Production of Valuable Materials from Rice Bran Biomass Using Subcritical Water

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Chapter 1

Introduction

1. General background

1. 1. Introduction to rice bran biomass

1. 1. 1. Biomass

The worldwide demand for fossil fuels continues to rise at a rapid pace while supplies are finite. As developing nations increase their needs for petroleum products, plastics, etc., supplies will become even tighter. In addition due to excessive use of hydrocarbon products environmental issues such as global warming have become considerably serious in the world. It is therefore important to develop alternative forms of energy and feed stocks based on renewable resources. Biomass is the most abundant renewable resource in the world.

Biomass is defined as matter produced through photosynthesis. Biomass contains three primary constituents: cellulose, hemicellulose, and lignin, and can contain other compounds (for example, extractives). Cellulose is a polymer of glucose, hemicellulose is an oligomer of both C_6- and C_5- sugars (mainly glucose and xylose), and lignin is a highly cross-linked polymer. The common molecular formula of cellulose, hemicellulose, and lignin are $[C_6(H_2O)_5]_n$, $[C_5(H_2O)_4]_n$, and $[C_{10}H_{12}O_3]_n$, respectively *[Petrus and Noordermeer, 2006]*. Lignin and (hemi)cellulose together form a sort of fiber reinforced composite structure, in which cellulose is the fiber part and lignin forms a cross-linked three-dimensional resinous structure. Such lignocellulose gives strength to trees and plants.

Biomass includes plant materials; agricultural, industrial, municipal wastes, and residues derived from them (such as rice bran, switch grass, sugar cane (bagasse), trees, paper waste, plastics, plant and tree clippings cardboard). In general, biomass can include anything that is not a fossil fuel that is bioorganic-based *[Lucia et al., 2006]*. Estimated amount of annual effectively available biomass (by type) in the USA (California) and Japan are shown in Figures 1 and 2, respectively *[Moller, online; The Asia Network of Organics Recycling, online]*.

With an annual production of up to 1.7-2.0 $\times 10^{11}$ ton, biomass has been identified as an important source for alternative fuels and valuable chemicals. Although a huge amount of biomass is annually produced in the world; however, only 6×10^9 tons of biomass is currently

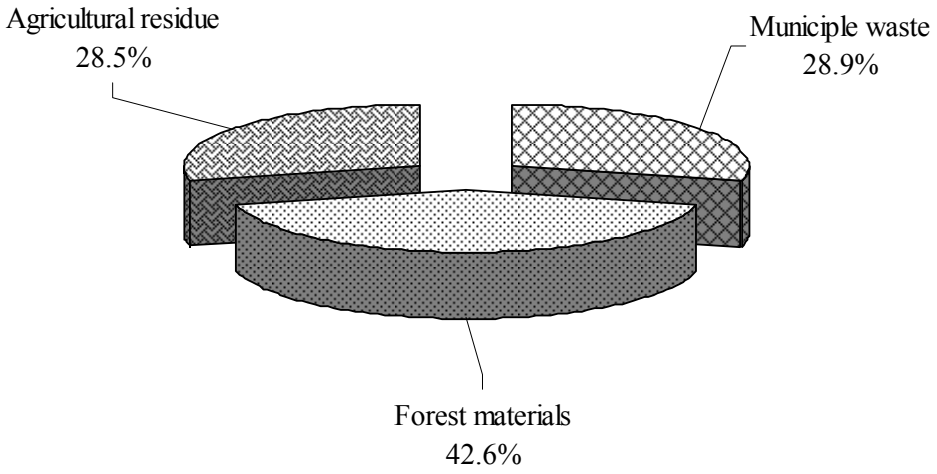


Figure 1. Amount of annually effectively available biomass in the USA (California) *[Moller, online]*.

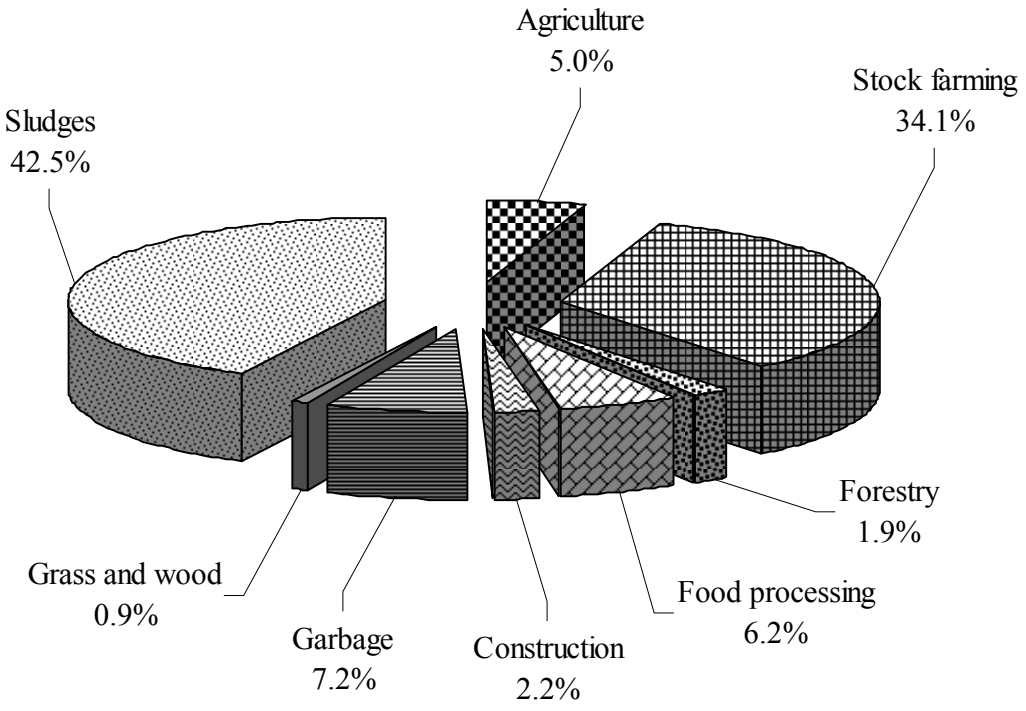


Figure 2. Amount of annually effectively available biomass in Japan *[The Asia Network of Organics Recycling, online]*.

used for food and non-food applications *[Girisuta et al., 2008]*. Therefore, it is indispensable to develop the environmentally friendly technologies to produce fuels, power, heat, and high-value chemicals from biomass with the lowest impact to the environment.

1. 1. 2. Rice bran

Rice (*Oryza sativa*) is one of the most important biomass in the world. It is a staple diet for two-thirds of the world's population *[Kaimal et al., 2002]*. About 617 million tons of rice is annually produced worldwide *[Watchararuji et al., 2008]*. Rough rice or paddy consists of a white, starchy endosperm kernel surrounded by a tight adhering bran coat that is enclosed by a looser outer hull or husk *[Prakash, 1996]*. Figure 3 shows the structure of rice *[Rice solution, online]*. In order to make rice susceptible for human consumption, several sequential processes must be carried out, such as cleaning, dehulling, milling, and polishing *[Danielski, 2007]*.

Rice hulls, which comprise about 25% by weight of paddy, are composed mainly of cellulose, lignin, and siliceous ash, and have feed and other industrial uses but no food value *[Saunders, 1985-86]*.

Rice bran is the major by-product of rice milling process. It is a brown layer which is nearly 8% of milled rice *[Sereewatthanawut et al., 2008]*. The production amount of rice bran is about 50-60 million tons per year *[Renuka Devi and Arumughan, 2007]*, which is mostly utilized as an animal feed ingredient, fertilizer, and fuel *[Pan et al., 2005; Zullaikah et al., 2005]*. Japan produces about 900 thousand tons of rice bran per year *[Tanaka et al., 2006]* which is used for different purposes. In Japan, approximately 34.0% of the produced rice bran is used to extract its oil, and nearly 80 thousand tons of rice bran oil is annually consumed *[Danielski et al., 2005]*. As shown in Figure 4, other estimated utilizations of rice bran are: 30.0%, 28.5%, 5.0%, 2.0%, and 0.5%, as waste and unknown, animal food, mushroom production, pickle preservation, and fertilization, respectively.

1. 1. 3. Composition of rice bran

Rice bran is a natural source of oil, carbohydrates, lignin, phenolic compounds,

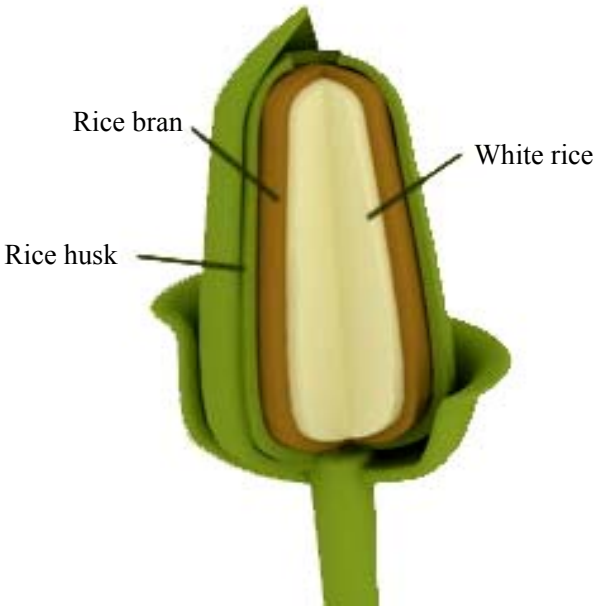


Figure 3. Diagrammatic representation of rice.

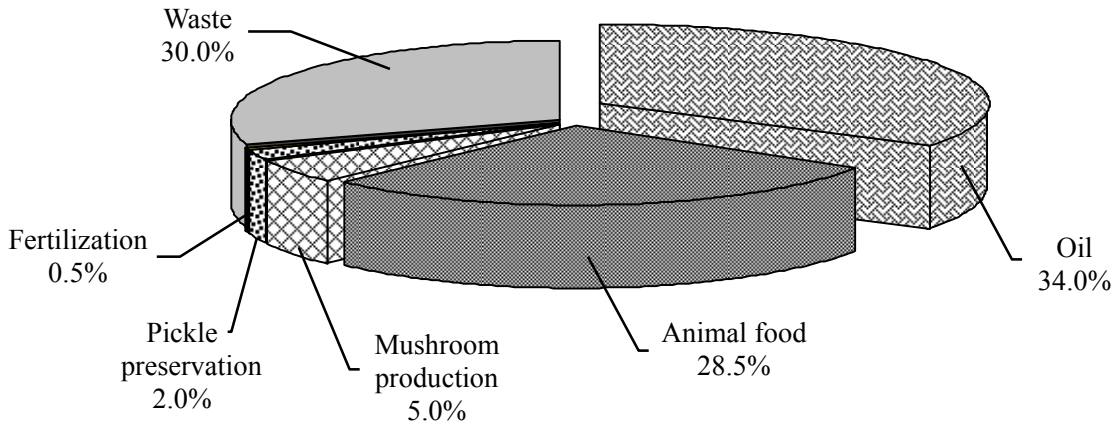


Figure 4. Utilizations of rice bran in Japan.

proteins, enzymes, vitamins, and dietary minerals *[Luh, 1980]*.

Depending on milling procedure, bran contains 10.0 to 26% oil *[Prabhakar and Venkatesh, 1986]* which has vast food and industrial applications. The commercial production of rice bran oil in year 2000 was estimated to be about 783 thousand tons *[Danielski et al., 2005]*. Rice bran oil contains triglycerides, diglycerides, monoglycerides, free fatty acids, wax, glycolipids, and phospholipids *[McCaskill and Zhang, 1999]*.

