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Valorisation of *Reutealis Trisperma* Seed from Papua for the Production of Non-Edible Oil and Protein-Rich Biomass

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Abstract. The valorisation of *Reutealis trisperma* seed for the production of non-edible oil and protein was investigated. *Reutealis trisperma* fruits contain approximately 60-61 wt%, d.b. mesocarp, 26-28 wt%, d.b. endosperm and 13 wt%, d.b. endocarp. The endosperm of ripe *Reutealis trisperma* fruit contains about 54-59 wt%, d.b. non-edible oil whereas the mesocarp contains only 3-9 wt%, d.b. oil. The cake obtained after the extraction of oil from the endosperm was mixed with the endocarp (20 wt% cake and 80 wt% endocarp) and used as feed (50 mg/larva/d) for the cultivation of *Hermetia illucens* larvae in a rearing container. The feed contains 39.2 wt%, d.b. hemicellulose, 10.9 wt%, d.b. cellulose and 29.9 wt%, d.b. lignin and 0.2 wt%, d.b. ash. The protein content of the feed was 19.1 wt%, d.b. A prepupal dry weight of approximately 50 mg/larvae was obtained after 12 d of treatment with an estimated productivity of 10.2 $kg_{prepupae}/m^3_{container}.d$. The estimated efficiency of black solider fly larvae in converting digested food was 21.6% with an assimilation efficiency of 27.7%. The prepupae of *Hermetia illucens* contains approximately 37.6 wt%, d.b. protein and 33.2 wt%, d.b. fat. As such indicates that the remaining biomass from *Reutealis trisperma* seed after the oil extraction has the potential to be used as feed for the production of protein-rich biomass.

Keywords: valorisation, *reutealis trisperma* seed, non-edible oil, extraction, *hermatia illucens* larvae, protein-rich biomass

1. Introduction

The island of New Guinea is extremely rich in biodiversity with almost 20.000 flora species [1]. Papua is one of the major provinces in the island which comprises a large mountainous area, forest lowlands and large areas of coastal mangrove swamps. It is reported that there are approximately 1030 flora species in Papua from which 55% of them are endemic species [2]. Papua has the highest number of flora species in Indonesia with a huge potential for bioproduct development [3] but most of the species are yet to be valorised by the local people.

Among the species that has received little attention is *Reutealis terisperma* (Blanco) Airy Shaw that belongs to the family of Euphorbiaceae. *Reutealis terisperma* which is locally known as 'kemiri sunan' in Indonesia is native to Philippines and cultivated in small scale in several Asian countries such as Indonesia, Malaysia and Philipinnes. Several studies about the potential of *Reutealis terisperma* as a non-edible source for the production of biodiesel have been reported [4], [5]. However literature about *Ruetealis trisperma* from Papua is very scarce in comparison to the same species from other regions of Indonesia such as West Java [4], [6].

The *Reutealis terisperma* seeds contain approximately 38-40 wt% oil. The oil can be valorised to produce biodiesel by a two stage process particularly acid esterification and alkali catalysed trans-esterification [5]. The remaining biomass (approximately 60 %) which is normally discarded as waste contain protein and lignocellulose. The biomass can be valorised as a substrate for the cultivation of black soldier fly larvae (BSFL) (*Hermetia illucens* L.) to produce higher value added products such as protein-rich

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biomass. BSFL which originates from the USA and spread throughout the tropics and sub-tropics have been considered as efficient converters for organic waste and nutritious feed for poultry [7]. BSFL which is a non-pest fly has a relatively high protein (40-45 wt%) and fat (27-35 wt%) content which depends on the substrate consumed by the larvae [7], [8].

This study aims to explore the valorisation of *Reutealis terisperma* fruits obtained from Papua as a potential source for the production of non-edible oil and protein-rich biomass. *Reutealis terisperma* oil was isolated from the seed and can be used for the production of biodiesel. The remaining biomass after the extraction of oil from *Ruetealis trisperma* seeds was valorised as a substrate for the cultivation of BSFL and the protein and fat content of the prepupae of *Hermetia illucens* L. were determined, which is an absolute novelty of this study.

