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論　　文　　名	「Utilization of Oil-Rich Biomass for the Production of Biodiesel Fuel and Exploration of the Multi-Beneficial Components」
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論文要旨

The great attentions have been received on biomass energy due to its benefits on sustainable development. It gives opportunities in energy security, social and economic development, climate change mitigation, and reduction of environmental and health impacts. Biomass has a large potential, which meets the goal of reducing greenhouse gases and could insure fuel supply in the future. The oils come from the oil-rich biomass divided in two groups: edible and non-edible oil. Focus should be shifted to exploit not only non-edible resources that do not compete with edible vegetable oil in market, but also multi-beneficial compounds in oil plants. It is imperative to have as much information on biochemical applications and pharmaceutical uses as possible, so that the potential of plant could be utilized efficiently. Candlenut tree (*Aleurites moluccana*) of which seeds contained 20 – 30 % oil is a resource of non-edible oils, and its oil has been used for pharmaceutical, cosmetic, industrial, and dietary purpose. *Jatropha curcas* is a multi-purpose and perennial plant belonging to a spurge Euphorbiaceae family. Its seed kernels contain up to 60 % oil that is non-edible. Rice bran contains not only an edible oil (20 %), but also high levels of various antioxidants, such as vitamin E, γ-oryzanol, and phenolic acids (especially ferulic acid (FA)). In Vietnam, an estimated 4.2 million tons of rice bran are generated each year. Much of rice bran has been discarded, and thus represents a large untapped potential source of a valuable commodity. Knowing their multi-beneficial compounds in oil-rich seeds and crops must be necessary for evaluating the price of raw materials, the quality of BDF and improving its economic efficiency.

In this study, the exploration of antioxidants in rice bran, and utilization of valuable compounds of by-products (soapstock) from rice bran oil processing is discussed. In addition, the BDF production from candlenuts oil, the behavior of toxic phorbol esters (PEs) in *Jatropha curcas* oil and BDF, and how to analyze these kinds of toxic compounds are also concerns. The utilization of oil-rich biomass is an important driving force for the socio-economic development by means of efficient uses of these resources from the point of view of biodiesel production associating with the exploration of multi-beneficial compounds. More specific discussions are outlined below.

In Chapter 1, general considerations on renewable energy, especially on biomass energy are summarized to highlight how they are important for human life and climate changes in aspect of sustainable developments. Then, the overview of biomass-based oil and biodiesel production is presented. Markedly, the utilization of biomass to improve its economy by exploring multi-beneficial compounds including the potential pharmaceutical compounds in biomass sources (rice bran) and toxic ones in non-edible oil seed (candlenut and *Jatropha curcas*) are summarized. The final part of this chapter presents the objective and organization of this thesis.

In Chapter 2, various methods for simultaneous extraction of tocols (tocopherols and tocotrienols) and γ-oryzanol, and FA from rice bran were examined using binary solvents of ethyl acetate and water, The former tocols and γ-oryzanol were distributed into the upper organic phase, and the latter FA was into aqueous phase. The procedures examined were as follows: alkaline treatment (Alk), ultrasound-assisted extraction (UAE), and Alk combined with UAE (Alk+UAE). The experiments were conducted by varying the temperature and the extraction duration. The maximum yields of tocols and γ-oryzanol were obtained at 25 ºC over a time span of 30 min. Upon raising the temperature to 80 ºC, the yield of FA increased dramatically, while the recovery of γ-oryzanol slightly decreased. Employing the Alk+UAE procedure at 25 ºC, the concentrations of tocols, γ-oryzanol, and FA from various species of white rice brans in Vietnam and Japan were in the ranges of 146 – 518, 1591 – 3629, and 352 – 970 µg / g, respectively.