Bran is rich in carbohydrates and lignin. Major carbohydrates in commercial bran are cellulose, hemicellulose, and starch. Lignin, cellulose, hemicellulose, and starch contents in bran ranged from 7.7 to 13.1%, 9.6 to 12.8%, 8.7 to 11.4%, and 5 to 15%, respectively *[Saunders, 1985-86]*. Phenolic compounds are useful substances with nutraceutical and antioxidant properties which are extensively bounded to carbohydrates and lignin in the cell wall of rice bran *[Wiboonsirikul et al., 2008]*. Rice bran is a potential source of antioxidants (such as phenolic compounds) for food, pharmaceutical, and cosmetic industries *[Iqbal et al., 2005]*.

The protein content of full-fat rice bran is about 14% *[Prakash, 1996]*. The major protein fractions in bran are albumin and globulin *[Luh, 1980]*. Protein content is influenced by variety, environment, and nitrogen fertilization *[Saunders, 1985-86]*.

Rice bran contains numerous enzymes. Among the enzymes, lipase has merited most attention because it is responsible factor in the nonutilization of rice bran as foodstuff and extent of industrial utilization of bran *[Luh, 1980; Saunders, 1985-86]*.

Vitamins found in rice bran are listed in Table 1. The range of vitamins depends on the degree of milling and processing, and possible contamination with hull *[Marshall and Wadsworth, 1994]*.

Rice bran is a good source of minerals; it contains aluminum, calcium, chlorine, iron, magnesium, manganese, phosphorous, potassium, silicon, sodium, and zinc. The ranges of mineral contents in rice bran are depicted in Table 2. The mineral content is impacted by variety, soil conditions and growing environment, and by the milling process used *[Luh, 1980; Saunders, 1985-86; Marshall and Wadsworth, 1994]*.

Table 1. Vitamins in rice bran.

Vitamin	Content [ppm]
Vitamin A	4
Thiamine	10-28
Riboflavin	2-3
Niacin	236-590
Pyridoxine	10-32
Pantothenic acid	28-71
Biotin	0.2-0.6
Myoinositol	4600-9300
Choline	1300-1700
p-Aminobenzoic acid	0.7
Folic acid	0.5-1.5
Vitamin B_{12}	0.005
Vitamin E	150

Table 2. Minerals in rice bran.

Mineral	Content [ppm]
Aluminum	53-369
Calcium	140-1310
Chlorine	510-970
Iron	190-530
Magnesium	8650-12300
Manganese	110-877
Phosphorus	14800-28700
Potassium	13650-23900
Silicon	1700-16300
Sodium	0-290
Zinc	80

1. 2. Review of related literatures

To date, numerous researches on treatment and extraction of useful compounds of rice bran have been reported. Also, many studies have been conducted for production of valuable compounds from rice bran biomass using solvent extractions and chemical processes like hydrolysis and decomposition. Some of these reports are classified and mentioned in the following.

1. 2. 1. Conventional methods

Over the years, conventional methods have been used for production of useful compounds of rice bran. There are many academic reports and patents on the application of conventional methods for treatment and extraction of valuable materials from rice bran, and increasing their production yield.

Many scientists have focused on the extraction of rice bran oil using conventional methods. Most of these methods are based on the choice of solvent with the use of heat and/or agitation *[Wang and Weller, 2006]*. Soxhlet and direct solid-solvent extraction techniques are as examples of the conventional methods which have been used for rice bran oil extraction. Generally organic solvents, especially hexane, have been used to extract its oil. Mamidipally and Liu *[2004]* extracted rice bran oil using hexane and d-limonene at their respective boiling points at various solvent-to-meal ratios; it was found that the optimum solvent-to-meal ratio was 5:1. Hu et al. *[1996]* have studied the effects of solvent-to-bran ratio and extraction temperature in direct solid-solvent extraction method; they used hexane and isopropanol solvents, and reported that increasing the solvent-bran ratios and extraction temperature increased the extraction yield of oil.

Rice bran oil is decomposed extraordinary quickly into free fatty acids and glycerol by lipase enzyme soon after milling process, which makes it unfit for edible use. The process of rancidity development can be avoided either by rapid oil extraction or by inactivation the enzyme, known as stabilization process *[Goffman et al., 2003]*. Numerous attempts have been made for inactivation of lipase enzyme and stabilization of oil. Prabhakar and Venkatesh *[1986]* could inactivate lipase enzyme by lowering pH from 6.9 to 4.0, and no considerable

increase was observed in free fatty acids concentration. Randall et al. *[1985]* stabilized rice bran by extrusion cooking process, and free fatty acids concentration remained constant for at least 30-60 days. Tao et al. *[1993]* applied microwave heating method for inactivation of lipase enzyme, and free fatty acids content in the treated samples slightly increased during storage. In another report, application of ohmic heating for stabilization of oil was studied *[Rao Lakkakula et al., 2004]*, and ohmic method could effectively inactivate enzyme.

Rice bran protein is favorable for human consumption. The most common method for production of rice bran protein is alkali hydrolysis followed by acid precipitation *[Sereewatthanawut et al., 2008]*. Jiamyangyuen et al. *[2005]* could recover rice bran protein in alkaline medium, and they showed that production yield depended on pH and extraction time; the optimum pH and time were 11 and 45 min, respectively. In another study, Shih et al. *[1999]* have treated rice bran using enzyme, and they could prepared protein-enriched products. Parrado et al. *[2006]* could also extract soluble proteins, peptides, and free amino acids from rice bran by enzymatic treatment technique.

Conventional extraction techniques have also been implemented for recovery of phenolic compounds from rice bran. Extraction of these valuable compounds has been performed using organic solvents. Generally, acetone, ethanol, ethyl acetate, methanol, propanol and/or their combinations have been applied *[Naczk and Shahidi, 2006]*. Chotimarkon et al. *[2008]* and Iqbal et al. *[2005]* could extract phenolic compounds with methanol from different types of rice bran using direct solid-solvent extraction method. Renuka and Arumughan *[2007]* have studied the extraction of phenolic compounds from rice bran by using organic solvents and application of soxhlet technique. Taniguchi et al. *[1994]* have patented a method for production of ferulic acid by hydrolysis of waste materials of rice bran oil production industries at 373 K, pH of 10, and reaction time from 8 to 10 hours; the produced ferulic acid was extracted using hexane solvent.

1. 2. 2. New methods

Conventional methods have several drawbacks; they are time-consuming, are of low selectivity, give low extraction yield, and use large amount of expensive, explosive, and sometimes toxic organic solvents *[Wang and Weller, 2006]*. These disadvantages can be

overcome by application of the new environmentally friendly techniques like supercritical and subcritical water.

Supercritical and subcritical water are performed by utilization of water as treatment medium which is abundant and green solvent. Water at near critical point has properties that are different from water under normal conditions. An example is the relative low dielectric constant, which is comparable with that of methanol or ethanol under ambient conditions. Because of these properties, along with the higher concentration of hydrogen and hydroxide ions *[Herrero et al., 2006]*, it seems to be a good medium for application in various fields of chemical reaction and material cycling. Supercritical and subcritical water, as green alternative techniques to conventional methods have attracted growing attention recently with a range of different applications such as oxidation of waste *[Dinaro et al., 2000]*, extraction *[Kubatova et al., 2001]*, hydrolysis and synthesis of organic compounds *[Galkin and Lunin, 2005; Herrero et al., 2006; Kruse and Dinjus, 2007]*. Yoshida et al. *[1999]* could produce organic acids and amino acids from fish waste using subcritical water hydrolysis. Salak Asghari and Yoshida *[2006]* performed decomposition reaction of fructose to 5-hydroxymethyl furfural over a temperature range of 473-593 K in a batch subcritical water system. Sasaki et al. *[1998]* have shown that cellulose could be rapidly hydrolyzed in subcritical and supercritical water in the range of temperature from 563 to 673 K. Hydrothermal conversion of municipal waste *[Goto et al., 2004]*, catalytic reduction *[Jennings et al., 2000]*, recovery of harmful metal ions from squid waste *[Tavakoli and Yoshida, 2005],* conversion of scallop viscera wastes to valuable compounds *[Tavakoli and Yoshida, 2006],* oxidation of alkyl aromatics *[Holliday et al., 1998]*, production of lactic acid from carbohydrates *[Bicker et al., 2005]*, and decomposition of plastics *[Shibasaki et al., 2004]* have been also reported.

Recently, increasing attention has been paid to the hydrolysis, conversion, and decomposition of biomass (especially in subcritical and supercritical water medium) for energy and synthesis of materials and chemical *[Lucia et al., 2006; Peterson et al., 2008]*. However, there are very few reports for treatment of rice bran biomass under subcritical water conditions. Wiboonsirikul et al. *[2007a]* have studied the production of functional substances from defatted black rice bran by subcritical water treatment; protein, carbohydrates, and radical scavenging activity of the products were investigated in detail. In another study, Wiboonsirikul et al. *[2007b]* could treat defatted rice bran in subcritical water medium in

order to extract phenolic and other antioxidant compounds at 323 to 523 K for 5 min reaction time. Wiboonsirikul et al. *[2008]* have produced phenolic compounds from defatted rice bran using subcritical water at 293 to 533 K for 5 min, and also at 473 and 533 K for 5 to 120 min; total phenolic content and antioxidant activity of the obtained solution after subcritical water treatment were investigated. Sereewatthanawut et al. *[2008]* have investigated defatted rice bran under subcritical water conditions; highest yield of protein and amino acids were obtained after 30 min of reaction at 473 K. Hata et al. *[2008]* evaluated antioxidant activity and total soluble sugar yield after subcritical water treatment of the defatted rice bran at the temperature range of 453 to 553 K for 5 min.

1. 3. Subcritical and supercritical water

Water, like other solvents, has "critical point" that occurs at a high temperature where liquid and vapor can coexist in the same container. The critical point of water has been reported at P_c = 22.1 MPa and T_c= 647.15 K *[Galkin and Lunin, 2005]*. At the critical point the two classic phases of vapor and liquid become indistinguishable. Supercritical water is described as water in temperature and pressure state over the critical point. The term of subcritical water refers to liquid water between its boiling point (373.15 K) and its critical temperature (647.15 K) under pressure high enough to maintain the water in the liquid phase. The positions of supercritical and subcritical water regions are shown on the phase diagram in Figure 5.

Generally water under subcritical or supercritical conditions, possesses properties very different from those of ambient liquid water. It has been demonstrated that supercritical water can be an effective technique for destruction of hazardous organic waste and sludge. During this process, most organic compounds are converted to CO_2, N_2, and water. However, supercritical water treatment has some disadvantages which can be overcome by using subcritical water treatment. There are three main important reasons to use subcritical water instead of supercritical water technique. Firstly, the main aim in supercritical water is decomposition of wastes to CO_2, N_2, and water which are not valuable compounds. On the other hand, subcritical water hydrolysis produces many valuable organic compounds. Secondly, subcritical water acts as a green powerful solvent which can be used for extraction

