称号及び氏名	博士（工学）　Nguyen Huynh Phuong Uyen
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論　文　名	「A New Co-Solvent Method Using FAME for Green Production of Biodiesel Fuel – Optimization and Practical Application （脂肪酸メチルエステルを利用したバイオディーゼル燃料生産の新規低環境負荷共溶媒法　－　最適化と実用化　－）」
論文審査委員	主査　古田　雅一 副査　松浦　寛人 副査　川又　修一 副査　田中　良晴

論文要旨

Biodiesel fuel (BDF) has a lot of benefits related to the environment, society, and energy security. The raw feedstocks for producing BDF are vegetable oils (edible and inedible one), animal fats, waste cooking oils and algal oils. Currently, edible oils such as soybean, rapeseed, sunflower, palm, coconut, olive and et al. are the main resources for BDF production in the world. However, there is the competition between the cultivation of plants producing edible oils for biodiesel production and the nurture of agricultural plants using for crops of human foodstuffs. The change of land use has been taken place by the energy policy from cultivation of plants for food stock to that for BDF production. The consequence could decrease carbon emissions along with BDF expansion

to such a level that the life-cycle greenhouse gas (GHG) emissions were lower than that with fossil fuels. However, cost of BDF from edible oils is more expensive than that of petrol. Therefore, biodiesel from non-edible oils is alternative feedstocks to overcome the associated problems with edible oils. The use of non-edible plant oils is very significant because of the tremendous demand for edible oils as a food source.

This study was designed to find an effective method to produce BDF from the oils, that was complied with BDF standard quality. This research was conducted to develop a new co-solvent method using FAME (fatty acid methyl ester) for green production of BDF. The thesis approaches following issues:

In **Chapter 1**, introduction of biofuels and biodiesel, the biodiesel chemical reaction, some advantages and disadvantages, the sources materials for the production of biodiesel, and some current methods for the production of biodiesel are discussed.

In **Chapter 2**, the production of biodiesel from canola oil by transesterification using FAME as co-solvent is described. The co-solvent method for BDF production has an advantage such as less energy, smaller amounts of reagents required, and high quality product of BDF could be obtained. In this chapter, a new co-solvent method was developed using FAME as co-solvent instead of acetone. We investigated the optimum condition of transesterification using FAME as co-solvent by varying the FAME amount of co-solvent, reaction temperature, catalyst amount, and the ratio of MeOH amount to oil. The parameters affecting FAME yields are as follows; FAME amount was in the range of 0–20 % to oil, and reaction temperature in the range of 20–50 °C, and the oil/MEOH molar ratio in the range of 1/3–1/6, and the KOH amount to oil in the range of 0.5–2.0 wt. %. With increasing initial FAME contents of 0, 5, and 10 wt. %, the FAME yields were accelerated, and attained 81.6 %, 89.1 %, 97.0 % in 60 min, respectively. Particularly, with the content of 20 wt .%, the yields were 95.4% in 20 min,

97.4 % in 40 min, and 99.4 % in 60 min, respectively. The temperature is also an important factor affecting the FAME yields. The reaction rate proceeded rapidly and the yield of FAMEs reached 97–99 % at the temperature of > 30 °C in 40 min. The optimal molar ratio of methanol to oil was also investigated and showed that ratios of 5/1 and 6/1 gave FAME yields of 98–99 % in 40 min, whereas the yield was 80 % with the stoichiometric molar ratio of 3/1. The molar ratios of methanol to oil of 5/1 was the optimal molar ratio for transesterification. The effect of the amount of KOH catalyst on the yield of FAMEs was examined. When the KOH amount was 1 wt. %, the 99.5 % yield of FAME was obtained. The optimum conditions for transesterification using co-solvent of FAME were as follows; the initial FAME was 20 wt. %, the molar ratio of MeOH/oil 5/1, KOH 1 wt. %, and reaction temperature 25 °C. The physicochemical properties of the BDF produced satisfy the BDF quality criteria prescribed in EN 14214 /JIS K2390 standards. The procedure contributes to the cost performance of BDF production and is an environmentally friendly manner by recycling of FAME product as co-solvent and reducing the amount of wastes.

In **Chapter 3**, enzymatic hydrolysis of vegetable and seed oils by enzyme of lipase was examined. Lipase contained in vegetable and seed oil could hydrolyze a lipid to produce fatty acids (FA). To produce a FAs from oil by hydrolysis reaction using enzyme as a catalyst, one must understand their physiological functions as well as their activity in vitro. In this study, , we examined the mechanism of lipase hydrolysis in *in vitro* experiments. Three kinds of enzymes, (1) AYS Amano from *Candida cylindracea*, (2) AK Amano from *Pseudomonas fluorescens*, and (3) PS Amano SD from *Burkholderia cepacia* LP-7 were selected to study the effect of enzyme type on the hydrolysis of oil. The lipase enzyme solution was added into the emulsions of oil and aqueous buffer solution of pH 7.0 at different concentrations of 6, 9, 11 %mg/g and the reaction temperate was

maintained in the range of 25–60 °C. During the process of reaction, the reaction mixture was taken out at certain interval and the glyceride components of triglycerides (TGs, oil), di-glyceride (DG), mono-glycerides (MG) and FAs were determined using high performance liquid chromatography (HPLC). The results showed that among three kinds of lipase, lipase of (1) gave the highest activity on the hydrolysis oils and 60 % of TG was hydrolyzed in 7 h at 40 °C. The reduction of 60 % TG was observed in 7 h at 40 °C. The number of products (DG, MG, and FA) from TGs was rapidly decreased in 2 h where 50% of TG was lost, and then gradually decreased.