In Chapter 3, the alkaline hydrolysis of γ-oryzanol was investigated using a homogeneous reaction system in order to obtain the maximum yield of FA. The parameters examined were as follows: ratios of potassium hydroxide (KOH) to γ-oryzanol and ratios of ethanol (EtOH) to ethyl acetate (EtOAc). Experiments were conducted by varying temperature, duration, and assisting with ultrasonication (26, 78, and 130 kHz, 50 W, a planar type). Under homogeneous conditions (concentration of γ-oryzanol 12 mg / mL, KOH / γ-oryzanol ratio (wt / wt) of 10 / 1, and EtOH / EtOAc ratio (v / v) of 5 / 1), a 45 % yield of FA was obtained at 60 °C for 3 hours, and 73 % yield at 75 °C, respectively. With assistance of 78 and 130 kHz irradiation, the yield increased 1.6 fold at 60 ºC, while it was only 1.2 fold with assistance of lower frequency of 26 kHz. Further with raising temperature to 75 °C (78 and 130 kHz), the yield reached to 90%. From soapstock, the 74.3% yield of FA was obtained without assistance of ultrasonication. During one month storage of reaction mixture, 20% of the *trans*-FA from γ-oryzanol was transformed into its *cis*-form.

In Chapter 4, we investigated the effect of temperatures and ultrasonic irradiation (20 kHz, a horn type) with different powers (50 and 180 W) on the base-catalysed hydrolysis of γ-oryzanol in a homogeneous reaction system (EtOH / EtOAc / H_2O). The experiments were conducted with various ratios (wt / wt) of KOH to γ-oryzanol amount (concentration of γ-oryzanol 6 mg / mL), temperature, and ultrasonic irradiation powers. Without assistance of ultrasonic irradiation, 83.2 % yield of FA was obtained under the conditions as follows: KOH / γ-oryzanol ratio (wt / wt) of 20 / 1, temperature of 75 ºC, duration of 3 hours. The yield of FA incredibly increased to 93.3 % in 2.5 h at 60 °C using ultrasonic irradiation of a horn-type 20 kHz with power of 180 W. These results indicated that using the high frequency of horn-type 20 kHz, the high yield (more than 90 %) of FA was obtained at relatively low temperature and in short time. The method can be used to produce FA from γ-oryzanol in soapstock from rice bran oil processing efficiently.

In Chapter 5, biodiesel (BDF) production from candlenut oil (CNO) was investigated by two-step co-solvent method in order to obtain the high quality of BDF. Firstly, esterification of FFAs (free fatty acids) (7 wt%) contained in CNO was conducted using a co-solvent of acetonitrile (30 wt%) and H_2SO_4 catalyst. The content of FFAs was reduced to 0.8 wt% in 1 hour at 65 °C. Subsequent transesterification of the produced crude oil was conducted using a co-solvent of acetone (20 wt%) and 1 wt% KOH. The BDF content of 99.3 % was obtained at 40 °C in 45 minutes. After cleaning-up under vacuum condition at 5 kPa, the water content in BDF was attained 0.023 %. The components of CNO BDF were characterized using a Fourier-transform infrared (FTIR) spectrometry and gas chromatograph-flame ionization detector (GC-FID). The physicochemical properties of BDF satisfied the ASTM D6751-02 standard. The gaseous exhausts from the diesel engine by combustion of the BDF blends (B0 − B100) with petro diesel were examined. The emissions of carbon monoxide (CO), and hydrocarbons (HC) were clearly lower, but that of nitrogen oxides (NO_x) was higher in comparison to those from petro diesel.

In Chapter 6, we investigated a series of PEs extracted from *Jatropha curcas* seed kernels cultivated in Trang Bang, Vietnam with MeOH, and identified more than seven components contained in the PEs. The isolation of main five components of a series of PEs was conducted using a semi-preparative reversed phase HPLC analysis of ODS-3 column. The five peaks of components were successfully isolated, and peaks of J2, J3, J5, and J7 were assigned to be Jatropha factors C_1, C_2, C_3, and $C_{4/5}$, that has been already reported by M. Hirota *et al.* in 1988 and W. Haas *et al.* in 2002, but J6 was a mixture of Jatropha factor C_6 and its isomer based on the data of UV and LC-tandem mass spectrometry (LC-MS/MS), and J2 was identified using ^{1}H NMR analysis. By characterization using LC-MS/MS analysis, all components of a series of PEs were elucidated to be the 12-deoxy-16-hydroxyphorbol esters composed of isomeric form of dicarboxylic groups with same m / z value of 380.

