称号及び氏名	博士（工学）　Le Anh Bang
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論文審査委員	主査　　竹中　規訓 副査　　久本　秀明 副査　　小川　昭弥 副査　　興津　健二

論文要旨

Energy is a basic need for social development and economic growth in modern society. The global energy demand has been increasing in recent years. Annual growth of global primary energy consumption from 1985 to 2016 was 1.0 – 2.5 %. According to the Energy Statistical Yearbook 2017 of Enerdata, the global energy demand has been expected to increase up to 31.2 % by 2030. Currently, over 80 % of the primary energy demand is covered by fossil fuels. The fossil fuels have been rapidly depleted due to their non-replenishable nature. In addition, consumptions of fossil fuel-based energy have also taken their toll on the environment, climate change, and health risks. Therefore, renewable energy sources, namely biomass, are attracted much attention as an alternative to fossil fuels. Using energy sources from waste biomass contribute also to reduce pressure of kind of this waste on environment. Despite the advantages of renewable energy sources over fossil fuels, the relatively high cost is remained as the main obstacle that hinders the wide spread use of renewable energy

sources in the world. The purpose of this thesis is to reduce the amount of agricultural waste, namely Roselle (*Hibiscus Sabdariffa*) and Jatropha (*Jatropha curcas)* seeds, and to recover valuable materials such as oil, vitamin E, and carbohydrate. Oil can be used as food or to produce biodiesel fuel. Vitamin E and carbohydrate, groups of antioxidants, can be used in medicine and food industries. Cascading use of valuable materials from the waste biomass have a part in reducing price of biofuel and speeding up the replacement of fossil fuel to renewable fuel. The thesis is divided into the following six Chapters:

In Chapter 1, general considerations on energy demand and role of renewable energy of biomass in the world were introduced. As suitable candidates of biomass resources for renewable energies in Vietnam, Roselle seeds and Jatropha seeds were selected for evaluation. Firstly, physicochemical properties of Roselle and Jatropha plants and applications to their oil were described. In addition to the main components of the oil, minor components contained in the seeds, vitamin E, carbohydrate, and others were reviewed. Finally, effective extraction methods conventionally used to recover valuable materials from plant materials were presented.

In Chapter 2, as Roselle seeds contain 21.3 – 26.6 wt% contents of carbohydrate in addition to the oil, ultrasound-assisted extraction (UAE) of saccharides using the extraction solvent of water was investigated by varying the frequency of 26, 78, and 130 kHz in the power range of 0.09 – 0.15 W. For comparison, a conventional mechanical stirring extraction (CMSE) was also conducted. The concentrations of sucrose, fructose, and glucose in an extract solution were monitored for 7 h. The concentration of di-saccharide of sucrose increased and reached to the peak at 10 min, and then decreased. On the contrary, the concentration of mono-saccharide of fructose and glucose increased gradually during 7 h. Using gas chromatography-mass spectrometry (GC-MS), main components of saccharides observed were fructose, glucose, and galactose in water extract, and sucrose and raffinose in methanol one. The results indicated that the enzymatic hydrolysis of sucrose was occurred simultaneously during extraction with water. The UAE under the applied conditions did not seriously affect the yield of total saccharides. However, ultrasound irradiation affected positively on enzymatic hydrolysis of sucrose.

In Chapter 3, on the basis of the results in Chapter 2, the effects of kinds of solvent, temperature, and UAE at 40 kHz with power of 0.7 W and 20 kHz with power of 25.5 W on the yield of total saccharide, and enzymatic hydrolysis of saccharides during extraction were investigated in detail. The solvents used for extraction were three kinds of solvents, water, methanol, and ethanol, and their mixtures. The saccharides were extracted effectively by water, methanol, ethanol and their mixtures. However, with increasing the water content in the solvent, the sucrose and raffinose were hydrolyzed enzymatically to be fructose, glucose, and galactose under the process of extraction. The enzymatic hydrolysis was inhibited when saccharides were extracted with water at the temperature of higher than 70 ºC and with water-alcohol mixtures of more than 50 vol% of alcohol in water. The temperature did not affect the yields of total saccharides, but the saccharides components extracted varied by activity of enzyme which depended on the temperature. The suitable temperature for enzyme activity was 50 ºC. Using UAE at 40 kHz with a power of 0.7 W at solid-liquid ratio of 1:10 (g:mL), the yield of total saccharides was 20 % higher than that by CMSE, and UAE at 20 kHz with power of 25.5 W at solid-liquid ratio of 1:25 (g:mL) did not increase the yield of saccharides in comparison with those of CMSE. However, this condition enhanced the yield of enzyme extracted from Roselle seeds.

