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Exploring the potential of grease from yellow mealworm beetle (*Tenebrio molitor*) as a novel biodiesel feedstock

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HIGHLIGHTS

- ▶ Yellow mealworm beetle grease could be a potential feedstock for biodiesel.
- ▶ Most of the properties of yellow mealworm beetle biodiesel met the EN 14214 standard.
- ▶ This study further indicated the promising use of insect fat to produce biodiesel.

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ABSTRACT

Biodiesel has been considered as one of the promising non-fossil fuels, but its development also have promoted a drastic debate due to its current production status, such as oilseeds dependency, arable land requirement, high cost and long-term impact on food prices. Therefore alternative resources with considerable lower cost that could be used for biodiesel production have been studied. Immature life stage of some insects is able to consume various organic wastes for fat accumulation. This high fat containing insect has the potential to serve as biodiesel feedstock. In this study, larval grease extracted from yellow mealworm beetle (*Tenebrio molitor* L.) (YMB), a post-harvest scavenger, was investigated for finding its potential as a substitute of oilseeds. Decayed vegetables were used to feed YMB and after 9 weeks, then the grease was extracted for biodiesel production. About 34.2 g biodiesel was obtained from 234.8 g dried YMB larval biomass. The main fatty acids of YMB biodiesel were linolenic acid (19.7%), palmitic acid (17.6%), linoleic acid (16.3%) and stearic acid (11.4%). Most of the properties of the YMB biodiesel fed on decayed vegetables met the standard EN 14214, including ester content (96.8%), density (860 kg/m^3), flash point (127 °C), cetane number (58), water content (300 mg/kg), and methanol content (0.2%). From comprehensive analysis on the effect to society, economy and environment, it can be concluded that YMB can recycle organic wastes into clean energy with low cost.

1. Introduction

With the rapid development of the global economy, energy crisis and environmental degradation have become a severe challenge that needs our whole human being to face together. For the last decades, many countries and scientific and technical workers have been involved in the effort to discover biofuels, such as biodiesel [1] and bioethanol [2]. Biodiesel has been increasingly recognized as one of the most promising renewable biofuels [3]. However, biodiesel also has aroused a drastic debate due to its current production status, such as oilseeds dependency, arable land requirement, high cost and long-term impact on food prices. Presently, biodiesel is mainly from edible oils; for example rapeseed oil is the major material for producing biodiesel in Europe, soybean oil in the USA, palm oil in Southeast Asia [4,5]. Large-scale production of biodiesel from edible oil could cause global imbalance to the food supply and bring more serious problem to the world's famine [6]. The main obstacle of biodiesel application is still the high cost in which about 75% comes from the feedstock, causing a tremendous restriction of its economic feasibility [7]. To mitigate this situation and reduce the biodiesel cost, many studies have been conducted to find new nonfood and cheap feedstock for biodiesel production,

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such as *Jatropha curcas* [8], *Chinese tallow* [9], and microalgae [10,11]. However, most of the researches are yet at the laboratory scale, due to the problem of high costs or unsolved technical barriers.

Insecta, which are rated as the most diverse animal group, are distributed extensively in nearly every corner of the world, from the equator to the poles. Insecta have attracted the attention of researchers for its potential as energy resource in recent years, in which the scavengers that can recycle organic wastes for fat accumulation are the most being investigated. Black soldier fly larvae (BSFL), *Hermetia illucens* L. which can colonize a wide range of organic matters including animal manure, domestic and municipal refuse, can use the waste nutrition for development in which high protein and fat were synthesized. This larval grease is a low cost, promising, non-food feedstock for biodiesel [12]. The grease derived from BSFL which is converted from different kinds of organic wastes has been proved a novel and available feedstock for biodiesel [13–15]. BSFL can convert organic wastes into biodiesel and protein feedstuff, which is in accordance with the concept of circular economy in short lifecycle, while the energy plants need long lifecycle and plenty of land which do avoid conflict with food necessary for human beings [16]. These scavengers may make waste profitable and provide a new choice for the feedstock of biodiesel. However, more candidate insects need to be evaluated based on their different feeding habits of all wastes.

