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Bioconversion of *Pandanus tectorius* using black soldier fly larvae for the production of edible oil and protein-rich biomass

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Abstract
The valorisation of *Pandanus tectorius* fruits for the production of edible oil and protein-rich biomass using a bio-refinery approach was investigated. The characteristics of *Pandanus* fruits and the total content of β–carotene, oil, and lignocellulose composition were determined. *Pandanus* fruits contain approximately 97-99 wt%, d.b. mesocarp, 0.8-2 wt%, d.b. endosperm and 0.7-1 wt%, d.b. endocarp. The mesocarp of *Pandanus* fruit contains approximately 1.8- 6.2 µg/g ß-carotene. The endosperm contains about 46-52 wt%, d.b. edible oil whereas the total lignocellulose content in the endosperm was approximately 63-67 wt%, d.b. Bioconversion of the exocarp of *Pandanus* fruits using the larva of *Hermetia illucens* (black soldier fly) for producing protein-rich biomass was also carried out. The effect of feeding rate on the efficiency of conversion of digested feed and productivity of prepupae was determined. At a feeding rate of 100 mg/larvae.d, the efficiency of conversion of digested feed was 27.4% with an estimated productivity of 4.7 $g_{prepupae}/m^2_{container}$.d. The produced prepupae has a protein content of 37.7 wt%, d.b. which may find suitable application as animal feed.

Keywords: *Pandanus tectorius*, β-carotene, *Hermetia illucens*, feeding rate, protein-rich biomass, productivity of prepupae

1. Introduction

Papua has the highest number of flora species in Indonesia with a tremendous potential for medicinal and food development [1] but most of the species are yet to be explored for greater benefits. It is stated that around 55% of more than 1000 flora species in Papua are endemic species [2]. One of the endemic species in Papua that has received little attention is the screw-palm (*Pandanus*) fruits. *Pandanus* (Pandanaceae) is a very important genus to highland people of Papua which comprises of approximately 600-750 species [3-5]. The leaves and roots of *Pandanus* are valorized for traditional house roof and basket. The fruits are regularly consumed by the local people as a source of carbohydrate and protein as well as for medicinal purposes [6-8].

Pandanus tectorius is one of the *Pandanus* species that is recognized as a highly variable species complex which is widespread on strandline and near coastal forests in Southeast Asia including Papua. Currently there are very little studies that report on the availability and composition of *Pandanus* in Papua which is locally known as “woromo” or “tukhe” [1, 7, 8]. It has been reported that the fleshy receptacle (mesocarp) of *Pandanus* is rich with carbohydrate (74%), protein (10%) and β-carotene (1.6 µg/g) [8]. The seeds are reported to have a lower carbohydrate content (25%) but higher protein (15.4%) and β-carotene (4.4 µg/g). In addition, the seeds also have a considerable amount of fat (48.5%).

Although the fruits are regularly consumed by the local people as staple food, a significant portion of the fruits is non-edible and normally discarded as waste. The remaining biomass contain relatively high amount of protein and lignocellulose that can be valorized as a substrate for the cultivation of black soldier fly larvae (BSFL) (*Hermetia illucens* L.) to produce protein-rich biomass. Recently BSFL have been considered as efficient converters for organic waste and nutritious feed for poultry [9-12]. BSFL is a non-pest fly with a relatively high content of protein (40-45 wt%) and fat (27-35 wt%) that can be valorized as animal feed [10].

This study aims to explore the valorisation of *Pandanus* fruits obtained from Papua as a potential source for the production of edible oil and protein-rich biomass using a biorefinery approach which aims to optimize the use of resources, minimize waste and maximize benefits

[13]. *Pandanus* oil was isolated from the seed and can be used for the production of edible oil with a relatively high content of β-carotene. The non-edible part of the fruit (exocarp) was valorized as a substrate for the cultivation of BSFL and the protein content of the prepupae of *H.illucens* L. were determined, which is an absolute novelty of this study.