2. Materials and Methods

2.1. Materials

Ripe and overripe *Ruetealis trisperma* fruits were obtained from Papua, Indonesia whereas black soldier eggs were obtained from Sumedang, Indonesia. Hexane (99 vol%) was obtained from Bratachem (Bandung, Indonesia).

2.2. Determination of Total Moisture Content

The total moisture content of the samples used in this study was determined bas on the procedures suggested by Abduh [9]. It involves heating the samples in the oven at 103 ^{O}C until constant weight.

2.3. Extraction of Oil from the *Ruetealis Trisperma* Seeds

Ruetealis trisperma fruits were dehulled to separate the fleshy content (mesocarp), shell (endocarp) and kernel (endosperm). The oil from the endosperm was extracted using a Soxhlet extractor as described by Abduh [9]. The samples were dried overnight at 103 ^{O}C and later grinded using a coffee grinder. Approximately 10 g of sample was extracted in a Soxhlet thimble with n-hexane for at least 5 h. The solvent was evaporated in a rotary evaporator and later dried in an oven at 60 ^{O}C until constant weight. The amount of oil in the seed is reported as gram oil per gram sample on a dry basis (d.b.)

2.4. Cultivation of Black Soldier Fly Larvae with the Biomass of *Ruetealis Trisperma*

Black soldier fly eggs obtained from Sumedang, Indonesia were hatched and the larvae was reared with chicken meal for 7 d before fed with the remaining biomass obtained from *Ruetealis trisperma* seeds after oil extraction as a substrate. Twenty black soldier fly larvae were placed inside a rearing container (diameter of 7 cm and 12 cm height) and fed with the remaining biomass from *Ruetealis trisperma* seeds. The cake obtained after the oil extraction was mixed with the endosperm (20 wt% cake and 80 wt% endocarp) before grinded and sieved (12 mesh). The mixed biomass was added with water (40 wt% residues and 60 wt% water) and used as a substrate for the cultivation of BSFL. The feeding rate was set at 50 mg/larva/d and the treatment was carried out until the larvae have developed into prepupae (approximately 12 d). The larvae were weighed every 3 d and moved into a new container containing fresh feed. The residues in the container which comprises of the excretory products and unconsumed feed were dried at 103 ^{O}C. The weight of the faeces was determined before and after the drying process. Data measurement was carried out until 50% of the population of larvae in the rearing container had developed into prepupae. After that the prepupae were inactivated by drying at 103 ^{O}C until constant weight was achieved.

2.5. Data Analysis

The efficiency of BSFL in converting the remaining biomass of *Ruetealis trisperma* seed into protein-rich biomass was accessed by calculating the assimilation efficiency, efficiency of conversion of digested-feed and waste reduction index as suggested by Scriber and Slansky [10] and Diener et al [7].

Assimilation efficiency (AE) provides information about the ability and effectiveness of BSFL in digesting the feed which can be calculated by the following equation:

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

where

I initial dry weight of feed (mg)

F dry weight of residue (mg)

Efficiency of conversion of digested-feed (ECD) provides information about the effectiveness of BSFL in converting the digested feed into biomass which can be calculated by the Eq. (2).

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

where

B dry weight of prepupae (mg)

I initial dry weight of feed (mg)

F dry weight of residue (mg)

The amount of feed digested during the period of treatment can be expressed as waste reduction index (WRI) and obtained by dividing the AE with the period of treatment (d).

2.6. Analytical Methods

The lignocellulose content of the sample used in this study was determined based on the procedures suggested by Chesson-Datta [11]. The fatty acid composition of the oil obtained from *Ruetealis trisperma* seeds was determined by gas chromatography-mass spectrometry (GC-MS) at the Chemical Laboratory, Indonesian Institute of Sciences, Bandung. The protein and fat content of the samples were analysed by Kjedahl method (SNI-01-2891-1992) and Gerber method (SNI-01-2981-1992), respectively at the Analytical Laboratory, University of Padjajaran, Jatinangor.