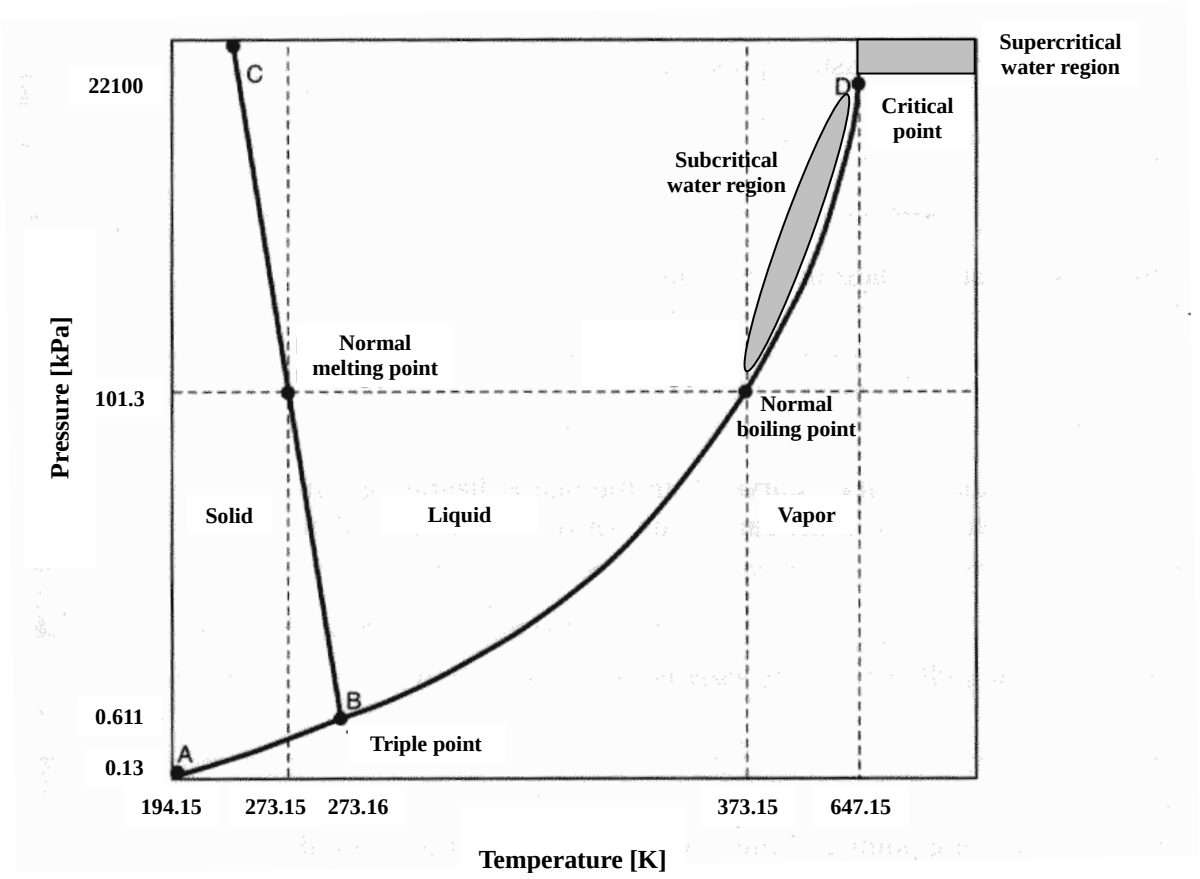


Figure 5. Phase diagram of water in P-T plane.

of useful materials. Thirdly, the preparation of supercritical water, needs higher temperature and pressure than subcritical water; in the other words, working under critical point of water is much more economic and feasible than above its critical point. In this work we focused on water applications under its critical point.

1. 3. 1. Physicochemical properties of water

At a temperature of 295 K and a pressure of 0.1 MPa, water is a polar solvent with a density of 1000 $kg.m^{-3}$, a dielectric constant , ε, of 79.73, and an ion production constant, K_w, of 1×10^{-14} *[Aki et al., 2001]*. Raising the temperature and pressure causes significant changes in the properties of water. The properties of water vary owing to variation of its dielectric constant, conductivity, ionic product, and the structure of H bond network. Changes in viscosity, heat capacity, diffusion coefficients, density influence the transport characteristics of aqueous solution *[Galkin and Lunin, 2005]*. Two main parameters of water are illustrated in the following.

1. 3. 1. 1. Ion production constant of water

Water, as acid and base, is both giver and taker of protons. When water reacts with its own kind, the hydronium and hydroxide ions are produced. The scheme of water dissociation is shown in Figure 6 *[Harvey, 2000]*.

The ion production constant of water is defined as $K_w = [H^+][OH^-]$; the concentration at room temperature and atmospheric pressure is 1×0^{-7} mol/l for both, and the value of K_w is 1×10^{-14} mol^2/l^2. Under high temperature and pressure conditions, the value of the ion production increases considerably from 10^{-14} to 10^{-11} mol^2/l^2 at about 520 K, and decreases sharply at temperatures higher than that temperature. The effect of temperature on ion production of water is shown in Figure 7 *[Akiya and Savage, 2002]*. Water has maximum ion production at around 523 K under saturation vapor pressure. This indicates that subcritical water may possess the effect of an acid catalyst *[Fukushima]*.

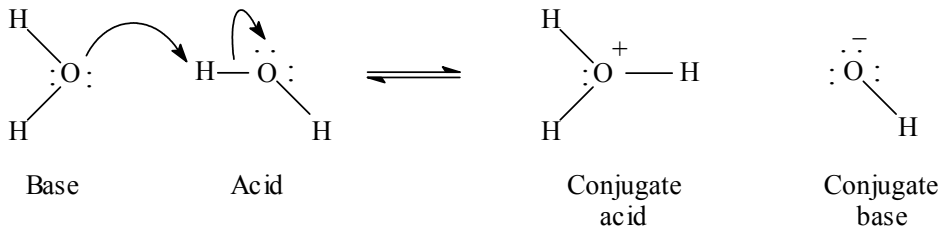


Figure 6. Scheme of water dissociation.

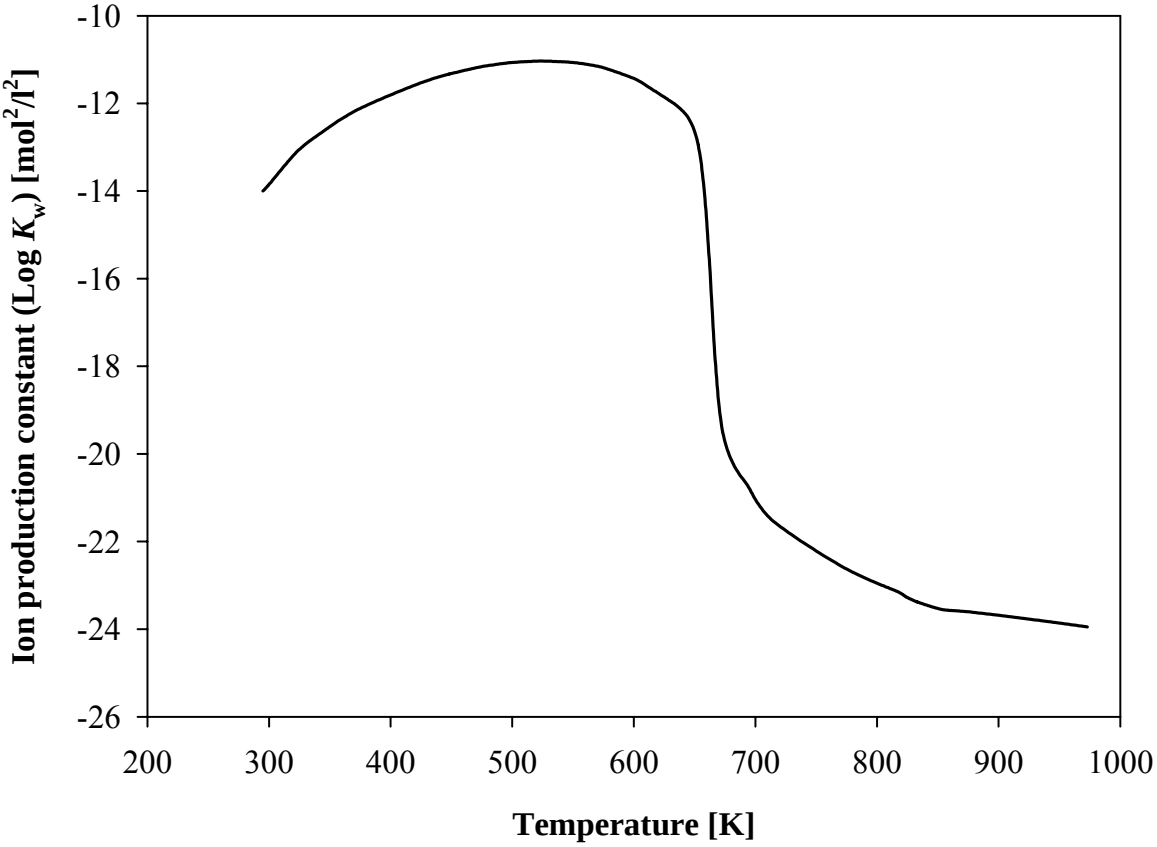


Figure 7. Effect of temperature on ion production constant of water.

1. 3. 1. 2. Dielectric constant of water

Dielectric constant (ε) expresses the affinity of water, as a reaction media, to reaction materials. When water is heated at temperatures above 373.15 K, under sufficient pressure to remain as liquid, its dielectric constant can be changed; changing temperature and pressure can control this value. Figure 8 shows the effect of temperature on dielectric constant *[Uematsu and Franck, 1980]*. For instance, water dielectric constant decreases from 80 (at room temperature) to 27 (at 523 K) almost equaling to that of ethanol at ambient temperature *[Luque de Castro et al., 1999]*.

These considerable manipulations of physicochemical parameters with pressure and temperature should be important in any application sensitive to the thermodynamic properties of water. These variations offer the possibility of using pressure and temperature to tune the properties of water to optimal values for a given chemical reaction. High temperatures and pressures actually induce a nonpolar solvent behavior of water. As sequence, organic compounds are completely miscible with water. As mentioned above, subcritical water is not only more economic and feasible process than supercritical water, but also is capable to produce and extract valuable organic compounds.

2. The aim of the thesis

The aim of this study is to develop an efficient environmentally friendly technique for hydrolysis and conversion of rice bran, a low-cost and abundant biomass, to valuable compounds (such as phenolic compounds, soluble sugars, organic acids, and amino acids) in subcritical water medium. Furthermore, efficient extraction of rice bran oil as favorable edible oil by subcritical water, and simultaneous lipase enzyme inactivation by hydrolysis reaction in subcritical water medium are investigated.

This thesis contains five chapters. The main focus of each chapter is summarized as follows:

Chapter 1 provides general background of this thesis. In the first part of this chapter, a general introduction about rice bran biomass is given, and its composition is presented. In the next part, conventional methods related to this thesis are described. In addition, application of

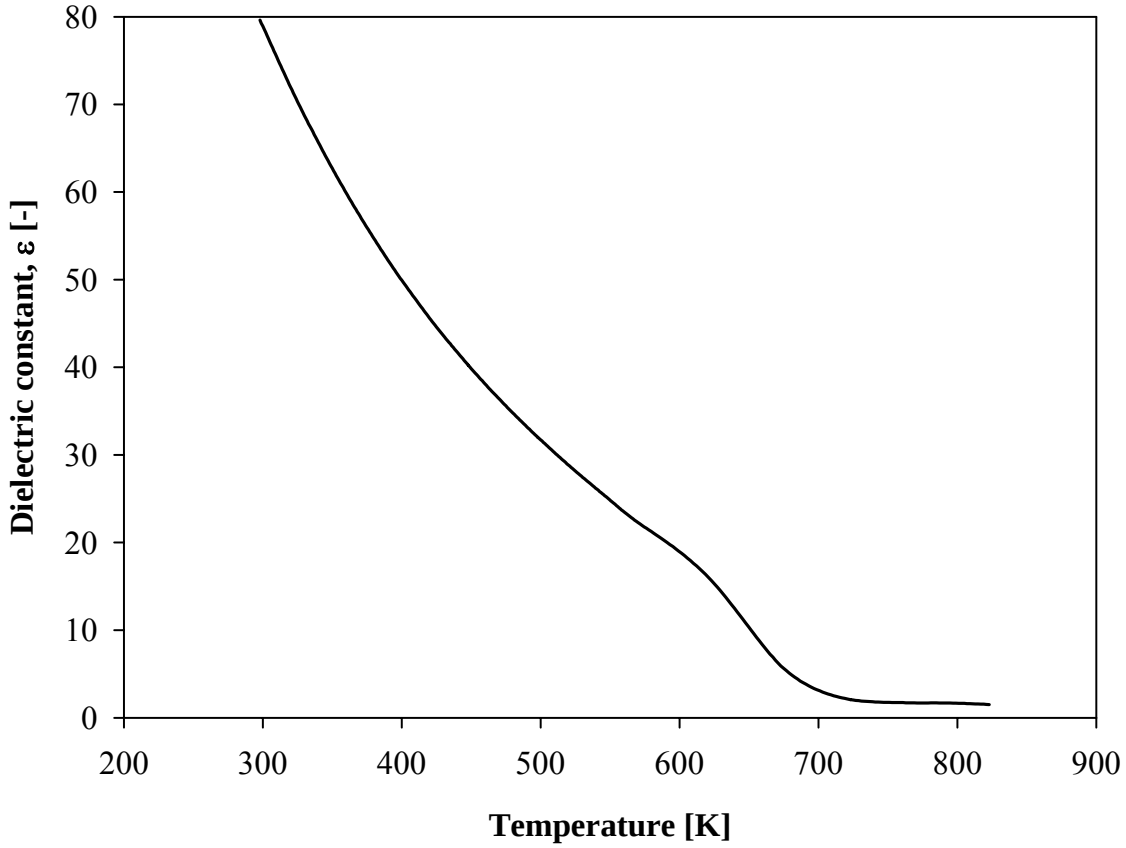


Figure 8. Effect of temperature on dielectric constant of water at 30 MPa.

subcritical and supercritical water as new green methods in various fields of chemical engineering and material cycling, and related researches on rice bran are reviewed. Finally, properties of water under and above its critical point are described.