2. Materials and Methods

This study was conducted from January to July 2015. Unripe, ripe and overripe *P. tectorius* fruits were obtained from Papua, Indonesia whereas black soldier eggs were obtained from Sumedang, Indonesia. Hexane, KOH, Na_2SO_4, petroleum ether and acetone were obtained from Bratachem (Bandung, Indonesia).

2.1 Determination of total moisture content

The total moisture content of the samples was determined at Institut Teknologi Bandung, Indonesia which involves heating the samples in the oven at 103 ^{O}C until constant weight was obtained [14].

2.2 Extraction of oil from the *Pandanus* seeds

Pandanus fruits were dehulled to separate the mesocarp (fleshy content), endocarp (shell) and endosperm (kernel). The oil was isolated from the endosperm by Soxhlet extraction as described in the literature [14]. It involves drying the samples overnight at 103 ^{O}C and later ground using a coffee grinder. About 10 g of the sample was placed in a Soxhlet thimble and extracted with n-hexane for at least 4 h. The solvent used in the extraction procedure was evaporated in a rotary evaporator before dried in an oven at 60 ^{O}C until constant weight was obtained.

2.3 Determination of β-carotene content

Pandanus fruits were cut into smaller pieces and and later ground using a coffee grinder. Approximately 30 g of sample was extracted in a Soxhlet thimble with acetone for at least 4 h. The solvent was evaporated in a rotary evaporator and later dried in an oven at 60 ^{O}C before further extracted with petroleum ether using a separatory funnel. The extract was then desaponified with KOH (15%) for 12 h. After that, the mixture was washed with water before separated and dried with anhydrite Na_2SO_4. The extract was then added with additional petroleum ether before analyzed with UV Spectrophometer. The concentration of β-carotene (in mg/L), $A_{\beta\text{-carotene}}$ was estimated using Eq. (1) [15].

$$A_{\beta\text{-karoten}} = 4{,}367\ A_{450} - 2{,}947\ A_{503} \quad (1)$$

Where A_{450} and A_{503} are the absorbance of the sample at wavelength 450 nm and 503 nm, respectively.

2.4 Cultivation of black soldier fly larvae with the exocarp of *Pandanus* fruits

Black soldier fly eggs acquired from Sumedang, Indonesia were hatched and the larvae were reared with chicken meal for 7 d before fed with non-edible part of *Pandanus* fruits particularly the exocarp. Twenty black soldier fly larvae of 7-d old were placed inside a rearing container (diameter of 7 cm and 12 cm height) and fed with the exocarp of *Pandanus* fruits (mixture of all different level of ripeness) that have been ground and sieved (12 mesh) as a substrate. The substrate was mixed with water at a ratio of 2:3 (weight per volume) [9]. The feeding rate was varied from 25-100 mg/larvae/d and the treatment was carried out for 27 d. The larvae were weighed and moved into a new container containing fresh feed every 3 d whereas the residues in the container were dried at 103 ^{O}C. The weight of the residues was determined before and after the drying process. After 27 d of treatment, the prepupae were inactivated by drying at 103 ^{O}C until constant weight was obtained.

2.5 Data analysis

The performance of BSFL in converting the exocarp of *Pandanus* fruits into protein-rich biomass was evaluated by calculating the efficiency of conversion of digested-feed as suggested by Diener *et al* (2009) and Manurung *et al* (2016). Efficiency of conversion of digested-feed (ECD) suggests the effectiveness of BSFL in converting the digested feed into biomass. The ECD value was calculated by dividing the dry weight of prepupae with the difference between initial dry weight of feed and dry weight of residue as suggested by Manurung *et al* (2016).

2.6 Analytical methods

The lignocellulose content of the samples was determined based on the procedures by Manurung *et al* (2016). 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 *Pandanus* oil was determined using gas chromatography-mass spectrometry (GC-MS) carried out at the Chemical Laboratory, Indonesian Institute of Sciences, Bandung.