3. Results and Discussion

Valorisation of *Reutealis terisperma* fruits obtained from Papua for the production of non-edible oil and protein-rich biomass has been studied as illustrated in Fig. 1. *Reutealis terisperma* seeds 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. The remaining cake which contain a relatively high amount of protein and lignocellulose was mixed with the remaining endocarp (20 wt% cake and 80 wt% endocarp) as well as water before used feed for the cultivation BSFL in a rearing container. The feeding rate was set at 50 mg/larva/d and the treatment was carried out until most of the larvae have developed into prepupae (protein rich biomass). The characteristic of *Ruetealis trisperma* fruit and the composition its oil as well as the cultivation of BSFL to produce protein rich biomass (prepupae) are discussed in the following sections.

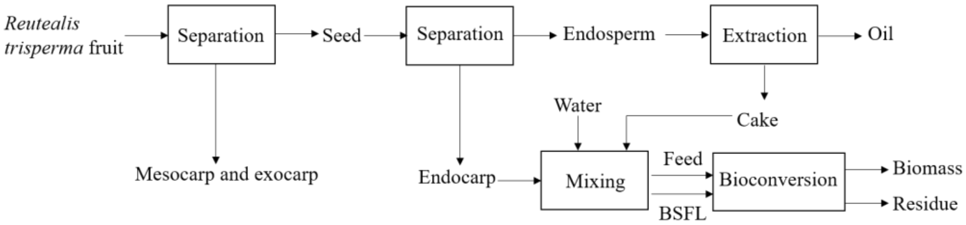

Fig. 1: Valorisation of *Reutealis trisperma* fruit for the production of non-edible oil protein rich biomass (prepupae)

3.1. Characteristics of *Ruetealis Trisperma* Fruits

The experiments were carried out using *Ruetealis trisperma* fruits obtained from the Papua, Indonesia. The fruits have a pale brown-greenish appearance with an average diameter of 4.5 cm. Each fruit contains 1 to 2 seeds which comprise of a pale brown endosperm surrounded by a relatively smooth and thin endocarp as shown in Fig. 2. A ripe *Ruetealis trisperma* fruit contain approximately 61.1 wt%, d.b. mesocarp, 26 wt%, d.b. endosperm and 13 wt%, d.b. endocarp. An overripe *Ruetealis trisperma* fruit had a relatively higher composition of endosperm particularly 27.5 wt%, d.b and lower content of endocarp; 13.0 wt%, d.b. The composition of the mesocarp was 59.5 wt%, d.b. which resembles the composition of a ripe *Ruetealis trisperma* fruit. The initial moisture content of the mesocarp as received was in the range of approximately

69 to77 wt% on a wet basis (w.b.) whereas the moisture content of endosperm was in range of 14.2 to 39.4 wt%, w.b.