Chapter 2 is devoted to evaluate the hydrolysis and decomposition of rice bran under subcritical water conditions in order to obtain value-added materials. Effect of temperature (over the whole temperature range of subcritical water) on the hydrolysis reaction is studied. In this chapter, production of various water-soluble compounds such as amino acids, organic acids, and soluble sugars is studied, and their optimum production conditions are described. In addition, extraction of hexane soluble (mainly rice bran oil) and acetone soluble substances after subcritical water reaction is also evaluated.

Chapter 3 deals with extraction of high quality edible oil from rice bran. In the first half of this chapter, effect of lipase enzyme on the quality of oil during storage of rice bran is experimentally analyzed, and lipase enzyme inactivation using subcritical water technique is studied. In the second half of this chapter, extraction of rice bran oil simultaneous with oil stabilization under subcritical water conditions is investigated. Furthermore, the production yield and quality of the extracted oil is compared with the oil obtained by conventional extraction methods.

Chapter 4 describes the production of phenolic compounds as well as other valuable substances from rice bran using subcritical water treatment. The effect of temperature (over the whole temperature range of subcritical water) and reaction time on the decomposition of lignin/phenolics-carbohydrate complexes of rice bran and production of phenolic compounds and water-soluble sugars are investigated, and the optimum temperature and reaction time for each phenolic compound are presented.

Chapter 5 summarizes the conclusions of this thesis.

Nomenclature

ε	Dielectric constant
K	Kelvin
K_w	Water ion production constant
MPa	Mega Pascal

P_c	Critical pressure
T_c	Critical temperature

References

Akai, S. N. V. K., Feng, J., Chateauneuf, J. E., Brennecke, J. F., Generation of xanthenium and 9-phenylxanthenium carbocations in subcritical water and reactivity with amylamine, *The Journal of Physical Chemistry A,* 105, 8046-8052, (2001).

Akiya, N., Savage, P. E., Roles of water for chemical reactions in high-temperature water, *Chemical Reviews,* 102, 2725-2750, (2002).

Bicker, M., Endres, S., Ott, L., Vogel, H., Catalytical conversion of carbohydrates in subcritical water: a new chemical process for lactic acid production, *Journal of Molecular Catalysis A: Chemical,* 239, 151-157, (2005).

Chotimarkon, C., Benjakul, S., Silalai, N., Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand, *Food Chemistry,* 111, 636-641, (2008).

Danielski, L., Extraction and fractionation of natural organic compounds from plant materials with supercritical carbon dioxide, *PhD Thesis,* Technische Universität Hamburg, Hamburg, Germany, (2007).

Danielski, L., Zetal, C., Hense, H., Brunner, G., A process line for the production of raffinated rice bran oil from rice bran, *The Journal of Supercritical Fluids,* 34, 133-141, (2005).

Dinaro, J., Howard, J. B., Green, W. H., Tester, J. W., Bozzelli, J. W., Analysis of an elementary reaction mechanism for benzene oxidation in supercritical water, *Proceeding of the Combustion Institute,* 28, 1529-1536, (2000).

Fukushima, Y., Application of supercritical fluids: a review, *R&D Review of Toyota CRDL,* 35, 1-9.

Galkin, A. A., Lunin, V. V., Subcritical and supercritical water: a universal medium for chemical reactions, *Russian Chemical Reviews (English Translation),* 74, 21-35, (2005).

Girisuta, B., Danon, B., Manurung, R., Janssen, L. P. B. M., Heeres, H. J., Experimental and kinetic modeling studies on the acid-catalysed hydrolysis of the water hyacinth plant to levulinic acid, *Bioresource Technology,* 99, 8367-8375, (2008).

Goffman, F. D., Pinson, S., Bergman, C., Genetic diversity for lipid content and fatty acid profile in rice bran, *Journal of the American Oil Chemists' Society,* 80, 485-490, (2003).

Goto, M., Obuchi, R., Hirose, T., Sasaki, T., Shibata, M., Hydrothermal conversion of municipal organic waste into resources, *Bioresource Technology,* 93, 279-284, (2004).

Harvey, D., *Modern Analytical Chemistry,* McGraw-Hill Companies, Inc., the USA, (2000).

Hata, S., Wiboonsirikul, J., Maeda, A., Kimura, Y., Adachi, S., Extraction of defatted rice bran by subcritical water treatment, *Biological Engineering Journal,* 40, 44-53, (2008).

Herrero, M., Cifuentes, A., Ibanez, E., Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae: a review, *Food Chemistry,* 98, 136-148, (2006).

Holliday, R. L., Jong, B. Y. M., Kolis, J. W., Organic synthesis in subcritical water oxidation of alkyl aromatics, *The Journal of Supercritical Fluids,* 12, 255-260, (1998).

Hu, W., Wells, J. H., Shin, T. S., Godber, J. S., Comparision of isopropanol and hexane for extraction of vitamin E and oryzanols from stabilized rice bran, *Journal of the American Oil Chemist's Society,* 73, 1653-1656, (1996).

Iqbal, S., Bhanger, M. I., Anwar, F., Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan, *Food Chemistry,* 93, 265-272, (2005).

Jennings, J. M., Bryson, T. A., Gibson, J. M., Catalytic reduction in subcritical water, *Green Chemistry,* 2, 87-88, (2000).

Jiamyangyuen, S., Srijesdaruk, V., Harper, W. J., Extraction of rice bran protein concentrate and its application in bread, *The Songklanakarin Journal of Science and Technology,* 27, 55-64, (2005).

Kaimal, T. N. B., Vali, S. R., Rao, B. V. S. K., Chakrabarti, P. P., Vijayalakshmi, P., Kale, V., Rani, K. N. P., Rajamma, O., Bhaskar, P. S., Rao, T. C., Origin of problems encountered in rice bran oil processing, *European Journal of Lipid Science and Technology,* 104, 203-211, (2002).

Kruse, A., Dinjus, E., Hot compressed water as reaction medium and reactant properties and

synthesis reactions, *The Journal of Supercritical Fluids,* 39, 362-380, (2007).

Kubatova, A., Miller, D. J., Hawthorne, S. B., Comparison of subcritical water and organic solvents for extracting Kava lactones from Kava root, *Journal of Chromatography A,* 923, 187-194, (2001).

Lucia, L. A., Argyropoulos, D. S., Adamopoulos, L., Gaspar, A. R., Chemical and energy from biomass, *Canadian Journal of Chemistry,* 84, 960-970, (2006).

Luh, B. S., *Rice: Production and Utilization,* AVI Publishing Company, Inc., the USA, (1980).

Luque de Castro, M. D., Jimenez-Carmona, M. M., Fernandez-Perez, V., Towards more rational technique for the isolation of valuable essential oils from plants, *Trends in Analytical Chemistry,* 18, 708-716, (1999).

Mamidipally, P. K., Liu, S. X., First approach on rice bran oil extraction using limonene, *European Journal of Lipid Science and Technology,* 106, 122-125, (2004).

Marshall, W. E., Wadsworth, J. I., *Rice Science and Technology,* Marcel Dekker, Inc., the USA, (1994).

McCaskill, D. R., Zhang, F., Use of rice bran oil in foods, *Food Technology,* 53, 50-53, (1999).

Moller, R. M., *Brief on biomass and cellulosic ethanol,* California biomass commission, California Research Bureau, ISBN: 1-58703-206-6, Available from http://www.library.ca.gov/crb/05/10/05-010.pdf.

Naczk, M., Shahidi, F., Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis: a review, *Journal of Pharmaceutical and Biomedical Analysis,* 41, 1532-1542, (2006).

Pan, Z., Cathcart, A., Wang, D., Thermal and chemical treatments to improve adhesive property of rice bran, *Industrial Crops and Products,* 22, 233-240, (2005).

Parrado, J., Miramontes, E., Jover, M., Gutierrez, J. F., Teran, L. C., Bautista, J., Preparation of a rice bran enzymatic extract with potential use as functional food, *Food Chemistry,* 98, 742-748, (2006).

Peterson, A. A., Vogel, F., Lachance, R. P., Froling, M., Antal, M. J., Tester, W. J., Thermochemical biofuel production in hydrothermal media: a review of sub- and

supercritical water technologies, *Energy & Environmental Science,* 1, 32-65, (2008).

Petrus, L., Noordermeer, M. A., Biomass to biofuels, a chemical perspective, *Green Chemistry,* 8, 861-867, (2006).

Prabhakar, J. V., Venkatesh, K. V. L., A simple chemical method for stabilization of rice bran, *Journal of the American Oil Chemist's Society,* 63, 644-646, (1986).

Prakash, J., Rice bran: properties and food uses, *Critical Reviews in Food Science and Nutrition,* 36, 537-552, (1996).

Randall, J. M., Sayre, R. N., Schultz, W. G., Fong, R. Y., Mossman, A. P., Tribelhorn, R. E., Saunders, R. M., Rice bran stabilization by extrusion cooking for extraction of edible oil, *Journal of Food Science,* 50, 361-364, (1985).

Rao Lakkakula, N., Lima, M., Walker, T., Rice bran stabilization and rice bran oil extraction using ohmic heating, *Bioresource Technology,* 92, 157-161, (2004).

Renuka Devi, R., Arumughan, C., Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment, *Bioresource Technology,* 98, 3037-3043, (2007).

Rice solution, Available from http://www.ricesolution.com/img/rice_diagram.jpg.

Salak Asghari, F., Yoshida, H., Acid-catalyzed production of 5-hydroxymethyl furfural from *D*-fructose in subcritical water, *Industrial & Engineering Chemistry Research,* 45, 2163-2173, (2006).

Sasaki, M., Kabyemela, B., Malaluan, R., Hirose, S., Takeda, N., Adschiri, T., Arai, K., Cellulose hydrolysis in subcritical and supercritical water, *The Journal of Supercritical Fluids,* 13, 261-268, (1998).

Saunders, R. M., Rice bran: composition and potential food uses, *Food Reviews International,* 1, 465-495, (1985-86).

Sereewatthanawut, I., Prapintip, S., Watchiraruji, K., Goto, M., Sasaki, M., Shotipruk, A., Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis, *Bioresource Technology,* 99, 555-561, (2008).

Shibasaki, Y., Kamimori, T., Kadokawa, J., Hatano, B., Tagaya, H., Decomposition reactions

of plastic model compounds in sub- and supercritical water, *Polymer Degradation and Stability,* 83, 481-485, (2004).

Shih, F. F., Champagne, E. T., Daigle, K., Zarins, Z., Use of enzymes in the processing of protein products from rice bran and rice flour, *Nahrung,* 43, 14-18, (1999).

Tanaka, T., Hoshina, M., Tanabe, S., Sakai, K., Ohtsubo, S., Taniguchi, M., Production of *D*-lactic acid from defatted rice bran by simultaneous saccharification and fermentation, *Bioresource Technology,* 97, 211-217, (2006).