Yellow mealworm beetle (YMB), *Tenebrio molitor* L. which is an important post-harvest scavenger and distributed all over the world, with preference to eat decayed grain or milled cereals in damp and poor conditions; in some cases they even infest stored products. Each female adult YMB lays about 300 eggs, which could be hatched into larvae in 10–14 days. YMB is very voracious and highly resistant to low temperature [17,18]. It is routinely used as traditional protein feedstock especially in aquiculture. However, no documents had reported the application of YMB grease as the raw material for biodiesel. In this study, YMB was fed with decayed vegetables, and then the grease was extracted to produce biodiesel. The results indicated that biodiesel could be produced with lower cost from the organic wastes by YMB.

2. Materials and methods

2.1. Biomass

The YMB colony used in this study was maintained in the National Engineering Research Centre of Microbial Pesticides, Huazhong Agricultural University, Wuhan, China. The decayed vegetables, such as carrots, lettuce seeds and Chinese leaves, were obtained from the local market of Huazhong Agricultural University and used to feed YMB larvae. The main components in the decayed vegetables were cellulose (32.5%), hemicellulose (21.8%), lignin (8.6%), soluble sugar (8.7%), protein (1.2%), fat and grease (0.7%) and impurities (19.6%). The cultivation was conducted in the insectaries at 25–30 °C, humidity (60–75%). Decayed vegetables were provided to YMB as needed. After 9 weeks, YMB were harvested and inactivated at 105 °C for 5 min, and then dried at 60 °C for 3 days. After being grounded with micro-mill, the dried YMB biomass was stored at 4 °C until grease extraction could be performed.

2.2. Grease extraction from YMB biomass

To increase the grease yield from YMB larvae, preliminary trials were conducted to evaluate the extraction efficiency of different organic solvents including ethanol, acetone, and petroleum ether with Soxhlet extractor. It was found that petroleum ether (bp. 60–80 °C) hold the highest efficiency and lower energy input. Hence the grease was extracted from the dried YMB biomass in a Soxhlet extractor with petroleum ether. After 8 h of refluxing, the solvent was distilled off. The crude grease of dried YMB biomass was calculated by the weight loss before and after extraction.

2.3. Refinement of the YMB grease

The crude grease contained various kinds of impurities including phospholipids, and solid impurities. After extraction, the crude grease was treated with 1–1.5% (V/V) of concentrated H_3PO_4 (85%, V/V) at 30 °C and 2–4% of softened water, and mixed subsequently. YMB grease was gently stirred, settled for precipitation, and filtered to remove the pectin. After this refinement process, YWB grease with high purity was obtained in the end.

2.4. Characterization of YMB grease

The properties of grease, such as iodine number, saponification value, and peroxide value, were determined according to the standard method. Iodine number indicates the amount of unsaturation in the YMB grease. Saponification value is represented by the milligrams of KOH, and is used for the measurement of the average molecular weight of the YMB grease. Melt point refers to the freezing point of the grease. Peroxide value indicates the level of rancidity during storage, while acid value reflects the amount of free fatty acid in the grease. Biodiesel was derived from different sources, such as fats and oils; YMB grease was suitable for biodiesel production.

2.5. Production of biodiesel from YMB grease

Biodiesel was produced by a two step process because of the content of free fatty acids in the YMB grease [13]. The reaction was carried out in a reactor equipped with a reflux condenser, provided with thermometer, mechanical stirring and sampling out let. The free fatty acids were changed into biodiesel to decrease the acidity of the crude grease, which was reduced to less than 0.1% after acid-catalyzed esterification. The mixture was poured into a funnel for separation. Then the upper layer was transferred to a reactor for alkaline-catalyzed transesterification by a previously reported procedure, in which a 6:1 M ratio of methanol and 0.8% (w/w) NaOH were added. The mixture was placed in a 65 °C water bath for 30 min, with agitation by a magnetic stirrer. After the reaction, the mixture was separated by gravity in a funnel. The upper layer was then separated from the lower and purified by distilling at 80 °C to remove the residual methanol.

2.6. Analysis methods

The fatty acid composition of the YMB biodiesel was analyzed by GC/MS (Agilent, USA) equipped with a polyethylene glycol phase capillary column (Agilent, USA) [14]. The acid value, kinematic viscosity, cetane number and cloud point were determined according to the standard methods (ASTM). Oxidation stability was determined by EN 14112 method.

3. Results and discussion

3.1. Extraction of the grease from YMB

About 4000 YMB larvae (0.017 g, individual weight) were fed with 5000 g decayed vegetables (1.25 g/larva) within 9 weeks. About 704.1 g fresh YMB larvae (0.176 g, individual weight) were obtained after cultivation and 234.8 g of dried biomass was ob-

Table 1
Comparisons of properties of YMB grease, BSFL grease and rapeseed oil.