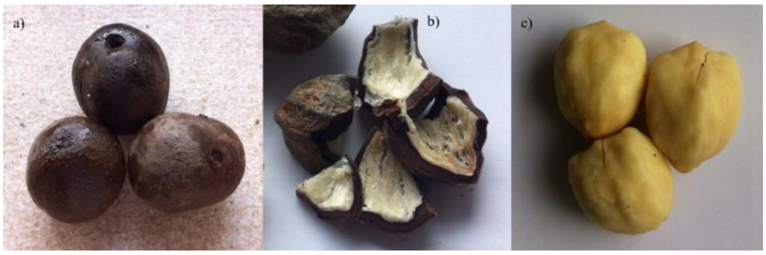

Fig. 2: Parts of Reutealis trisperma (a) fruits, (b) endocarp, and (c) endosperm

3.2. Composition of *Ruetealis Trisperma* Oil and Protein Content of Remaining Biomass

Ruetealis trisperma fruits were dehulled to separate the mesocarp, endocarp and endosperm. The oil content of mesocarp and endosperm of both ripe and unripe fruits was determined using a standardized Soxhlet extraction with n-hexane. For ripe fruits, the endosperm contains about 54 wt%, d.b. non-edible oil whereas the mesocarp contains only 3 wt%, d.b. oil. As for the overripe fruits, the endosperm and mesocarp contain a relatively higher amount of oil particularly 58.7 and 9 wt%, d.b., respectively. The total oil content in this study is slightly higher than the values reported by Holilah et al. [4] which lies in the range of 50-52 wt%. A slightly lower value obtained by the previous study most probably due to the hot pressing method used to isolate the oil. Soxhlet extraction with n-hexane as used in this study has been reported to be efficient in the extraction of oil content from oilseeds [9].

The fatty acid composition of the oil was determined and shown to consist mainly of palmitic acid (9.9 %), stearic acid (2.6 %), oleic acid (50 %) and linoleic acid (37.5 %). Scientific publication on the fatty acid composition of *Ruetealis trisperma* oil is rather limited in comparison to other non-edible oils such as jatropha oil and rubber seed oil [9]. The fatty acid composition of *Ruetealis trisperma* oil originated from Papua greatly differs from East Java which primarily composed of palmitic acid (22.9%), stearic acid (22%), oleic acid (30.2%), linoleic acid (13.6%), linolenic acid (1.8%) and archidic acid (0.79%) [4]. As such may be due to the different climate and soil conditions between Java and Papua. The relatively high content of oleic acid up to 50% in the *Ruetealis trisperma* oil promotes its suitable application as a promising feedstock for the production of biodiesel [4].

The protein content of remaining biomass from the *Ruetealis trisperma* seeds was also determined by Kjedahl methods. The protein content of the cake obtained after the extraction of oil from the ripe endosperm was 62.2% whereas the endocarp had a protein content of the 8.3%. As for the overripe *Ruetealis trisperma* fruits, the cake and endocarp had a relatively higher amount of protein particularly 73.4 and 9.8%, respectively. These results indicate that the remaining biomass after the extraction of oil from the *Ruetealis trisperma* seeds have a huge potential to be used as animal feed. The potential application of the remaining biomass of *Ruetealis trisperma* as a substrate for the cultivation for BSFL which is known to have a high protein and fat content suitable for animal feed application is discussed in the following section.

3.3. Bioconversion of *Ruetealis Trisperma* Biomass after Oil Extraction as Feed for the Cultivation of BSFL

Black soldier fly eggs obtained from Sumedang, Indonesia were hatched and the larvae was fed with chicken meal for 7 d before fed with the remaining biomass obtained from *Ruetealis trisperma* seeds after oil extraction as a substrate. The cake obtained after the extraction of oil from the endosperm was mixed with the endocarp (20 wt% cake and 80 wt% endocarp) and used as a feed for the cultivation of BSFL. The feed contain 39.2 wt%, d.b. hemicellulose, 10.9 wt%, d.b. cellulose and 29.9 wt%, d.b. lignin and 0.2 wt%, d.b. ash as determined based on the procedures suggested by Chesson-Datta procedures [11]. The protein content of the feed was 19.1 wt%, d.b. as determined by the Kjedhal method. The feeding rate was set at 50 mg/larva/d and the treatment was carried until the larvae have developed into prepupae (approximately 12 d).

Fig. 3 shows the weight profile of larvae in a rearing container fed with the remaining biomass from *Ruetealis trisperma* seeds. Initially the average weight of the larvae was approximately 37 mg/larva after fed with chicken meal for 7 d. At day 0, the chicken meal was replaced with the remaining biomass from *Ruetealis trisperma* seeds and the weight of larvae in the rearing container was continuously monitored. From Fig. 3, it can be observed that the average weight of larvae increased with time before it reached a plateau after 10 d (135.2 mg/larva). During this period the larvae continuously feed until they have obtained the amount of energy required to perform pupal development. After 10 d, the larvae secreted prothoracicotropic hormone (PTHH) which caused the larvae to stop feeding and developed into prepupae [7]. This is reflected in Fig. 3 that the average weight of the larvae decreased from 135.2 to 125.5 mg/larva when the larvae was continuously fed with the remaining biomass from *Ruetealis trisperma* seeds until 12 d.