In Chapter 7, the behaviors of toxic PEs in the *Jatropha curcas* oil were investigated during BDF production in order to certificate the safety use of the produced biodiesel fuel (BDF). LC-MS/MS and photo-diode array detector (PDA) analyses revealed that the PEs contained in *Jatropha curcas* oil had a tigliane skeleton. The partition coefficients ($K_{MeOH/oil}$) of several kinds of PEs between MeOH and the oil ranged from 2.4 to 20. As a result, the PEs in the oil were largely partitioned into the MeOH phase. The PEs in the oil were converted stoichiometrically into one molar phorbol and the corresponding three molar fatty acid methyl esters via a transesterification reaction in a KOH/MeOH solution. The phorbol produced predominantly partitioned into the glycerin phase. A small amount of phorbol residue contained in the BDF could be removed by washing with water. These results suggest that it is safe to use BDF produced by the aforementioned transesterification reaction and purification process. However, phorbol contamination of glycerin and wastewater from the production process should not be ignored.

In Chapter 8, conclusion of this thesis and summary of the previous chapters are described.

審査結果の要旨

　本論文は、バイオディーゼル燃料(BDF)を合成するにあたり、油の豊富なバイオマス中の有用物質の利用および非食用油に含まれる油中の毒性物質の BDF 製造過程での挙動について詳しく調べた論文であり、次のような成果を得ている。
１）食用に供することができるがその多くが未利用で世界で最も多くの潜在的生産量を有する米ぬか中の抗酸化剤の tocol（α、β、γトコフェロールとα、β、γトコトリエノールの総称)、γ-オリザノールとフェルラ酸の抽出方法を確立した。アルカリ条件下で超音波照射することで、米ぬか中に含まれる抗酸化剤の全量に対して、ほぼ８０％－９０％の抽出が可能となることを明らかにした。
２)上記の研究においてアルカリ条件下の抽出でγ-オリザノールから抗酸化剤として有用なフェルラ酸への加水分解を発見し､超音波照射下で 2 種の混合溶媒(エタノール/酢酸エチル)を用いることで 70%～９０％がフェルラ酸に加水分解できることを見出した。
３）Candle nuts 油を原料とした BDF の製造を検討した。Candle nuts 油には油分の分解した遊離脂肪酸が７－１０％も含まれているため、通常のアルカリ触媒法のトランスエステル化では純度のよい BDF が製造できなった。しかし、遊離脂肪酸を硫酸触媒によるエステル化でメチルエステルを生成し、分離後共溶媒法によるトランスエステル化を KOH 触媒で行なうことで高純度の BDF 製造を確立した。
４）非食用油である Jatropha curcas は BDF 原料として期待されていたが、この実には毒性の強いフォルボールエステルが含まれていることが問題であった。本研究では、まずこのフォルボールエステルの分析方法を検討し、確立した。この確立した分析法を用いることで、フォルボールエステルがアルカリ触媒によるトランスエステル化中で毒性の低いフォルボールに変化すること、生成した BDF 中にはフォルボールエステルは存在しないことを明らかにした。

　以上の諸成果は、経済的理由で未だに軽油の代替となっていない BDF を、その製造過程で高価な薬効成分を抽出・利用し、毒性物質を含まない非食用油－BDF を合成することで、安心して軽油と競合できる BDF とするためのブレークスルーに繋がる極めて重要な知見を得たものである。石油の枯渇が懸念される中、人類の未来の燃料として使うことができ、さらに地球温暖化対策にも繋がるため、人類の持続可能な発展に対して貢献するところ大である。また、申請者が自立して研究活動を行うのに必要な能力と学識を有することを示したものである。