In **Chapter 4**, distributions of tocols contained in the oil and in parts of rice cultivars and their UVB sensitivity were investigated. The tocols (or Vitamin E) are antioxidants contained in the vegetable and seed oil, and plants for protection from environmental factors such as sunlight and air. Tocols (tocopherol (T) and tocotrienol (T3)) is a group of eight methyl substitutional isomers, namely four homologues of α-, β-, γ-, and δ-tocopherol (T) and four homologues of α-, β-, γ-, and δ-tocotrienol (T3) that are composed of the polar chromanol ring and hydrophobic aliphatic side chain. There have been great interests in the photosensitivity (or photo-resistance) of Japonica rice cultivars and tocols synthesized in rice plants. In the present study, distributions of phytochemicals of tocols contained in four parts of six Japonica rice cultivars were investigated. A relationship between tocol components of cultivars and their ultraviolet B (UVB) sensitivity was elucidated. The six rice cultivars cultivated in a greenhouse were collected at harvest age of early September and separated into four parts of leaves, stems, seeds, and roots. The total tocols contained in leaves was the highest of α-T (230±126 mg/fresh-g) and decreased in the order of seed (48.6±6.6mg/fresh-g), stem (13.0±11.4 mg/fresh-g), and root (0.97±0.37 mg/fresh-g). In leaves and stems, the highest component was 85 % of α-T following with ca. 6 % of γ-T. In seed, the main components

were 38 % of δ-T3 following with 32 % of α-T, and 20 % of α-T3. In root, the main components were 55 % of α-T following with 14 % of γ-T, 13 % of δ-T3. By the correlation analysis, the total tocols (and content of α-T) in root among four parts, exhibited a negative-correlation with UVB-sensitivity index (SI) of the cultivars predominantly ($P<0.03$).

In **Chapter 5**, the obtained results were summarized and the future study was discussed for promoting and developing the method of production BDP by large scale and applying to the industrial process.

審査結果の要旨

地球温暖化の主な原因とされている CO_2 の主要な排出源とされる化石燃料に代わる環境調和型燃料として、バイオディーゼル燃料（BDF）が強く期待されている。カーボンニュートラルと見なされる植物バイオマス由来の油脂は BDF の原料として期待されているが、製造コスト、食用油との農地の競合など、実用化に向けての課題が多い。本研究は農業生産と競合することなく荒れ地の緑地化にも貢献できる東南アジア産の非食用の植物油脂に着目し、新たに脂肪酸メチルエステル（FAME）を共溶媒としたより実用的な製造法を開発し、種実に含まれる油脂の安定性に関わるリパーゼによる油脂成分の分解劣化挙動、さらにバイオマスの高度利用を目指した有用物質単離などについても検討し，以下の成果を得ている。

(1) カノーラ油を原料とし、従来のアセトンに代わる共溶媒として FAME の利用を発想し、触媒としてのメタノール、KOH の量比，温度などを最適化し，FAME20％、MeOH/oil 5/1、KOH 1wt％，反応温度 25℃で 99.5％以上の転換効率を達成し、国内外の製造基準に適合する BDF の製造法を確立した。

(2) 種実中の油脂の劣化の原因となっている種実中のリパーゼの反応機構を探るために市販の微生物由来のリパーゼによる油脂の分解を試験管内で経時的に調べたところ，トリグリセリドから順次ジグリセリド、モノグリセリドを生じ、最終的に脂肪酸が生じることを確認した。またリパーゼのガンマ線照射により活性は減少することを明らかにした。

(3) アジア地域における食料源として不可欠なイネのバイオマス利用高度化を図るために、光合成系を UV-B から防御するトコール（ビタミン E）類に着目し、我が国で栽培されている 6 品種について食用に供される種実以外の葉、茎、根の含量を分析すると共に UV-B に対する抵抗性との関連性について検討した。その結果、従来注目されてこなかった根にもトコール類が検出され、品種間の根のトコール含量の差は UV-B に対する感受性と負の相関性を示すことを明らかにした。

以上の研究結果は東南アジア地域における BDF 製造技術の実用化に不可欠な有用性の高い実践的知見を与えており、また未利用生物資源の高度利用という点においても今後の研究の発展に寄与するところ大である。これらの成果はアジア地域における低炭素社会の実現を促進する鍵となることを大いに期待させるものであり、また、申請者が自立して研究活動を行うに必要な能力と学識を有することを証したものである。