3. Results and Discussion

Valorisation of *Pandanus* fruits obtained from Papua for the production of edible oil and protein-rich biomass has been studied as illustrated in Fig. 1. *Pandanus* fruits were first separated from the remaining mesocarp and exocarp. The seeds were then dehulled to remove endosperm from the endocarp. The endosperm which contain a significant amount of oil was subjected to Soxhlet extraction to isolate the oil which contain a relatively high amount β-carotene. The non-edible part of the fruit (exocarp) which contain a considerable amount of protein and lignocellulose was mixed with water before used feed for the cultivation BSFL in a rearing container. The feeding rate was varied from 25- 100 mg/larvae/d and the treatment was carried out until most of the larvae had developed into prepupae (protein rich biomass). The characteristic of *Pandanus* fruits and the composition of its oil as well as the cultivation of BSFL to produce protein-rich biomass (prepupae) are discussed in the following sections.

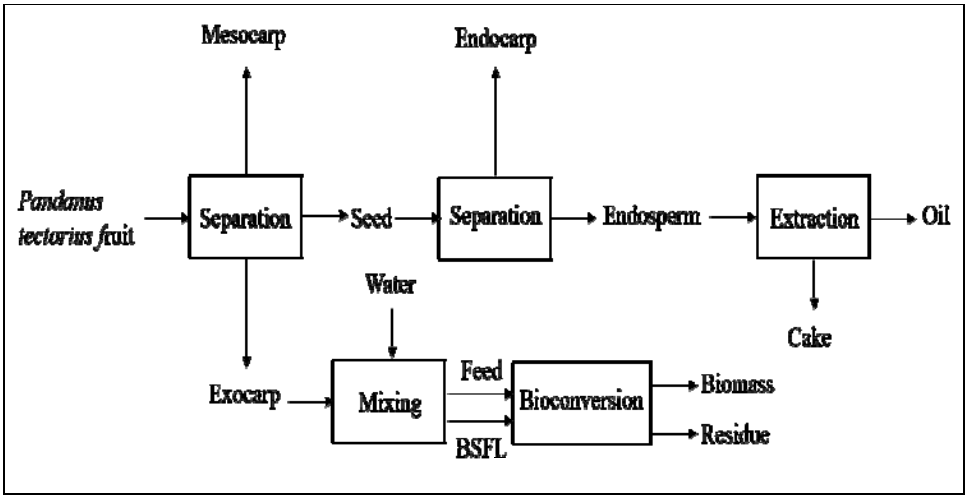

Fig 1: Valorisation of *Pandanus* fruit for the production of edible oil and protein rich biomass (prepupae)

3.1 Characteristics of *Pandanus* fruits

Pandanus fruits with three different level of ripeness particularly unripe, ripe and overripe were obtained from Papua, Indonesia. A typical ripe *Pandanus* fruit has an average diameter of 25 cm. The fruits consist of approximately 38 phalanges with an average length of 10 cm and each phalange has 5 to 10 seeds. The dehulled seeds have an average width and length of 0.7 cm and 1.9 cm as shown in Figure 2. Overripe fruits have approximately similar number of phalanges and seeds. Unripe *Pandanus* fruits have an average diameter of 12 cm. The fruits have approximately 35 phalanges with an average length of 6 cm. Each phalange has 3 to 5 seeds with an average width and diameter of 0.5 and 1.2 cm. The color of the basal section of the phalanges varies from pale to dark yellow depending on the level of ripeness.