Property	YMB grease	BSFL grease [14]	Rapeseed oil [19]
Acid value (mg KOH/g)	7.6	8.7	1.14
Iodine value (gI/100 g) (wt.%)	96	84	115.5
Saponification value (mg KOH/g)	162	157	188.6[a]
Cloud point (°C)	3.7	5	−3.9
Peroxide value (meq/kg)	0.27	0.03	0.75[b]

[a] Reported by Shi and Bao [20].
[b] Reported by Bandonien et al. [21].

tained from 5000 g decayed vegetables, yielding about 41.6 g of YMB grease. Grease accounted for about 17.7% of the insect biomass. The yield of YMB grease from decayed vegetables is around 0.8% (w/w).

3.2. Characteristics of YMB grease

The properties of YMB grease and rapeseed oil were given in Table 1. Acid value of the YMB grease (7.6 mg KOH/g) and BSFL grease (8.7 mg KOH/g) was much higher than that of rapeseed oil (1.14 mg KOH/g). The iodine number of YMB grease and BSFL grease were lower than that of rapeseed oil, indicating that insect grease may have lower degree of unsaturation. Saponification value of YMB grease (162 mg KOH/g) was determined, and the result was very close to that of BSFL grease (157 mg KOH/g). The cloud point of YMB grease and BSFL grease were similar (3.7 °C and 5 °C), which was much higher than that of rapeseed oil (−3.9 °C). Peroxide value of YMB grease and BSFL grease were lower than that of rapeseed oil [14,19–21]. Different fats or oils as feedstock were used to produce biodiesel with different compositions and properties in different production processes. One can see from Table 1 that the characteristics of YMB grease were similar to BSFL grease and suitable to be used for biodiesel production.

Table 2
Fatty acids compositions of YMB biodiesel, BSFL biodiesel and rapeseed biodiesel.

Fatty acids	YMB biodiesel	BSFL biodiesel [14]	Rapeseed biodiesel [22]
Saturated fatty acids			
Capric acid (10:0)	1.2	3.1	–
Lauric acid (12:0)	1.3	35.6	–
Myristic acid (14:0)	8.1	7.6	–
Palmitic acid (16:0)	17.6	14.8	3.49
Stearic acid (18:0)	11.4	3.6	0.85
Unsaturated fatty acids			
Palmitoleic acid (16:1)	9.3	3.8	–
Oleic acid (18:1)	1.6	23.6	64.40
Linoleic acid (18:2)	16.3	2.1	22.30
Linolenic acid (18:3)	19.7	–	8.23
Odd carbochain fatty acids			
Pentadecanoic acid (15:0)	1.5	–	–
Heptadecanoic acid (17:0)	1.7	1.0	–
Nonadecanoic acid (19:0)	2.6	1.4	–

3.3. Chemical compositions of the YMB biodiesel

In this research, about 34.2 g of biodiesel was produced from 41.6 g of YMB grease fed on decayed vegetables by the two-step method. The total yield of biodiesel was about 0.7% (w/w) from decayed vegetables by YMB.

The chemical composition of fatty acid methyl ester in YMB biodiesel was analyzed by GC/MS, and the result was shown in Table 2. The data of BSFL biodiesel and rapeseed biodiesel were also compared [22]. The number of carbons in the YMB biodiesel ranged from 14 to 18, which was similar to that of fossil diesel. The structural characteristic of fatty acids showed that YMB biodiesel was a feasible substitute for petro-diesel. Raw materials that contain large proportions of fatty acid triglycerides are preferred. For example, rapeseed oil was found to contain more fatty acid triglycerides with little free fatty acids. The materials of low-cost oils and fats, such as restaurant waste grease in some areas are available; however, the major problem of using these low-cost oils or fats is that they often contain large amounts of free fatty acids which are difficult to be converted to biodiesel. Our results indicated that fatty acid compositions of YMB biodiesel were similar to that of BSFL biodiesel, and the YMB grease was a suitable feedstock of biodiesel.