Fig. 4 shows the appearance of BSFL at the beginning (day 0) and at the end (day 12) of the development time. At day 0, the length the BSFL was approximately 1 cm and continuously increased during the development time to reach approximately 2 cm after 12 d fed with the remaining biomass from *Ruetealis trisperma* seeds. Taking into account the initial 7 d period of the larvae fed with chicken meal, the total growth period for the larvae to develop into prepupae was approximately 19 d. Hence, the larvae in the rearing container had an average weight increase of 6.6 mg/larva/d. After 19 d, the prepupal dry weight was determined as 50 mg/larva. With a rearing volume 0.00041 m^3, the estimated productivity of the biomass was 10.2 $kg_{prepupae}/m^3_{container}.d$.

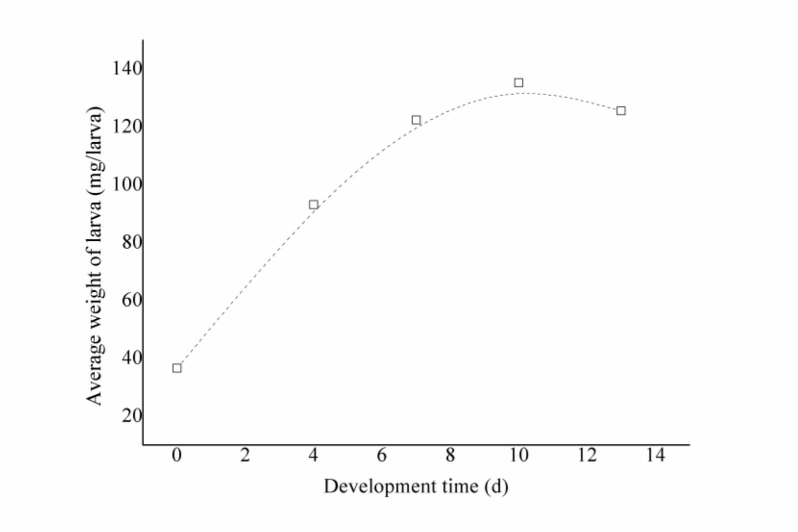

Fig. 3: Average weight of larvae with development time fed with the remaining biomass from *Ruetealis trisperma* seeds in a rearing container (feeding rate of 50 mg/larva/d)

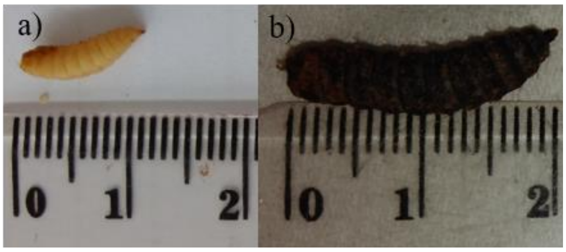

Fig. 4: Black solider fly larva at different development time fed with the remaining biomass from *Ruetealis trisperma* seeds in a rearing container (feeding rate of 50 mg/larva/d): a) Day 0 (b) Day 12

Fig. 5 shows the percentage of feed converted into biomass (prepupal weight), used for metabolism and remains as residual matter. A very large portion (72.3%) of the feed were categorized as residue which comprise of faeces excreted by the larvae or feed that had not been consumed at all by the larvae. A relatively small fraction of the feed (6.0%) was converted into prepupal biomass. Approximately 21.7% of the feed was used by the larvae for their metabolic needs which was calculated as the difference between residue and biomass to the total amount of feed provided during the development time.