Taniguchi, H., Nomura, E., Tsuno, T., Minami, S., Method of manufacturing ferulic acid, *US Patent,* 5288902, (1994).

Tao, J., Rao, R., Liuzzo, J., Microwave heating for rice bran stabilization, *Journal of Microwave Power and Electromagnetic Energy,* 28, 156-164, (1993).

Tavakoli, O., Yoshida, H., Conversion of scallop viscera wastes to valuable compounds using sub-critical water, *Green Chemistry,* 8, 100-106, (2006).

Tavakoli, O., Yoshida, H., Effective recovery of harmful metal ions from squid wastes using subcritical and supercritical water treatments, *Environmental Science & Technology,* 39, 2357-2363, (2005).

The Asia Network of Organics Recycling (ANOR), *The amount of generation of main organic wastes,* Available from http://www.jora.jp/anor/eng/jap.html.

Uematsu, M., Franck, E. U., Static dielectric constant of water and steam, *Journal of Physical and Chemical Reference Data,* 9, 1291-1306, (1980).

Wang, L., Weller, C. L., Recent advances in extraction of nutraceuticals from plants, *Trends in Food Science & Technology,* 17, 300-312, (2006).

Watchararuji, K., Goto, M., Sasaki, M., Shotipruk, A., Value-added subcritical water hydrolysate from rice bran and soybean meal, *Bioresource Technology,* 99, 6207-6213, (2008).

Wiboonsirikul, J., Hata, S., Tsuno, T., Kimura, Y., Adachi, S., Production of functional substances from black rice bran by its treatment in subcritical water, *LWT-Food Science and Technology,* 40, 1732-1740, (2007a).

Wiboonsirikul, J., Kimura, Y., Kadota, M., Morita, H., Tsuno, T., Adachi, S., Properties of extracts from defatted rice bran by its subcritical water treatment, *Journal of Agricultural and Food Chemistry,* 55, 8759-8765, (2007b).

Wiboonsirikul, J., Kimura, Y., Kanaya, Y., Tsuno, T., Adachi, S., Production and characterization of functional substances from a by-product of rice bran oil and protein production by a compressed hot water treatment, *Bioscience Biotechnology and Biochemistry,* 72, 384-392, (2008).

Yoshida, H., Terashima, M., Takahashi, Y., Production of organic acids and amino acids from fish meat by sub-critical water hydrolysis, *Biotechnology Progress,* 15, 1090-1094, (1999).

Zullaikah, S., Lai, C. C., Vali, S. R., Ju, Y. H., A two-step acid-catalyzed process for the production of biodiesel from rice bran oil, *Bioresource Technology,* 96, 1889-1896, (2005).

Chapter 2

Decomposition of Rice Bran and Production of Valuable Materials Using Subcritical Water

1. Introduction

Subcritical water treatment is an environmentally friendly technique with a wide range of applications, such as extraction, hydrolysis, and wet oxidation of organic compounds *[Holliday et al., 1998; Kruse and Dinjus, 2007]*. Subcritical water is defined as hot water at temperatures ranging between 373 and 647 K under high pressure to maintain water in the liquid state. Dielectric constant, which can be changed by temperature, is the most important when using water as an extraction solvent; it decreases from 80 (at room temperature) to 27 (at 523 K) which is almost equal to that of ethanol at ambient temperature *[Galkin and Lunin, 2005; Herrero et al., 2006]*. Thus, subcritical water can be used for extraction of organic compounds instead of using organic solvents which are environmentally unacceptable. On the other hand, subcritical water has been widely used for hydrolysis of organic compounds. Recently growing attention has led to extensive research activities using subcritical water for hydrolysis and conversion of biomass and carbohydrates to useful compounds *[Sasaki et al., 1998; Yoshida et al., 1999; Kruse and Gawlik, 2003; Yoshida and Tavakoli, 2004; Bicker et al., 2005; Tavakoli and Yoshida, 2005; Abdelmoez and Yoshida, 2006b; Salak Asghari and Yoshida, 2006; Tavakoli and Yoshida, 2006; Salak Asghari and Yoshida, 2007]*.

One of the most useful biomass is rice. Rice bran is a by-product of rice milling process which is nearly 8% of milled rice *[Danielski et al., 2005]*. The production amount of rice bran is about 50-60 million tons per year, which is normally used as animal feed *[Renuka Devi and Arumughan, 2007]*. Japan produces about 0.9 million tons of rice bran *[Tanaka et al., 2006]* which is used for different purposes.

Rice bran is a natural resource of oil, proteins, fibers, vitamins and antioxidants. In addition, it is a good resource of minerals such as silica, iron, calcium, and zinc *[Luh, 1980]*. There are many methods for treatment and extraction of its useful compounds, such as soxhlet extraction, direct solid-solvent extraction, and more recently supercritical CO_2 and subcritical water treatments *[Hu et al., 1996; Xu and Godber, 2000; Mamidipally and Liu, 2004]*. Mostly, organic solvents, such as methanol, ethanol, ethyl acetate, hexane, acetone and isopropanol *[Proctor et al., 1994; Proctor and Bowen, 1996; Chen and Bergman, 2005]* have been used for rice bran treatment.

The above-mentioned conventional methods have some disadvantages; e.g. they are

time-consuming, are of low selectivity, give low extraction yield and utilize large amounts of expensive and/or toxic organic solvents *[Wang and Weller, 2006]*. Moreover, some of organic solvents create problems of explosion, pollution, and fire escape. These disadvantages of organic solvents can be overcome by using subcritical water as a so-called green solvent.

Recently, increasing attention has been paid to subcritical water treatment of rice bran as a cheap and abundant biomass. For instance, Wiboonsirikul et al. *[2007a]* have studied the production of functional substances from defatted black rice bran by subcritical water treatment; protein, carbohydrates, and antioxidant activity of the products were investigated in detail. In another report, defatted rice bran has been treated in subcritical water in order to study the extraction of phenolic and other antioxidant compounds at 323 to 523 K for a 5 min reaction time *[Wiboonsirikul et al., 2007b]*. Sereewatthanawut et al. *[2008]* have investigated defatted rice bran under subcritical water; highest yields of protein and amino acids were obtained after 30 min of reaction at 473 K.

There are few available reports on subcritical water treatment of rice bran. To the best of our knowledge, there is no previous report on the study of rice bran over the whole temperature range of subcritical water. In this chapter, our objective is to develop and evaluate subcritical water in order to better understand its temperature effects (from 373 to 633 K) on rice bran.

2. Materials and methods

2. 1. Materials

A Japonica-type rice (*Oryza sativa L. japonica*) was used in this experimental study. Sodium carbonate, sodium hydrogen carbonate, and phenol were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). EDTA and Bis-Tris were bought from Dojindo (Japan); n-caprilic acid was purchased from Tokyo Chemical Industry Co. Ltd. (Japan). Mercaptoethanol and brij-35 (Polyoxyethyleneglycol dodecyl ether) were obtained from Pierce (USA). Potassium hydrogen phthalate, sulfuric acid and sodium hypochlorite were purchased from Chameleon Reagent (Osaka, Japan). All other reagents and solvents were purchased from Wako Pure Chemical Industries, Ltd. (Japan).

2. 2. Procedure

A batch reactor used for subcritical water treatment was a stainless steel tube (SUS316, i.d. 16.5 mm × 150.4 mm) with a Swagelok fitting (ready-made, from Swagelok AG). In a typical experiment, an accurately weighted amount (about 3.0 g) of rice bran (comminuted and sieved through a 590 μm-mesh sieve) and about 18 cm^3 of distilled water were charged into the reactor. Argon gas was used to force air out of the reactor before the reaction, and it was capped tightly. It was immersed in a preheated oil bath (Thomas Kagaku Co. Ltd., Celsius M type) with temperatures ranging from 373 to 453 K or in a preheated salt bath (Thomas Kagaku Co. Ltd., Celsius 600H) in the temperature range 453 to 633 K for 5 min. In this work, the reaction time (i.e. 5 min) mentioned above includes the heat-up time. The salt bath mixing speed and reactor shaking rate have great effects on the rate of heating-up. The combined effects of both reactor shaking and salt bath mixing speed can significantly increase heating-up rate to a steady state condition in a very short time (e.g. 25 s) *[Abdelmoez and Yoshida, 2006a]*. This reaction time was also suitable to investigate most of the desired and undesired reactions in subcritical water medium *[Yoshida et al., 1999 and 2003]*. The reactor was then removed from thermal bath and quickly quenched by soaking into a cold water bath at room temperature. Reactor content was washed into a test tube, taking particular care to prevent loss of any of the liquid. The reaction pressure was estimated from a steam table. The details were explained elsewhere *[Yoshida et al., 1999]*.

2. 3. Separation of produced phases after subcritical water treatment

After subcritical water treatment, all contents in the reactor were poured into a test tube and classified and isolated into four phases: hexane-soluble (HS), water-soluble (WS), acetone-soluble (AS), and remained solid phases. The separation procedure was as follows: Hexane (5 cm^3) was gently added to the test tube and allowed to stand for 5 min at 298 K, then centrifuged at 2500 g for 10 min and supernatant was separated. This procedure was repeated eight times. Then, aqueous phase and remained solid were separated by filtration. Hexane (5 cm^3) was added to the remained solid and this mixture was shaken for 5 min. After centrifugation, the supernatant was separated and added to the obtained HS phase from

water-soluble phase. This procedure was repeated four times. HS amount was calculated by weight after evaporation of hexane. Remained solid was also washed with 10 cm^3 of acetone, several times. AS amount was calculated by weight after evaporation of the solvent. Finally, remained solid was placed in an oven at 333 K to dry to constant weight. The solubility, remained solid, and rice bran conversion yields were calculated as follows:

$$\text{Solubility yield} = \frac{W_s}{W_i} \quad (1)$$

$$\text{Remained solid yield} = \frac{W_{rs}}{W_i} \quad (2)$$

$$\text{Rice bran conversion yield} = 1 - \text{Remained solid yield} \quad (3)$$

in which W_s, W_{rs}, and W_i are weights of soluble materials (into hexane, acetone, or water), remained solid, and initial dry sample, respectively.

2. 4. Analysis

Concentration of organic acids were determined by HPLC, using a pump (Shimadzu LC-10AD VP, Shimadzu Co., Japan) with two ion-exclusion chromatography columns (Shim-pack SCR-102H, 8 mm × 300 mm, Shimadzu Co., Japan) in series and their detection affected using post-column pH-buffered electroconductivity detection (Shimadzu CDD-6A, Shimadzu Co., Japan). The mobile phase was 5.5 mM p-toluensulfonic acid solution at a flow rate of 0.8 cm^3/min. Mixtures of 5.5 mM p-toluensulfonic, 20 mM Bis-Tris and 100 μM EDTA were used as post-column reagents, all at flow rates of 0.8 cm^3/min. The column (Shimadzu CTO-10AC VP, Shimadzu Co., Japan) temperature was kept at 318 K.

Amino acids concentration was determined by an HPLC system (Shimadzu LC-10AT VP, AM1NO-NA column) using a fluorescence detector (Shimadzu RF-10A XL, Shimadzu Co., Japan). The temperature of the column (Shimadzu CTO-10A VP, Shimadzu Co., Japan) was 333 K.

Two size-exclusion chromatography columns in series (Shodex-sugar KS-804 and KS-801, 8 mm × 300 mm, Shodex Co., Japan) in an HPLC system, in conjunction with a pump (Jasco PU-2080plus, Jasco Crop., Japan) coupled to a refractive index detector (Jasco RI-2031plus, Jasco Crop., Japan), were used for quantitative analysis of the products, which could not be detected using a UV detector. This HPLC system was operated at an oven (Jasco

CO-2065plus, Jasco Crop., Japan) temperature of 305 K using mili-Q water at 0.4 cm^3/min flow rate as a mobile phase.

Total organic carbon (TOC) and total nitrogen (TN) were measured by a TOC/TN analyzer (Shimadzu TOC-V CPH/CPN, Shimadzu Co., Japan). A double-beam UV-visible spectrophotometer (Shimadzu UV-1600, Shimadzu Co., Japan) was used for all spectrophotometric measurements.