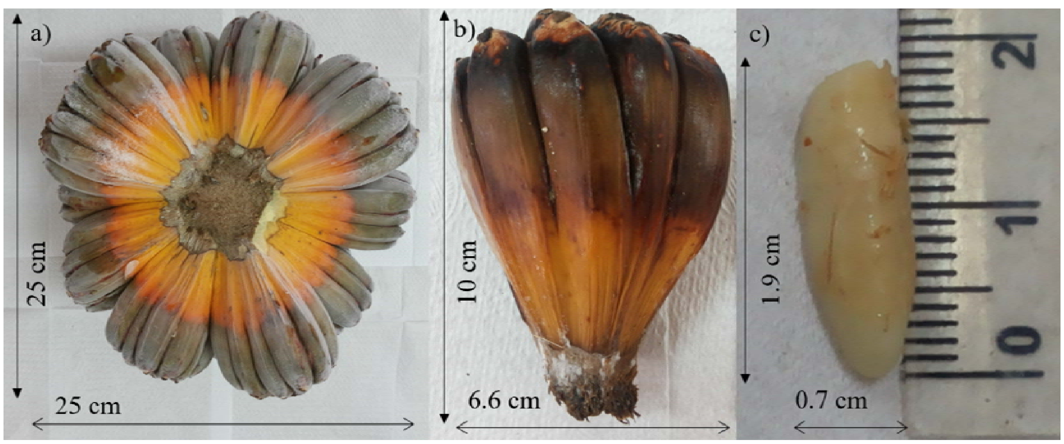

Fig 2: Parts of ripe *Pandanus tectorius* (a) fruits, (b) phalange, and (c) endosperm.

The mesocarp and exocarp constituted 97 to 99% on a dry basis (d.b.) with the remaining fraction belongs to the endosperm (dehulled seed) and endocarp (shell) as shown in Table 1. The total moisture content of the mesocarp and exocarp upon received varies from 41.6 to 58.8 % on a wet basis (w.b.) whereas the average moisture content of the endosperm varies from 18.1 to 27.2 % depending on the ripeness level.

Table 1: Mass fraction and moisture content of unripe, ripe and overripe *Pandanus*

Properties	Unripe	Ripe	Overripe
Weight (%, d.b.)			
Mesocarp and exocarp	98.2	97.3	98.9
Endosperm	0.8	2.0	1.0
Endocarp	1.0	0.7	0.1
Moisture content (%, w.b.)			
Mesocarp and exocarp	58.8	56.7	41.6
Endosperm	18.1	32.2	27.2

3.2 Composition of *Pandanus* biomass

Pandanus fruits were dehulled to separate the mesocarp, exocarp, endocarp and endosperm. The concentration of ß-carotene in the mesocarp was determined by first extracting ß-carotene from the mesocarp with solvents before analysed with UV Spectrophotometer. Based on the analysis, the mesocarp contain a considerable amount of ß-carotene which increases with the increasing level of fruit ripeness. The mesocarp from unripe fruits has a ß-carotene of 1.8 µg/g whereas the ripe fruits has a ß-carotene of 3.1 µg/g. The highest ß-carotene of 6.2 µg/g was obtained from overripe fruits. Miller (1956) reported that *Pandanus* fruit has a ß-carotene of 1.8 µg/g [16]. In another study by Englberger *et al* (2003), the ß-carotene content of *Pandanus* fruits lies in the range of 0.2 to 3.9 µg/g [17]. The study concluded that there was a great range in carotenoid content between different cultivars although all were fully ripe. This conclusion is supported by other studies which showed that the ß-carotene content of *Pandanus* fruits varies from 0.6 to 190 µg/g for nine different cultivars [17] and from 1.1 to 3.7 µg/g for nine different cultivars [18].

The oil content of the endosperm was determined using a standardized Soxhlet extraction with n-hexane. An overripe endosperm has a relatively high oil content of 52 wt%, d.b whereas the unripe endosperm has a lower oil content of 46.3 wt%, d.b. Kogoya *et al* (2014) have previously reported the oil content for different species of *Pandanus* (*P. julianeti, P. woromo, P. brosimos*) which varies from 47 to 50 wt%, d.b. Another two unidentified species of *Pandanus* have an oil content in the range of 33-48 wt%, d.b.

