称号及び氏名	博士（工学）　Omid Pourali
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論　文　名	Production of Valuable Materials from Rice Bran Biomass Using Subcritical Water
論文審査委員	主査　　吉田　弘之
	副査　　小西　康裕
	副査　　白井　正充

Summary

The worldwide demand for fossil fuels continues to rise at a rapid pace while supplies are finite. As developing nations increase their needs for the fossil resources and hydrocarbon products, supplies will become tighter. Furthermore, due to excessive use of fossil resources in recent decades, environmental issues such as global warming have become considerably serious in the world. Therefore, it is urgently required to look for alternatives to fossil resources to have sustainable economic growth. Biomass is the most abundant renewable resource in the world. As an annual production is up to 1.7-2.0 × 10^{11} ton, it has been identified as an important source for alternative fuels and valuable chemicals. However, only 6 × 10^{9} ton of biomass is currently used for food and non-food applications; therefore, it is indispensable to develop the environmentally protection technologies, such as biorefinery, to bring the lowest impact to the environment. Subcritical water is one of the biorefinery techniques which have been utilized in various fields of green engineering and material cycling.

The present study has been devoted to develop subcritical water treatment technique to obtain valuable and edible substances from rice bran which is inexpensive and abundant biomass. It contains oil, carbohydrates, proteins, vitamins, enzymes, phenolic compounds, and dietary minerals. The effect of several parameters of subcritical water was investigated in detail. This study had three main objectives. The first objective was to develop the possibility of rice bran decomposition and hydrolysis for production and/or extraction of valuable substances such as organic acids, amino acids, and water-soluble sugars. The second objective was utilization of subcritical water as extractive medium for extraction of rice bran oil simultaneous with the inactivation of lipase enzyme of bran in order to produce edible oil. The third objective was to clarify the feasibility of phenolic compounds production by hydrothermal degradation of lignin/phenolics-carbohydrate complexes of rice bran under subcritical water conditions. The major and important results of this thesis are summarized as follows:

In chapter one, the general introduction and importance of the present work as well as review of previous works were presented.

In chapter two, application of subcritical water as green medium for hydrolysis and decomposition of rice bran was investigated in order to obtain value-added bio-based substances. A batch type subcritical water reaction system was used to perform the decomposition of rice bran over the whole temperature range of subcritical water at the reaction time of 5 min. It was observed

that hydrolysis reaction was effectively carried out without utilization of any organic solvent, acid, base, and/or enzyme.

Significant increases of total nitrogen (TN) and total organic carbon (TOC) in the water phase proved that bio-macro polymers of rice bran such as proteins and polysaccharides were hydrolyzed using subcritical water; they showed peaks at around 553 and 505 K, respectively. Decomposition of proteins under subcritical water conditions produced several amino acids; eight essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine) and six non- and/or conditionally essential amino acids (alanine, asparatic acid, glutamic acid, glycine, serine, and tyrosine) were identified in water phase which generally showed highest yield at 400 K. It was found that lysine and glutamic acid had the highest yields among essential and non-essential amino acids. Besides amino acids, organic acids were also identified in water phase after subcritical water hydrolysis reaction; the identified organic acids were acetic, citric, formic, glycolic, and levulinic acids. They were produced with decomposition of carbohydrates and amino acids at temperatures higher than 463 K.

Production of acidic organic substances like organic acids and amino acids during decomposition process may autocatalyze hydrolysis reaction of rice bran and increase production of useful materials.

Results indicated that cellulosic parts of rice bran could be successfully decomposed to water-soluble sugars using subcritical water treatment; fructose, glucose, glyceraldehyde, and sucrose were four sugars identified in the water phase. Furthermore, total soluble sugars of the aqueous phase (including mixtures of poly-, oligo-, di-, and mono saccharides) were investigated. The optimum condition for the best yield of total soluble sugars (nearly 20% of initial dry matter) was achieved at temperature of 463 K.

It was understood that hexane-soluble compounds (mainly rice bran oil) and acetonesoluble substances (attributed to tar, carbonized biomass, and in general water- and hexane-insoluble compounds) were produced during hydrolysis reaction, and could be isolated after subcritical water reaction.

In chapter three, application of subcritical water for extraction of rice bran oil in order to produce edible oil was investigated. In the first part of this chapter, effect of lipase enzyme of bran on the rancidity of the rice bran oil during storage period was studied. Then, inactivation of enzyme was studied using subcritical water. Experiments were performed at temperature range of 393 to 513 K and reaction time of 10 and/or 20 min. The quality of the obtained oil was evaluated with respect to its total free fatty acids concentration over a 12 week period, and compared with the oil obtained by the conventional extraction methods.

Without any treatment of the oil obtained by the conventional extraction methods and due to the enzyme activity, it was realized that total free fatty acids concentration in untreated sample increased sharply from 5.6% to 36.0% over storage period. On the other words, in less than one week from the date of rice milling, rice bran oil deteriorated (total free fatty acids concentration was higher than 10.0% in untreated oil) and became unfit for human consumption.