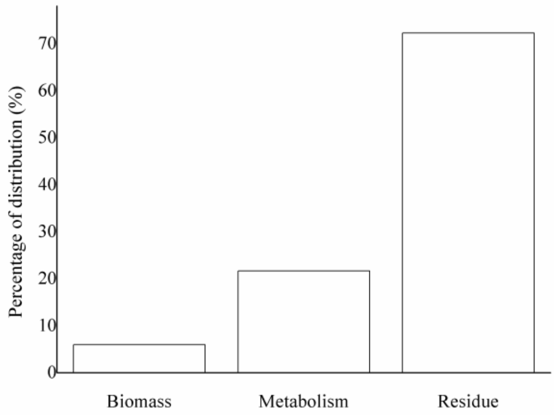

Fig. 5: The percentage of feed converted into biomass (prepupal weight), used for metabolism and remains as residue

The efficiency in converting digested (ECD) feed was approximately 21.6%. The ECD value indicates how efficient the BSFL in converting the feed into biomass. The assimilation efficiency (AE) of the BSFL was determined as 27.7%. This value provides a more accurate food conversion efficiency of the BSFL. In addition the waste reduction index (WRI) was also determined as 2.3. This index indicate the time that the larvae require to obtain a particular AE value. Diener et al [7] reported that the ECD and WRI values for BSFL reared on chicken feed at a rate of 50 mg/larvae/d were 26.2 % and 3.1, respectively. As such indicates that the values of ECD and WRI obtained in this study resembles the values reported in the literature.

Table 1 shows the comparison of lignocellulose composition of the feed and residue which comprises the excretory products and unconsumed feed as determined by the procedures suggested by Chesson-Datta procedures [11]. The hemicellulose content decreased from approximately 39.2 to 21.5 wt%, d.b and the cellulose content also decreased from 10.9 to 6.5 wt%, d.b. The results indicate that both hemicellulose and cellulose were digested during the bioconversion process. The lignin content increased from approximately 29.9 to 59.5 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 by Li et al [12] and Zheng et al [13] that BSFL has the ability the digest hemicellulose and cellulose but not lignin.

Table 1: Lignocellulosic composition of the feed and residue remains in the rearing container

Component	Percentage (wt%, d.b.)	
	Feed	Residue
Hot water soluble	19.8	12.0
Hemicellulose	39.2	21.5
Cellulose	10.9	6.5
Lignin	29.9	59.5
Ash	0.2	0.5

The protein and fat content of the prepupae were analysed and the results showed that the biomass contain 37.6 wt%, d.b. protein and 33.2 wt%, d.b. fat. These results resemble the protein and fat content (37.3 and 33.1 wt%, respectively) when BSFL were reared with chicken feed as reported by Diener et al [7] and St-Hilaire et al [14]. The protein and fat content obtained in this study also satisfies Indonesian National Standard for protein (SNI 01-3929-2006) and fat (SNI 01-3909-2006) requirement of animal feed for chicken (13.5 and 7 wt%, d.b.). Hence, the prepupae of black soldier fly reared on the remaining biomass of *Ruetealis trisperma* seed may find suitable application in the feed industry.

4. Conclusions

The potential of *Ruetealis trisperma* seed as a non-edible source for the production of plant oil and protein has been investigated. The endosperm contains a relatively high content of non-edible oil (54-59 wt%, d.b). The cake obtained after the extraction of oil from the endosperm was mixed with the endocarp (20 wt% cake and 80 wt% endocarp) and its application as feed for the cultivation of BSFL in a rearing container has been studied. A prepupal dry weight of approximately 50 mg/larvae was obtained after 12 d of treatment with an estimated productivity of 10.2 $kg_{prepupae}/m^3_{container}$.d. The estimated efficiency of black solider fly larvae in converting digested food was 21.6% with an assimilation efficiency of 27.7%. Bioconversion of the remaining biomass of *Ruetealis trisperma* seed (after the extraction of oil) using BSFL increased the protein content from 19.1 to 37.6 wt%, d.b. The fat content also increased significantly from 0 to 33.2 wt%, d.b. As such indicates that the remaining biomass from *Ruetealis trisperma* seed after the oil extraction has the potential to be used as feed for the production of protein-rich biomass.

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