A CHNS analyzer (Perkin-Elmer, model 2400) was used to calculate the carbon, hydrogen, nitrogen, and sulfur content of the solid samples.

3. Results and discussion

3. 1. Specifications of rice bran

Rice bran contains organic and mineral compounds. The contents and composition of rice bran depend on the internal (species) and external (soil, climate) conditions. The organic part of the rice bran was identified as 44.9% of carbon, 7.2% of hydrogen, 3.3% of nitrogen, and 1.2% of sulfur. Aluminum, calcium, chlorine, iron, magnesium, manganese, phosphorous, potassium, silicon, sodium, and zinc have already been reported as main inorganic compounds *[Marshall and Wadsworth, 1994]*. Water content was 8.8%. Figure 1 shows the effect of ignition temperature on the residue of the rice bran after 6 h of ignition. The loss of the sample weight increased up to 90% at temperatures higher than 823 K. Obviously, the lost and remaining amounts were attributed to organic and non-volatile inorganic compounds, respectively.

Figure 2 shows photos of products after subcritical water reaction for 5 min. Generally, rice bran is a water-insoluble biomass. However, after subcritical water treatment, at low temperatures (i.e. 373-433 K) a slurry phase was obtained. It was viscous and its color was little changed. However, at moderate temperatures (i.e. 433-553 K), it was slightly viscous and reddish to brown in color. At high temperatures (i.e. 553-633 K), it was a very viscous slurry with dark brown to black color.

3. 2. Isolated phases from rice bran slurry after subcritical water treatment

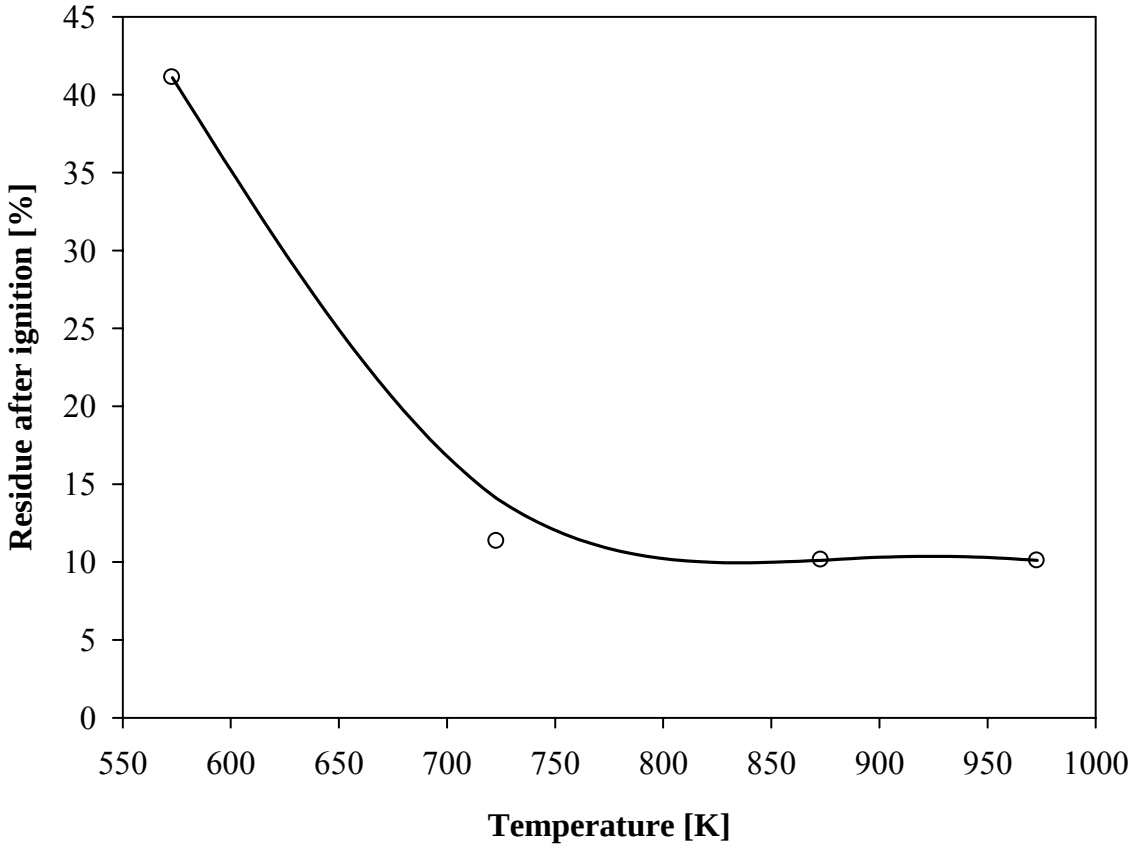


Figure 1. Effect of ignition temperature on residue of rice bran (ignition period 6 h).

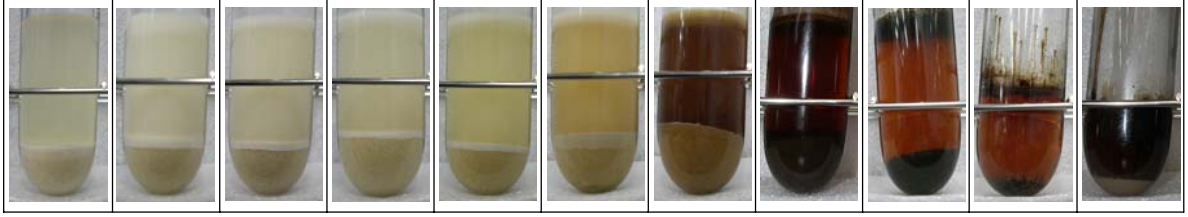
Control	373 K	393 K	413 K	433 K	453 K	473 K	513 K	553 K	593 K	633 K
										

Figure 2. Typical photographs of subcritical water treatment of rice bran as function of temperature for 5 min reaction time.

Figure 3 demonstrates the effects of reaction temperature on HS phase, AS phase and remained solid of rice bran after subcritical water treatment for 5 min. The amounts of HS and AS increased with increasing temperature and remained solid amount decreased consequently. HS phase was a yellowish viscose liquid, which was mainly rice bran oil *[Liu and Mamidipally, 2005]* with maximum yield of 27% (see Figure 3). On the other hand, any other extractive compounds, which may be soluble in hexane, can also be extracted into this phase. The extracted oil has a variety of applications. For instance, depending on its quality, it can be used as edible oil or as feed stock of biodiesel *[Zullaikah et al., 2005]*. The AS phase is ascribed to tar, carbonized sample, and any other compounds that can dissolve neither in water nor in hexane. Most of the remained solid contains un-reacted rice bran and mineral compounds. The aqueous phase contains mainly hydrolyzed products of proteins, cellulose, and hemicellulose parts of rice bran *[Sasaki et al., 1998; Wiboonsirikul et al., 2007a and b; Sereewatthanawut et al., 2008]*. These will be discussed in more detail later in this chapter and chapter 4. In this chapter, we mainly focus on the WS compositions.

3. 3. Water-soluble (WS) phase

3. 3. 1. General

As the most important measures of decomposition of rice bran by the subcritical water hydrolysis reaction, TOC and TN were investigated. Figure 4 shows the effect of subcritical water temperature on TOC and TN yields at a reaction time of 5 min. As both curves showed peaks at almost the same temperature as ion production of water-temperature curve, the soluble products were produced by hydrolysis reaction in subcritical water. TOC showed a peak at around 505 K, and then this decreased with increasing temperature, owing to a weak hydrolysis reaction, pyrolysis, and gasification of the organic compounds.

The shape of the TN profile is similar to the TOC curve. This profile showed a peak at 553 K, which decreased somewhat by increasing temperature due to final degradation of N-containing organic compounds to gaseous by-products such as NH_3. Generally, TN contents in the aqueous phase are a function of N-containing soluble proteins, peptides, and particularly of the amino acids and ammonia. The small amounts of the produced gases were not

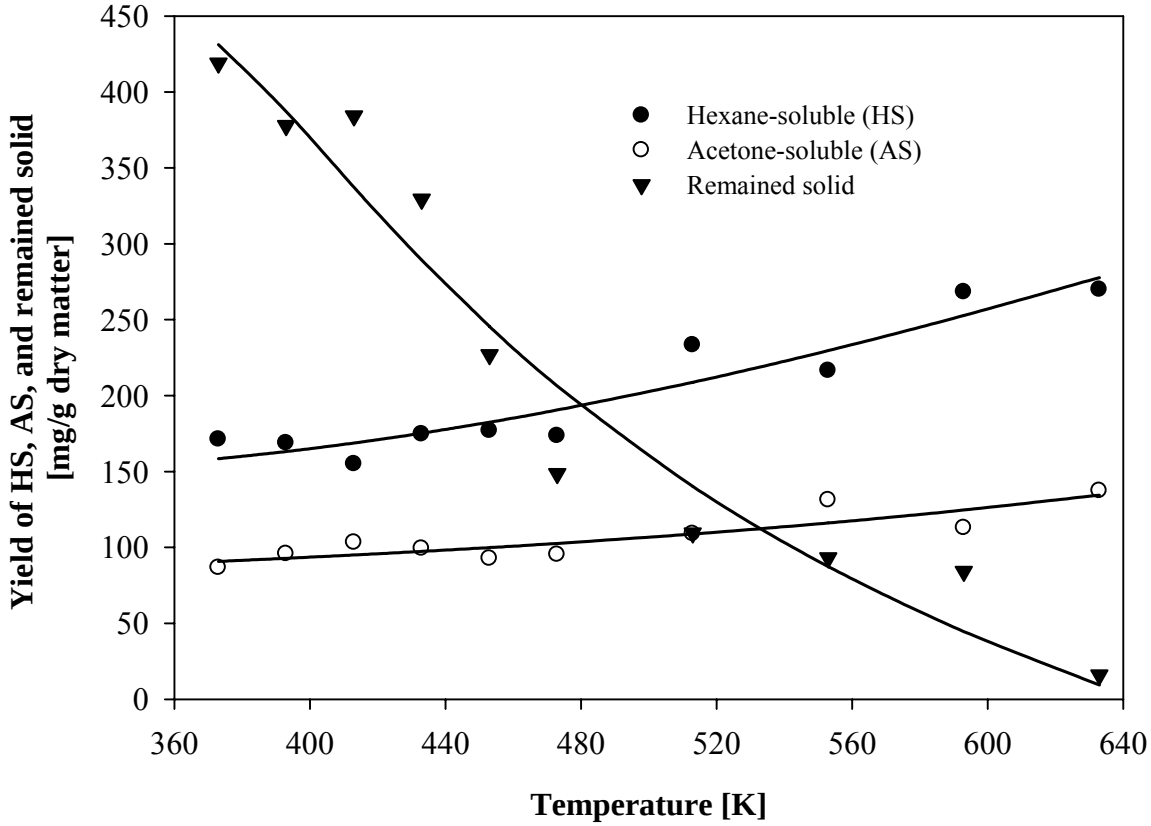


Figure 3. Effect of temperature on amounts of hexane-soluble (HS), acetone-soluble (AS), and remained solid obtained by subcritical water treatment of rice bran at reaction time of 5 min.