Experimental evidence showed that the enzymatic problem could be completely and irreversibly overcome using subcritical water treatment; total free fatty acids concentration remained constant in the stabilized oil during 12 weeks and no increase was observed. Total free fatty acids concentration in the treated oils was a function of subcritical water temperature and reaction time; the lowest and highest concentration of total free fatty acids were obtained at 433 K for 10 min and 473 K for 20 min, respectively. It was also observed that the conventional solid-solvent extraction methods, such as using hexane at ambient temperature, could not effectively inactivate enzyme, and total free fatty acids concentration increased in the course of oil storage.

Furthermore, the kinetic of free fatty acids formation in untreated sample was investigated. Based on the experimental data obtained from free fatty acids formation, a kinetic model was developed. Theoretical line matched well with the experimental formation curve of total free fatty acids. The kinetic model could accurately predict the rate of free fatty acids formation; therefore, this model can be used in the food industries to process the oil from rice bran prior to its deterioration. In the second part of this chapter, extraction of rice bran oil in subcritical water medium was investigated. Subcritical water could successfully extract oil in a short reaction time of 10 min simultaneous with oil stabilization. It was found that the temperature of subcritical water affected the oil extraction considerably; the higher the temperature was, the greater was the yield of oil extraction. Approximately 94% of total oil of rice bran was successfully extracted using subcritical water technique.

In chapter four, the possibility of phenolic compounds production as well as other useful substances from rice bran under subcritical water conditions was studied. Decomposition of rice bran was investigated at different temperatures ranging from 373 to 633 K at the reaction time of 10 min, and also at 493 K at reaction time of 2 to 30 min. Decomposition of lignin/phenolics-carbohydrate complexes of this biomass produced several phenolic compounds. The main products were found to be caffeic, ferulic, gallic, gentisic, p-coumaric, p-hydroxybenzoic, protocatechuic, sinapic, syringic, vanillic acids, and vanillin which showed antioxidative properties. Protocatechuic and vanillic acids were the major phenolic compounds among the identified ones.

Results demonstrated that phenolic compounds were mainly produced from decomposition of lignin/phenolics-carbohydrate complexes of rice bran and not from rice bran oil. In addition, production of these valuable materials was influenced by subcritical water temperature and reaction time. It was found that the phenolic compounds could be selectively and optimally produced with temperature and reaction time variations. From reaction time point of view, production of phenolic compounds could be rapidly achieved in subcritical water medium which was much less than those reported in conventional methods that increased the economic feasibility of this method.

Subcritical water could also efficiently decompose the released carbohydrate macromolecules of rice bran to water-soluble sugars. Significant amount of the produced soluble sugars can be used as an inexpensive feed stock for bioethanol production with vast food and industrial applications.

In chapter five, general conclusions of the present work were given.

From the results obtained in this thesis, it is concluded that the decomposition, hydrolysis, and treatment of rice bran, which is a low-cost and abundant biomass, to valuable and useful bio-based compounds using subcritical water as cheap, safe, clean, and environmentally friendly technique is a feasible process. In addition, it seems that the production of valuable compounds from rice bran in subcritical water medium can be scaled up to the industrial level. The author believes that the technical feasibility and other significant results of this thesis can eventually contribute to the further progress in the treatment of not only rice bran but also other biomass to recover and produce several valuable bio-based substances in order to replace fossil derive chemicals.

審査結果の要旨

米ぬかには、食用として最良と言われている脂質、炭水化物、蛋白質、セルロース、ビタミン、酵素、フェーノール系の薬効成分、ミネラルなど人体に有効な成分が多く含まれ

ているが、これらの多くは利用されていない。

本論文は、亜臨界水の強力な加水分解力を用いて、米ぬかの蛋白質、でんぷん、セルロースを分解し、有価物が生成することを明らかにした。蛋白質からは多数のアミノ酸が生成した。特にリジンとグルタミン酸の生成量が多く、400K付近で最大値を示すことを明らかにした。また、米ぬかのセルロースが分解して、大量の糖を生成させることにも成功した。463K付近で原料の 20%が糖に転換できることを示しており、今後エタノール発酵の原料としての適用の可能性を示した。

米は良質の脂質（米ぬか油）を表面付近に含有している。この米ぬか油は、玄米の間はほとんど劣化しないが、精米と同時に急速に劣化する。これは、玄米表面付近に存在する加水分解酵素リパーゼが精米と同時に活性化し短期間に劣化させてしまうためである。本論文では、亜臨界水により米ぬかから約 10 分という短時間で 94%という高い収率で米ぬか油を抽出できること、さらに、亜臨界水が油を抽出している間に、リパーゼも分解し完全に不活化できることを明らかにした。亜臨界水により抽出した油の劣化を 12 週間にわたって追跡したが、全く劣化が見られないことを示している。すなわち、亜臨界水処理という高速で安価な方法により、劣化しない良質の米ぬか油を生産できることが明らかとなり、今後の米ぬか油の食用としての利用促進に大きく道を開いた。

米ぬかには、フェノール系の多くの薬効成分が含まれている。亜臨界水により、これらが効率よく生産できることを示した。さらに、得られたフェノール系薬効成分が、単なる亜臨界水による抽出ではなく、リグニンと炭水化物の亜臨界水加水分解反応により生成することを実験的に証明した。