One can see from Table 2 that these fatty acids varied in carbon chain length and the degree of unsaturation. The main fatty acids identified from YMB grease-based biodiesel were linolenic acid, palmitic acid, linoleic acid and stearic acid, and their relative contents were 19.7%, 17.6%, 16.3% and 11.4%, respectively. The total saturated and unsaturated fatty acid compositions of YMB grease were 45.4% and 46.9%, respectively. Three kinds of fatty acids with odd carbon number were identified from YMB biodiesel, and two kinds from BSFL biodiesel. The odd carbon fatty acids do not exist in oilseeds plants but can be found in insect. For example, there were no odd carbon fatty acids in rape biodiesel, while there were 5.8% odd carbon fatty acids in the YMB biodiesel, and 2.4% in the BSFL biodiesel.

3.4. Properties of the YMB biodiesel

Biodiesel properties are influenced by the structure of the individual fatty acid ester, such as degree of unsaturation and fatty acid profile. The quality indexes of biodiesel prepared from YMB grease in this study were demonstrated in Table 3. The properties of the YMB biodiesel were compared with biodiesel from BSFL [14] and rapeseed oil [19], in aspects of density, kinematic viscosity, flash point, ester content, acid number and cetane number, which were then compared with the EN 14214. Most of the fuel properties of

Table 3
Comparisons of properties of YMB biodiesel, BSFL biodiesel, rapeseed biodiesel and specifications of EN 14214.

Properties	EN 14214	YMB biodiesel	BSFL biodiesel [14]	Rapeseed biodiesel [19]
Ester content (%)	96.5 min	96.8	97.2	–
Density (kg/m^3)	860–900	860	872	880
Viscosity at 40 °C (mm^2/s)	3.5–5.0	5.9	5.8	4.83
Water content (mg/kg)	500 max	300	300	300
Flash point (closed cup) (°C)	120 min	127	121	–
Cetane number	51 min	58	53	45
Acid number (mg KOH/g)	<0.5	0.9	0.8	0.31
Methanol or ethanol (wt.%)	0.2%	0.2%	0.3%	–
Distillation	–	92% at 360 °C	91% at 360 °C	91% at 352 °C

YMB biodiesel were found to be in reasonable agreement with the specifications of standard EN 14214 as shown in Table 3.

Flash point of biodiesel is generally higher than that of petro-diesel. Methanol content is an important factor of biodiesel, and the flash point will be reduced because of methanol. In addition, methanol has bad effects on rubber parts, and can reduce the biodiesel combustion efficiency. Flash point and methanol content of YMB biodiesel met the EN 14214. Viscosity of biodiesel is usually higher than that of diesel fuel, as we found with YMB biodiesel. But viscosity can be reduced and meet the requirement of diesel standard by blending it with petro-diesel or other fuel. The free fatty acid causes the increase of acid value. Acid number of YMB biodiesel is out of the EN 14214 which may be due to the low conversion rate in the first step of acid-catalyzed esterification. Thus, a further optimization of reaction conditions for biodiesel production of YMB grease is needed. Biodiesel refining process afterwards also can help to minimize this issue.

Non-edible oil has been considered as an ideal feedstock of biodiesel because it can be produced at a lower cost without either arable land or human food. As non-edible oil, YMB grease, which can be produced from organic wastes, has high reproductive capacity and avoid the land competition with food. YMB grease was suitable for biodiesel production, YMB could serve as an energy source and the biodiesel industry would be further diversified.

4. Conclusions

Biodiesel has been used as replacement for fossil fuel over the last 10 years. But biodiesel from oilseed presents many problems because it requires large arable land. Moreover, using the food grade feedstock for biodiesel when many people still suffering from hunger because of the shortage of sufficient farm land is unacceptable, so low-cost feedstock is urgently needed to enable the possibility of producing biodiesel from organic wastes. By converting the decayed vegetables into the grease of YMB, about 34.2 g biodiesel was obtained from 234.8 g dried YMB biomass. It is indicated that the YMB grease is a proper feedstock for biodiesel. The properties of the YMB biodiesel fed on decayed vegetables which has a desirable methyl ester profile met the standard EN 14214, including ester content (96.8%), density (860 kg/m^3), flash point (127 °C), cetane number (58), water content (300 mg/kg), and methanol content (0.2%). Biodiesel production from organic wastes has great potential to cut down energy consumption, reduce the environmental impact and lower the cost compared with biodiesel from oilseeds and petroleum diesel. This study further demonstrated that saprophagous and omnivorous insects, such as BSFL and YMB, could serve as recyclers of nutritious organic wastes for energy use, and insect fat deserves more social attention for its promising utilization in energy substitution.

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