In Chapter 4, a cascading use of oil (glycerides), vitamin E, and saccharides from Roselle seeds was investigated. The Roselle seeds contained 18.0 wt% of oil, 120.5 ± 0.2 μg/g of vitamin E, and 6.3 wt% of water-soluble saccharides. The solvent used for extraction of each components was as follows: (a) hexane for oil; (b) binary solvent (hexane to ethyl acetate (8:2 v/v)/ 3.3 % KOH in ethanoic aqueous solution) for tocopherols; and (c) water for total saccharides. UAE at 40 kHz with power of 0.8 W was conducted. The yield of each component was evaluated by varying the order of extraction procedure as follows: the order 1 was (c), and then (a); the order 2 was (b), and then (c); the order 3 was (a), and then (c). The most suitable extraction order was the order 3; the yields of oil and vitamin E were 100 % and 95.8 % in (a), and that of saccharides was 86.2 % in (c). By using the order 1, the yield of saccharides in (c) was 90.4 %, and the yields of oil and vitamin E in (a) were low as 13.1 % and 0.1 %, respectively. The low yields of these components were due to the hydrophilicity of residue when extracted with nonpolar solvent of hexane. Using the order 2, the yields of vitamin E and oil were 77.6 % and 15.0 % in upper phase of (b), respectively, and those of saccharides in lower phase of (b) and in (c) were 44.2 and 46.0 %, respectively. The low yield of oil was due to the partial saponification during extraction with alkali solution. The extraction efficiency of vitamin E by (a) was higher than that by (b). These results indicated that the extraction order 3, hexane and then water, was the most suitable for cascading use of these compounds. However, it was necessary to further exploit the separation procedure of vitamin E from main component of oil.

In Chapter 5, a cascading use of minor components of fatty acids, saccharides, and phytochemicals from Jatropha seeds was examined. The homogenized seed kernels were extracted with methanol, and the extract was distributed into ethyl acetate/water phase. The components of each layer were derivatized with TMS reagent of *N, O*-bis (tri-methyl silyl) trifluoroacetamide and the TMS derivatives obtained were screened by GC-MS. In hydrophobic phase of ethyl acetate, the degradation products from tri-glycerides to fatty acids (FAs), mono-saccharides, and glycerin were identified. In which, the four FAs of palmitic acid, oleic acid, linoleic acid, and stearic acid were identified as the main components with total content of 12 wt% of kernel. In addition, two tocochromanols of γ-tocopherol and γ-tocotrienol, and three phytosterols of campesterol, stigmasterol, and β-sitosterol were also identified. In hydrophilic phase of water, di-saccharide of sucrose was identified as the main component with content of 3 wt% of kernel. Furthermore, tri-saccharide of raffinose, sugar alcohol of sorbitol and *myo*-inositol, metabolites of amino acid, and a series of other metabolites were also identified in this layer. These results suggested that the *Jatropha curcas* seed kernel could be applied to a cascade use for metallic soap, liquid fuel, food and medical supplement, and cosmetics in addition to biodiesel fuel.

In Chapter 6, the conclusion of this thesis is summarized, and future perspective is described.

審査結果の要旨

本論文は、将来の化石燃料の枯渇や、温暖化対策として注目されているバイオマスの石油代替および有効利用に関する研究をまとめたものである。これまでのバイオマス燃料は、燃料の製造だけを目的として植物種子の油や動物の脂から製造されてきたが、現状では石油との価格競争のために製造・使用が断念される例が多く、石油の代替となることはできない。そこで、植物の種子に含まれる油以外の高価値有効成分（ビタミン E、糖類など）を段階的に効率よく回収できる技術を確立することを目的とし、その回収方法を詳細に調べ、次のような重要な結果を得ている。

１）Roselle 種子（ベトナム産）から抽出した糖類の抽出・回収方法を検討した結果、スクロースとラフィノースが存在していること、抽出時間や抽出液の放置時間が変化すると抽出液中の各種糖類の濃度は様々な値に変化することを見出した。さらに詳細に研究した結果、糖類の濃度変化の原因が酵素反応であることを明らかにし、抽出時の溶媒、温度、超音波抽出の最適化に成功した。

２）Roselle 種子から糖類、ビタミン E、植物油をカスケード的に抽出するために、まずヘキサンで抽出し、次に水で抽出を行なうことが最も効果的であることを示した。さらに本研究で得られた結果から、カスケード抽出における溶媒の選択は次の点を考慮して選択する必要があると結論付けた。1）所望の化合物の収率、2）使用される溶媒の環境への影響、3）抽出および精製プロセスに必要な総エネルギー。

３）Jatropha curcas の種子核粉末をメタノールで抽出し、抽出物を酢酸エチル/水相に分配し、抽出物の分析を行なった。その結果、酢酸エチル相（疎水相）においては、トリグリセリド、脂肪酸、単糖類、グリセリンを、水相においては、スクロース、ラフィノース、糖アルコール、アミノ酸の代謝物を同定した。これらの結果から、Jatropha curcas の種子からは、バイオディーゼルに加えて、金属石鹸、食品および医療補助食品、化粧品のカスケード用途に適用できることを示した。

以上の結果は、石油代替としてのバイオ燃料の可能性を示すものであり、人類の長い繁栄と成長を支えるエネルギー問題の解決に大きく貢献するものである。また、申請者が自立して研究活動を行なうのに十分な能力と学識を有することを示したものである。学位論文審査委員会は、本論文の審査ならびに最終試験の結果から、博士（工学）の学位を授与することを適当と認める。