The fatty acid composition of the extracted *Pandanus* oil was determined by GC-MS and the results are shown in Table 2. From the table, it can be observed that the fatty acid composition was not influenced by the ripeness level of the fruit. *Pandanus* oil is mainly composed of unsaturated fatty acid, particularly linoleic acid (37-41%) and oleic acid (25-30%). The oil also contain a considerable amount of palmitic acid (24-33%) and a relatively small of steric acid (4-7%).

Table 2: Fatty acid composition of *Pandanus* oil

Fatty acid	Percentage (wt%, d.b)		
	Unripe	Ripe	Overripe
Palmitic acid (C16:0)	33	24	31
Stearic acid (C18:0)	4	7	4
Oleic acid (C18:1)	25	30	29
Linoleic acid (C18:2)	38	41	37

The lignocellulose composition in the endosperm was also determined and the results are shown in Table 3. The total lignocellulose content in the endosperm slightly increased from 63 to 67 wt%, d.b. as the ripeness level increased from unripe to ripe. The total lignocellulose composition for overripe endosperm is approximately 66 wt%, d.b. As such indicates that the total lignocellulose composition in the endosperm remains approximately the same when the fruit has become ripe. For unripe and overripe endosperm, the hemicellulose content is always the highest (32% and 47%, respectively), followed by cellulose (20% and 10.5%, respectively) and lignin (11% and 8%, respectively). However for ripe endosperm, the lignin content (24%) is relatively high which is close to its hemicellulose content (26.5%).

Table 3: Lignocellulose composition of *Pandanus* endosperm

Lignocellulose	Percentage (%)		
	Unripe	Ripe	Overripe
Hemicellulose	32	26.5	47
Cellulose	20	16.5	10.5
Lignin	11	24.2	8

Apart from the mesocarp and endosperm, *Pandanus* fruits contain a considerable amount of parts that are not edible particularly the exocarp. This exocarp is normally discarded away as waste by the local people. The protein content of the exocarp was analyzed and the results showed that the exocarp contain only 3.7 wt%, d.b. protein. This study attempts to investigate the potential application of this exocarp (Fig. 1) as a substrate for the cultivation for BSFL to increase its value as protein meal which will be discussed in the following section.

3.3 Bioconversion of *Pandanus* tectorius biomass after oil extraction as feed for the cultivation of BSFL

Black soldier fly eggs obtained from Sumedang, Indonesia were hatched and the larvae were fed with chicken meal for 7 d before fed with non-edible part of *Pandanus* fruits particularly the exocarp. The exocarp contain 23 wt%, d.b. hemicellulose, 29.5 wt%, d.b. cellulose and 30.9 wt%, d.b. lignin using the Chesson-Datta procedures [18]. The feeding rate was varied from 25-100 mg/larvae/d and the treatment was carried out for 27 d.

Figure 3 shows the weight profile of larvae in a rearing container fed with the exocarp of *Pandanus* fruits at different feeding rates. Initially the average weight of the larvae was approximately 28 mg/larvae after fed with chicken meal for 7 d. At day 0, the chicken meal was replaced with the exocarp of *Pandanus* fruits and the weight of larvae in the rearing container was continuously monitored. From the figure, it can be observed that the average growth rate of the larvae varies with feeding rate. At a feeding rate of 100 mg/larvae/d, the average weight of the larvae continuously increased from approximately 28 to 59.9 mg/larvae after 27 d. A rather slower growth rate was observed when the larvae were fed with the exocarp of *Pandanus* fruits at a rate of 50 mg/larvae/d. After 24 d, the average weight of the larvae was approximately 40 mg/larva and remain constant until the end of treatment.