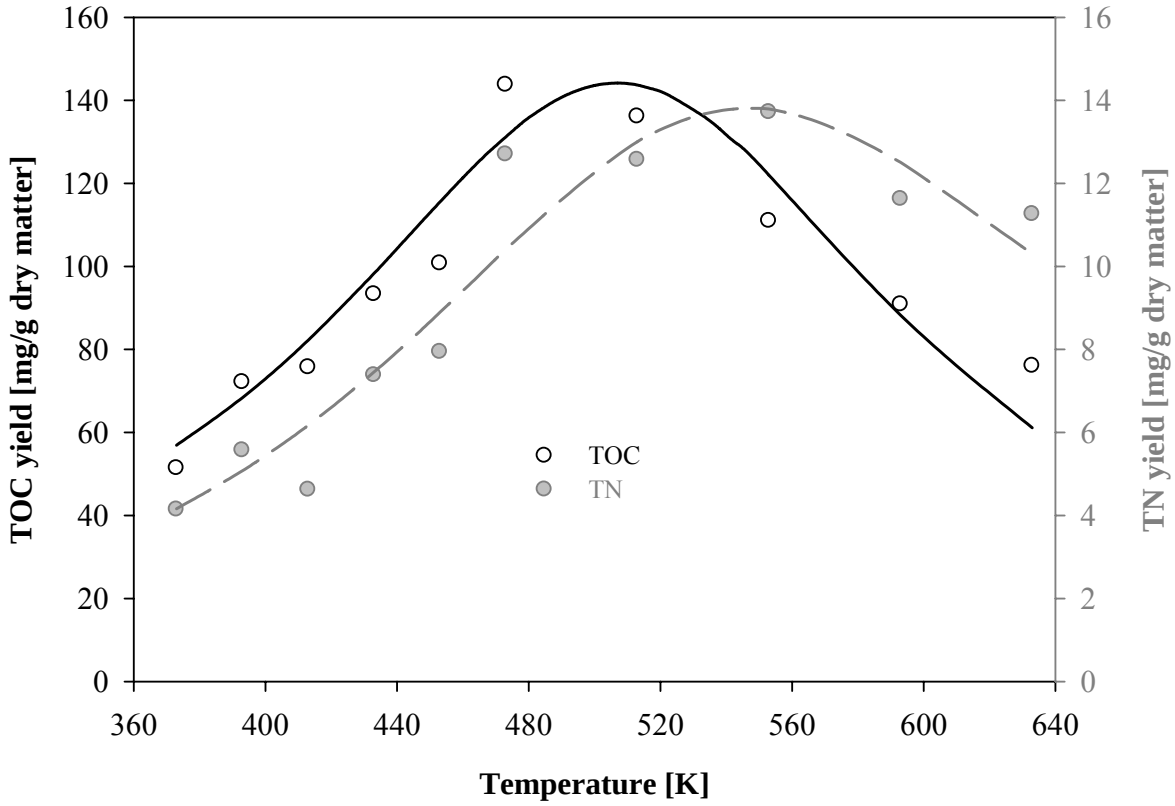


Figure 4. Effect of subcritical water temperature on TOC and TN yields at the reaction time of 5 min.

quantified in this research work.

3. 3. 2. Amino acids

Water-soluble amino acids were produced by subcritical water hydrolysis reaction of rice bran protein. Up to eight essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine) and six non- and/or conditionally essential amino acids (glutamic acid, alanine, tyrosine, serine, glycine, and asparatic acid) were found in WS phase. Figures 5 and 6 show the effect of reaction temperature on amino acid yields at the reaction time of 5 min. In general, peaks appeared around 400 K. Lysine and glutamic acid had the highest yield among the identified essential and nonessential amino acids, respectively. Due to decomposition of amino acids to low molecular weight carboxylic acids and gaseous products *[Yoshida et al., 1999; Abdelmoez and Yoshida, 2006b; Lamoolphak et al., 2006]*, amino acids were not identified at temperatures higher than 520 K. Yield and temperature differences between amino acid and TN peaks confirmed that other N-containing compounds (water-soluble proteins and peptides) were produced in the aqueous phase by hydrolysis reaction of rice bran under subcritical water conditions, and these may be main components of TN. These compounds were not analyzed in this research work.

3. 3. 3. Saccharides (total soluble sugars)

As rice bran is a rich source of polysaccharides, subcritical water hydrolyzed them to significant amounts of water-soluble sugars. Figure 7 shows the production yields of several quantified soluble sugars (sucrose, fructose, glucose, and glyceraldehyde) as a function of temperature at the reaction time of 5 min. Sucrose showed a peak at 413 K, and decomposed to fructose and glucose *[Haghighat Khajavi et al., 2005]* at higher temperatures. In fact, it must give equimolar amounts of fructose and glucose from hydrolysis of sucrose; on the other hand, fructose is less stable than glucose at the subcritical water condition *[Salak Asghari and Yoshida, 2006]*. Since production yield of fructose was higher than that of glucose (see Figure 7), it seems that another pathway must also exist for production of fructose (besides that obtained from hydrolysis of sucrose) *[Salak Asghari and Yoshida, 2006]*. The yield of

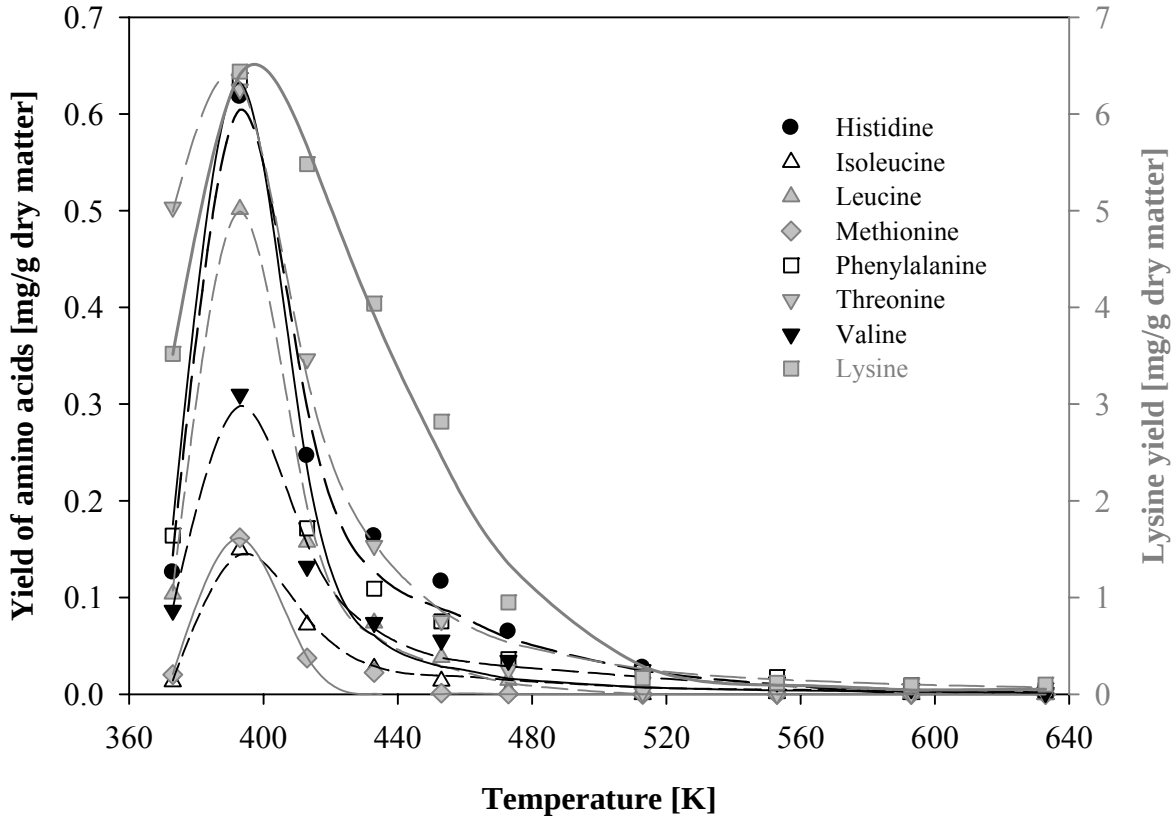


Figure 5. Yield of essential amino acids after subcritical water treatment of rice bran (identified in the aqueous phase) versus temperature at 5 min reaction time.

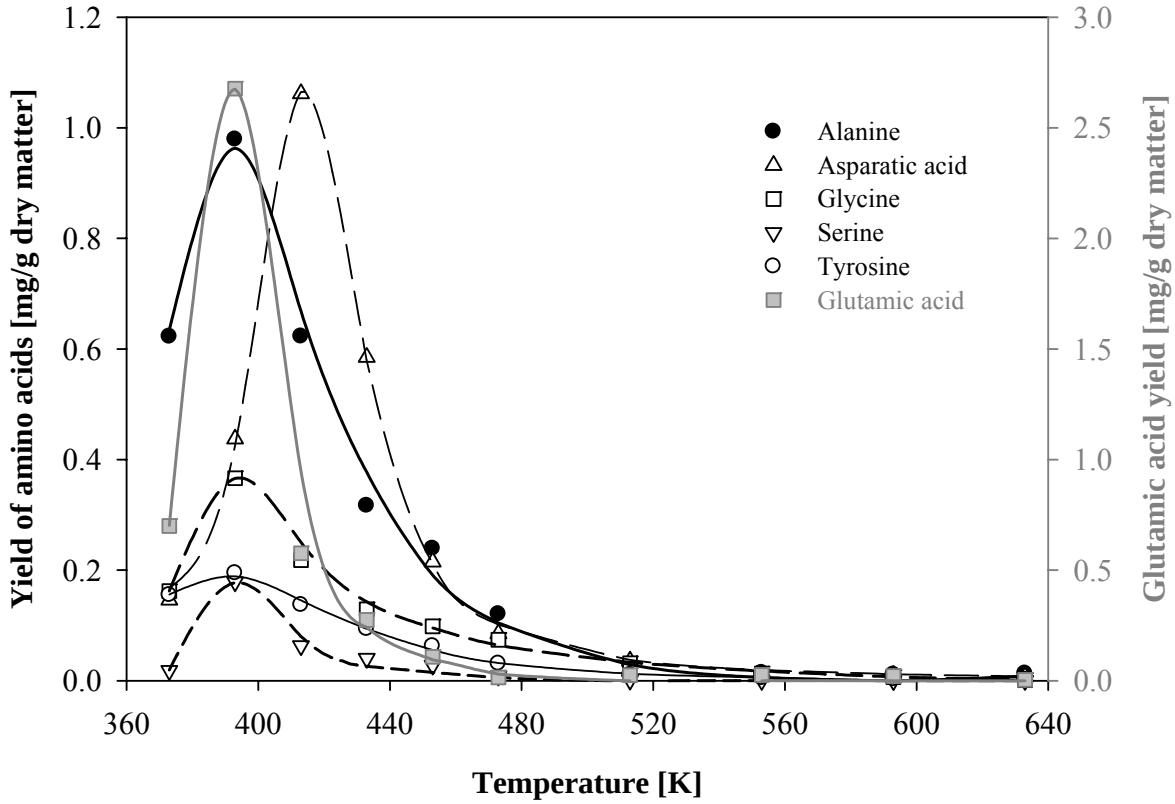


Figure 6. Yield of nonessential amino acids after subcritical water treatment of rice bran (identified in the aqueous phase) versus temperature at 5 min reaction time.

