m^2_{container}.d$ as shown Table 1). This data is in line with the amount of fat content in the prepupae (18.9 wt%) which was also the lowest as compared to other conditions. Similarly, the highest estimated productivity of prepupal biomass (124.2 $g_{prepupae}/m^2_{container}.d$) was obtained when the amount of fat content in the prepupae was also the highest (28.3 wt%). As such implies that the productivity of the prepupal biomass is highly influenced by the ability of the larvae to digest the feed and convert the substrate into their own biomass.

The protein and oil content obtained in this study satisfy the requirement of animal feed for chicken (13.5 and 7 wt%, d.b.) as outlined by the Indonesian National Standard for protein (SNI 01-3929-2006) and fat (SNI 01-3909-2006). Hence, the prepupal biomass of BSF fed with the remaining biomass from rubber seeds may be utilized for animal feed application.

The oil content in the prepupae produced from the cultivation of BSFL fed by the remaining biomass of rubber seeds was extracted using a Soxhlet extraction. The fatty acid composition of the extracted oil was determined using a GC-MS and the results are shown in Table 3.

The BSF oil obtained in this study consists of lauric acid (24.6%), oleic acid (24.5%), linoleic acid (19.3%), palmitic acid (18.4%) and stearic acid (8%) with minor amounts of myristic acid, capric acid and palmitoleic acid. These values are relatively similar to the fatty acid composition of BSF oil reported in previous studies [15, 24]. The presence of this

medium and long chain fatty acid in poultry diet may help to prevent enteric disease in poultry by stimulating natural immune response in the poultry, improve nutrient digestibility and increase the body weight and feed conversion ratio in broiler chicken [25].

Table 3: Fatty acid composition of BSF oil

Fatty acid	Composition (wt%)	
	this study[1)]	Literature[2)]
Saturated fatty acid		
Capric acid (10:0)	1.1	0-3.8
Lauric acid (12:0)	24.6	0-27.8
Myristic acid (14:0)	3.3	0-8.1
Palmitic acid (16:0)	18.4	10-14.2
Stearic acid (18:0)	8.0	7.6-8.9
Unsaturated fatty acid		
Palmitoleic acid (16:1)	0.8	0-4.1
Oleic acid (18:1)	24.5	11-22.5
Linoleic acid (18:2)	19.3	1.8-14.6

[1)] BSF oil extracted from the combined prepupae harvested at different conditions, [2)] [15, 24]

4. Conclusions

A co-conversion process using black soldier fly larvae and microbes to convert rubber seeds into prepupal biomass containing high amount of protein and oil has been investigated. The highest prepupal biomass productivity of 124.2 g/m^2.d on a dry basis with an assimilation efficiency of 28.7% and efficiency of conversion of digested feed of 25.9% was obtained when the larvae were cultivated using shade rate of 100% and fed with a substrate that had been pre-treated with a Biotaff solution containing 10^7 cell/ml. At this condition, the prepupal biomass has a protein content of 28.6 wt% and a fat content of 28.3 wt% which mainly consists of lauric acid (24.6%), oleic acid (24.5%), linoleic acid (19.3%) and palmitic acid (18.4%).

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