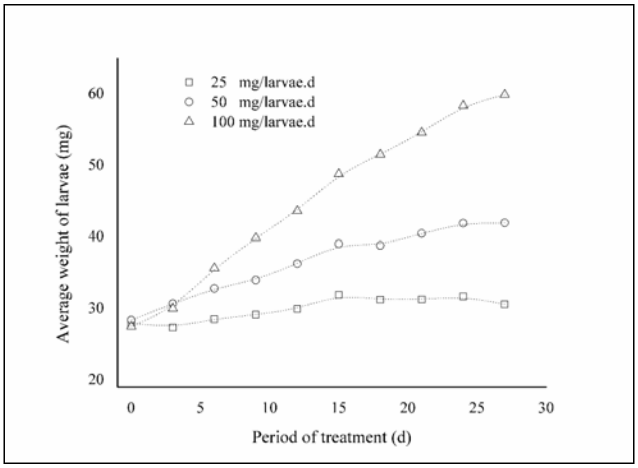

Fig 3: Average weight of larva with development time fed with the exocarp of *Pandanus* fruits in a rearing container

At a feeding rate of 25 mg/larvae/d, the average weight of the larva only slightly increased with time before it reached a plateau after 15 d (31 mg/larvae). In comparison to a relatively higher average growth rate reported at higher feeding rates (50-100 mg/larva/d), a feeding rate of 25 mg/larva/d might have caused a food stress to the larvae due to insufficient feed. At such conditions, the larvae continued to eat and grow until it reach its critical weight (approximately 30 mg) by prolonging its pupation period [9]. After 15 d, the larvae might have obtained the amount of energy required to perform pupal development and secreted prothoracicotropic hormone which caused the larvae to stop feeding and developed into pre pupae [9, 10].

The growth of larvae is highly dependent on the type of feed, temperature, relative humidity as well as water content in the feed [20, 21]. Diener *et al* (2009) had previously investigated the cultivation of BSFL using chicken feed as a substrate. A daily feeding rate of 50 mg chicken feed/larva resulted in a prepupal dry weight of 32.7 mg in 20 d which is equivalent to an average weight increase of 1.6 mg prepupae/d [9]. Manurung *et al* (2016) had also investigated the cultivation of BSFL using the residue from *Reutealis trisperma* seed. A daily feeding rate of 50 mg /larva resulted in a prepupal dry weight of 135.2 mg in 19 d which is equivalent to an average weight increase of 6.6 mg prepupae/d [10]. In this study, approximately 14 mg prepupal dry weight was obtained in 27 d (feeding rate of 50 mg /larvae/d) which is equivalent to an average weight increase of 0.5 mg prepupae/d. As such indicates that the type of feed determine the larval growth and consequently the final prepupal dry weight.

The amount of water content in the feed also plays an important role in the larval growth. In this study, the substrate was mixed with water at a ratio of 2:3 (weight per volume) as recommended by Diener *et al* (2009) to provide the required moisture in the feed [9]. The residues in the container which comprises of excretory products and unconsumed feed were dried at 103 ^{O}C to determine the water content in the residues and the results are shown in Figure 4. The amount of water content in the residues is highly influenced by the feeding rate. The texture of the substrate in a form of fibrous powder allows a higher water holding capacity with an increasing amount of substrate. This is reflected in Figure 4 that the water content in the residues increases from approximately 36 to 78% as the feeding rate increased from 25 to 100 mg/larvae/d. This is in agreement with the results reported by Guo-Hui *et al* (2014) that the feed should contain an optimum water content of 70% to increase the larval growth rate [21].

Figure 5 shows the relative proportion of feed that was converted into prepupal biomass, used for metabolism of the larvae and remains as residual matter. The results show that only a very small fraction of the feed (0.3-1.2%) was transformed into prepupal biomass. In contrast, a very large portion of the feed (95-96%) remains as residue which comprises of faeces excreted by the larvae or feed that had not been consumed at all by the larvae. Based on the proportion of feed that was converted into prepupal biomass and remains as residue, the proportion of feed that was used by the larvae for their metabolic needs lies in the range of 3-5%. The amount of feed that was converted into prepupal biomass obtained in this study is lower in comparison to the results presented by other studies. Diener *et al* (2009) stated that around 6-16.1% of chicken feed was transformed by BSFL into prepupal biomass [9] whereas Manurung *et al* (2016) reported that around 4-6% of the remaining biomass from *Reutealis trisperma* was transformed by BSFL into protein rich biomass [10]. As such may be due to the ability of BSFL to decompose different type of feed which contain different organic matters.

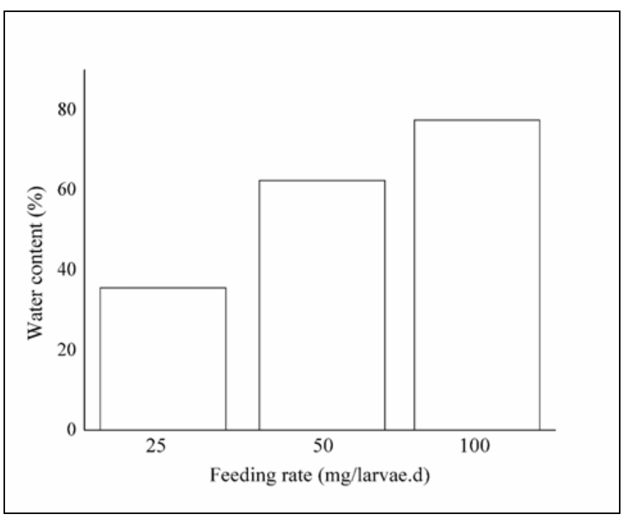

Fig 4: Water content of residues in a black soldier fly rearing container that comprises of excretory products and unconsumed feed

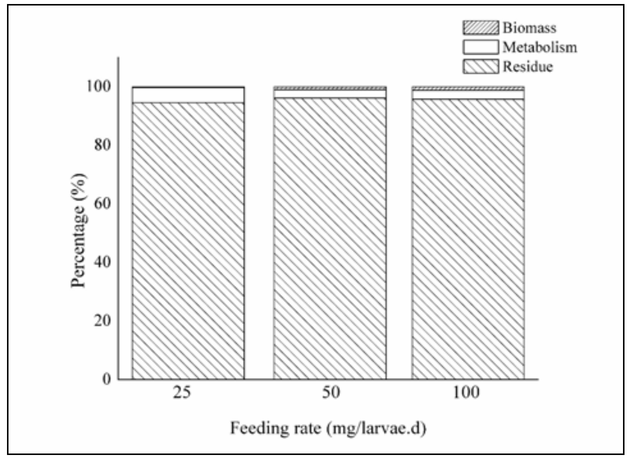

Fig 5: The percentage of feed converted into biomass (prepupal weight), used for metabolism and remains as residue

The efficiency of BSFL in converting the feed into its biomass can be estimated from the ECD value which is the ratio of dry weight of prepupae to the difference between initial dry weight of feed and dry weight of residue. From Table 1, it can be observed that the ECD increased from 6.3 to 26.3% as the feeding rate increased from 25 to 50 mg/larvae/d. Further increasing the feeding rate from 50 to 100 mg/larvae only slightly increased the ECD to 27.4%. Lower ECD value for feeding rate of 25 mg/larvae/d suggests that the larvae were experiencing food stress. At such conditions, the larvae consumed the feed only to reach its critical weight and obtained the amount of energy required to perform pupal development. At higher feeding rates, the ECD values agree with the results reported in the literature which lies in the range of 26 to 38% [9, 10].

The productivity of the prepupae at different feeding rates was estimated from the dry weight of the prepupae divided by the area of the container and growth period and the results are shown in Table 4. Taking into account the initial 7 d period of the larvae fed with chicken meal and 27 d of treatment with exocarp from *Pandanus* fruit as feed, the total development period from eggs to prepupae was approximately 34 d. Given that the rearing container used in this study has an area of 0.0024 m^2 and every container has 20 larvae, the productivity of the prepupae lies in the range of 1.4 to 4.7 g/m^2.d.

Table 4: Estimation of efficiency of conversion of digested feed and productivity of prepupae

Feeding rate (mg/larvae/d)	Efficiency of conversion of digested feed (%)	Dry weight of prepupae (mg)	Productivity of prepupae (g/m^2.d)
25	6.3	5.5	1.4
50	26.3	9.6	2.4
100	27.4	19.1	4.7

These results are lower than the previous findings reported by other studies. Manurung *et al* (2016) reported a productivity of 56.6 $g_{prepupae}/m^2$.d when BSFL were fed biomass from *Reutealis trisperma* whereas a higher productivity of 145 $g_{prepupae}/m^2$.d was estimated by Diener *et al* (2009) when fed which chicken feed [9]. Possible reasons for these differences are due to the different type of the feed and larval density used in these studies. Diener *et al* (2009) applied a larval density of 5 larva/cm^2 whereas this study applied a very much lower larval density particularly 0.86 larva/cm^2 which corresponds to the work done by Manurung *et al* (2016).

These results suggest that increasing the larval density may possibly increase the productivity of the prepupae.

Table 5 shows the comparison of lignocellulose composition of the feed and residue which comprises the excretory products and unconsumed feed as determined according to the Chesson-Datta procedures [19]. The hemicellulose content only slightly decreased from approximately 23 to 22 wt%, d.b and the cellulose content also decreased from 29.5 to 25.5 wt%, d.b. The results indicate that both hemicellulose and cellulose were digested during the bioconversion process. The lignin content slightly increased from approximately 30.9 to 32.4 wt%, d.b. which indicates that the BSFL was not able to digest lignin during the bioconversion process. These findings are in agreement with the results obtained in previous studies that BSFL has the ability the digest hemicellulose and cellulose but not lignin [10, 22].

Table 5: Lignocellulosic composition of the feed and residue remains in the rearing container for feeding rate of 100 mg/larvae.d

Component	Percentage (wt%, d.b.)	
	Feed	Residue[1]
Hemicellulose	23.0	22.0
Cellulose	29.5	25.5
Lignin	30.9	32.4

[1]feeding rate of 100 mg/larva/d

The protein content of the prepupae was analyzed and the results showed that the biomass contain 37.7 wt%, d.b. protein, a great increase in comparison to the protein content of the exocarp of *Pandanus* fruit (3.7 wt%, d.b.). Hence, bioconversion of the exocarp using BSFL successfully increased the added value of the remaining biomass from *Pandanus* fruits. The protein content of the prepupae obtained in this study resembles the protein content (37.3-37.6 wt%, d.b.) when BSFL were reared with chicken feed and residue from *Reutealis trisperma* as reported in previous studies [9, 10]. The protein content obtained in this study also satisfies Indonesian National Standard for protein (SNI 01-3929-2006) requirement of animal feed for chicken (13.5 wt%, d.b.). Hence, the prepupae of black soldier fly reared on the remaining biomass of *Pandanus* fruits may find suitable application as animal feed.

4. Conclusion

The total content of β–carotene, oil, and lignocellulose composition of *Pandanus* fruits have been determined. The mesocarp of *Pandanus* fruit contains approximately 1.8- 6.2 µg/g in ß-carotene. The endosperm contains about 46-52 wt%, d.b. edible oil whereas the total lignocellulose content in the endosperm was approximately 63-67 wt%, d.b. Bioconversion of the exocarp of *Pandanus* fruits using black soldier fly larva for producing protein-rich biomass has been carried out. At a feeding rate of 100 mg/larvae.d, the efficiency of conversion of black solider fly larvae in digesting the exocarp was 27.4% with an estimated productivity of 4.7 $g_{prepupae}/m^2_{container}$.d. The produced prepupae has a protein content of 37.7 wt%, d.b. which may find suitable application as animal feed.

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