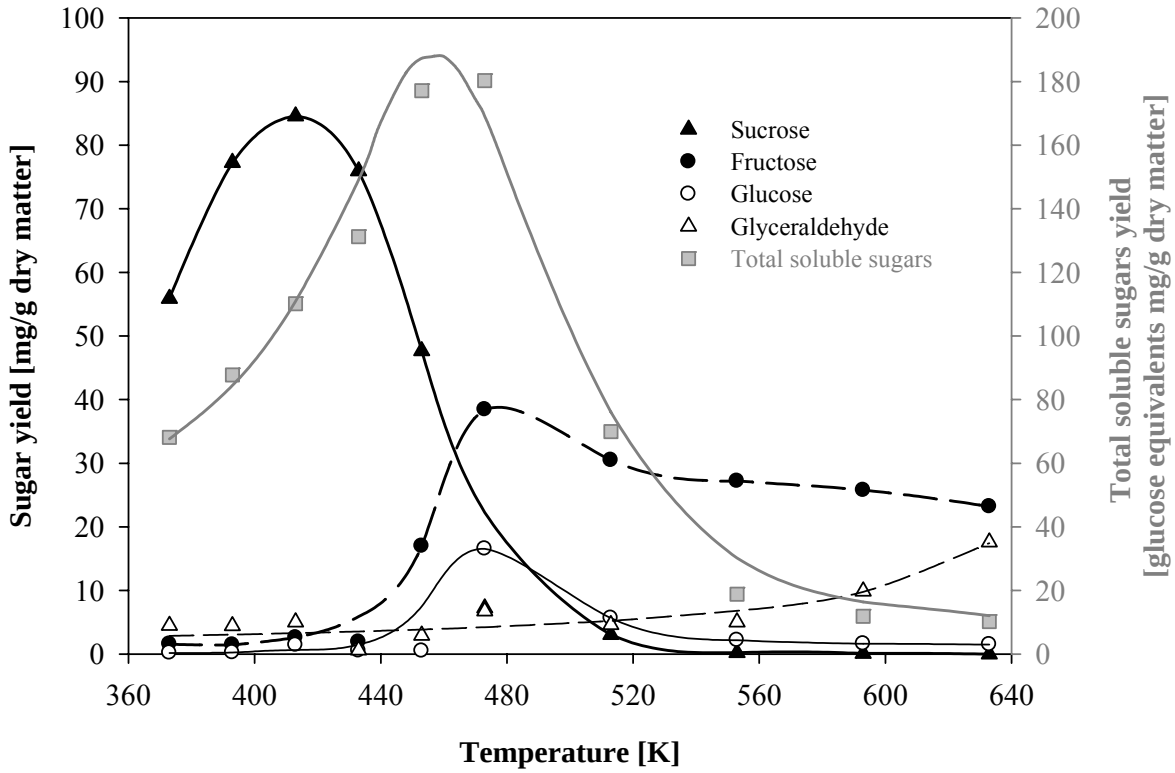


Figure 7. Effect of subcritical water temperature on sugar production yields in aqueous phase at the reaction time of 5 min.

glyceraldehyde increased almost linearly by increasing temperature.

Furthermore, total sugars of aqueous phase (including mixtures of poly-, oligo-, di-, and mono-saccharides) were quantified by a photometric method *[Hodge and Hofreiter, 1962]* and results are shown in the same Figure. About 20% of total soluble sugars in the aqueous phase is a very promising amount of rice bran hydrolysis by subcritical water. This amount decreased steeply from 463 to 633 K.

3. 3. 4. Organic acids

Organic acids can be produced by decomposition of biomass, carbohydrates, and amino acids *[Yoshida et al., 1999; Yoshida and Tavakoli, 2004; Abdelmoez and Yoshida, 2006b; Lamoolphak et al., 2006; Salak Asghari and Yoshida, 2006; Tavakoli and Yoshida, 2006]*. In this work, five WS organic acids were identified from decomposition of the rice bran. Figure 8 shows that acetic, formic, glycolic, and levulinic acids were produced at temperatures above 463 K. Acetic acid increased up to 553 K and then leveled off to a constant yield. Formic acid showed a peak at 513 K and decreased to zero at temperatures above 608 K. Glycolic and levulinic acid yields continuously rose with increasing temperature; however, glycolic acid showed a small amount of degradation at higher temperatures (i.e. 583 K). Citric acid was only identified at temperatures lower than 473 K. Due to the formation of WS organic acids, pH in the aqueous phase was changed, by increasing subcritical water temperature, to acidic values. The minimum pH was 4.6 at around 513 K. Then it was again increased to 5.1 at 593 K. This increment may be attributed to the decomposition of organic acids to other compounds, especially gaseous products and may also be due to buffering of the solution. Production of organic acids, and consequently, decreasing of the pH, led to the conclusion that autocatalysis may occur during subcritical water treatment of rice bran.

3. 4. Remained solid

In order to realize the changes in composition of remained solid by subcritical water treatment, CHNS (carbon, hydrogen, nitrogen, and sulfur) amounts in remained solid were

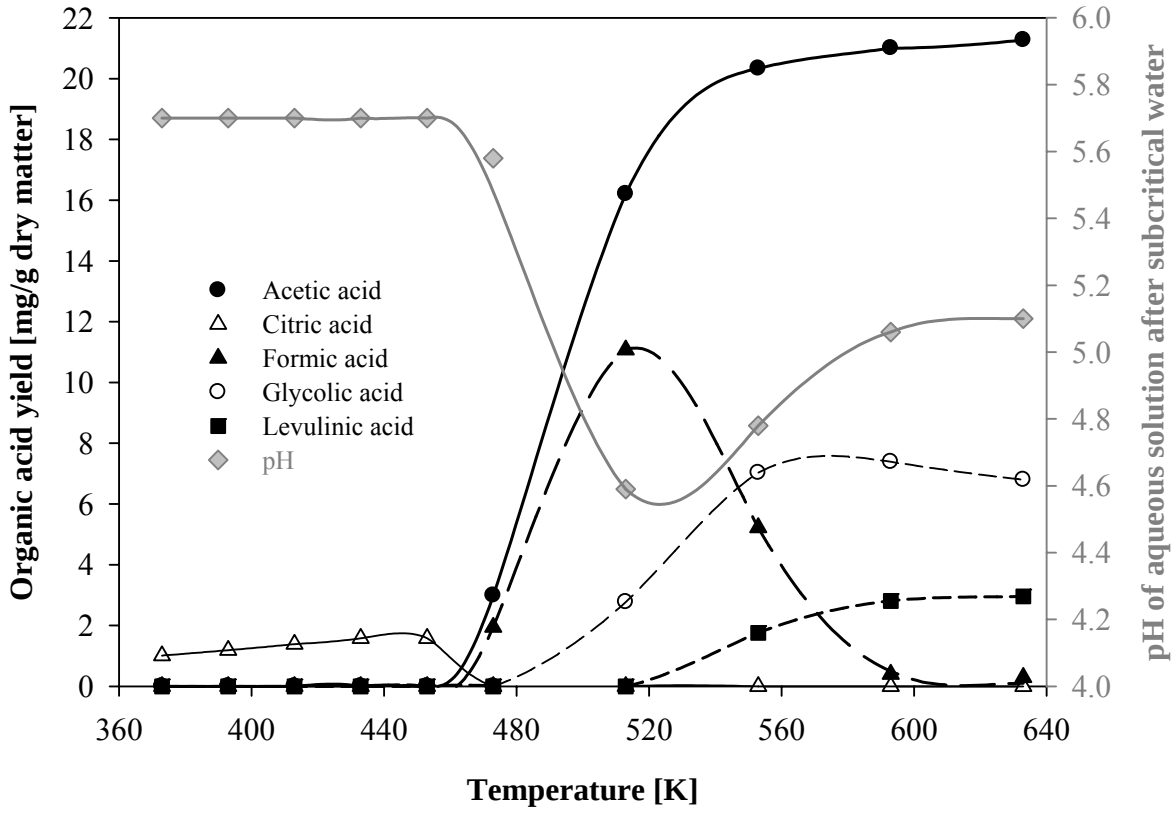


Figure 8. Organic acid yields of aqueous phase and its pH as a function of subcritical water temperature at 5 min reaction time.

evaluated. Figure 9 shows the effect of temperature on the ratios of H/C, N/C, and S/C in the remained solid for 5 min of reaction time. The H/C was decreased by increasing subcritical water temperature, particularly at higher temperatures, due to pyrolysis reactions. N/C ratio showed a minimum at 533 K and, the S/C ratio was gradually decreased by subcritical water temperature increase.

3. 5. Hexane, acetone, and water solubilities by rice bran conversion

Part of rice bran was dissolved in the three phases (HS, AS, and WS) by treating it under subcritical water conditions. Remained solid was the phase which was not dissolved in the above phases. Obviously the amounts of remained solid and dissolved materials depended on rice bran conversion. The solubilities of rice bran in hexane, acetone, and water phases, as functions of rice bran conversion by subcritical water were calculated by equations (1) to (3) and the results are shown in Figure 10. The greater the rice bran conversion, the greater were the amounts of HS, AS, and WS produced. Clearly, rice bran conversion and solubility yields depended on subcritical water reaction temperature (see Figure 3). Figure 10 also reveals that HS yield was always higher than that of AS, and WS yield was greater than those of HS and AS. This Figure shows that WS, HS, and AS solubilities were non-linear functions of rice bran conversion whilst total solubility was a linear function of rice bran conversion.

4. Conclusions

Subcritical water processing, as a green and environmentally friendly technique, has been successfully applied for rice bran treatment and production of valuable materials. The extraction of rice bran oil was found to be a feasible process. Rice bran oil was successfully extracted with higher yields than those obtained by conventional methods. It was apparent that subcritical water temperature influenced oil production. The higher the subcritical water temperature was, the greater was the amount of oil obtained. Maximum extracted rice bran oil, as HS phase, was nearly 27 % of the initial dry matter. In addition, temperature had considerable effects on the yield of the obtained tar and remained solid.

Another interesting finding was that of rice bran liquefaction by subcritical water

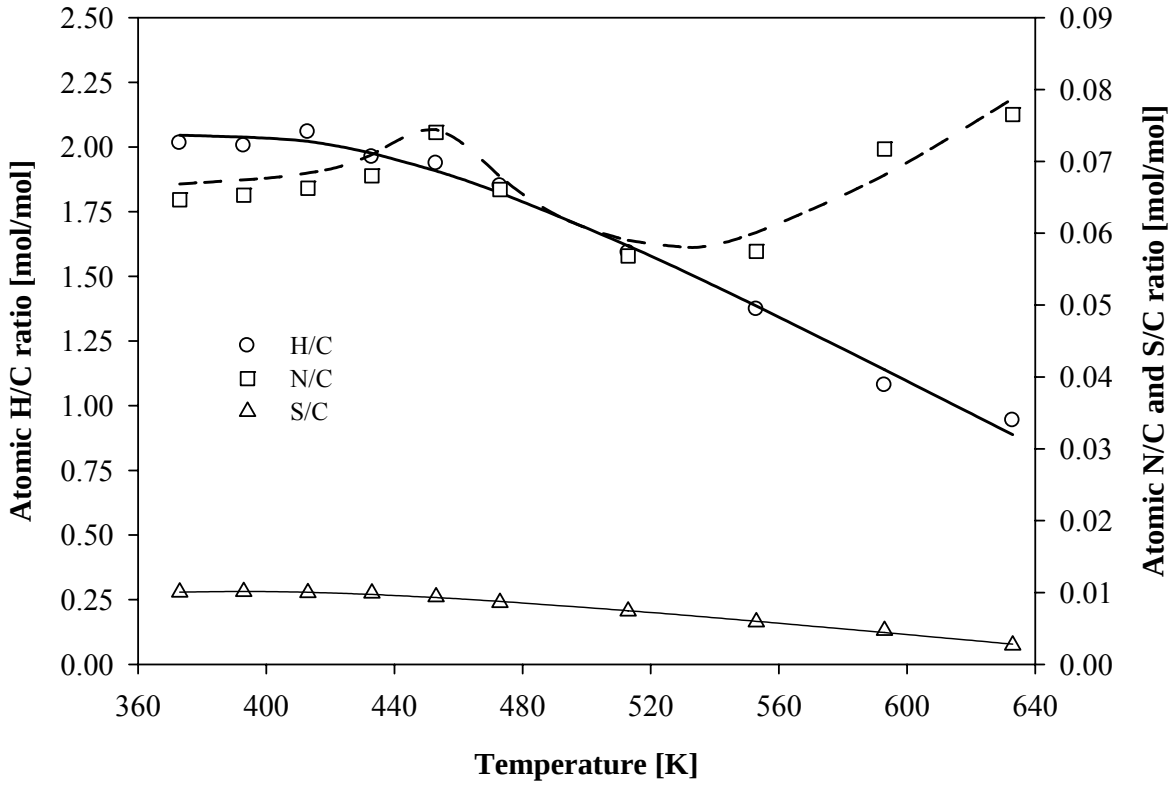


Figure 9. Effect of reaction temperature on the element composition of remained solid at 5 min reaction time.

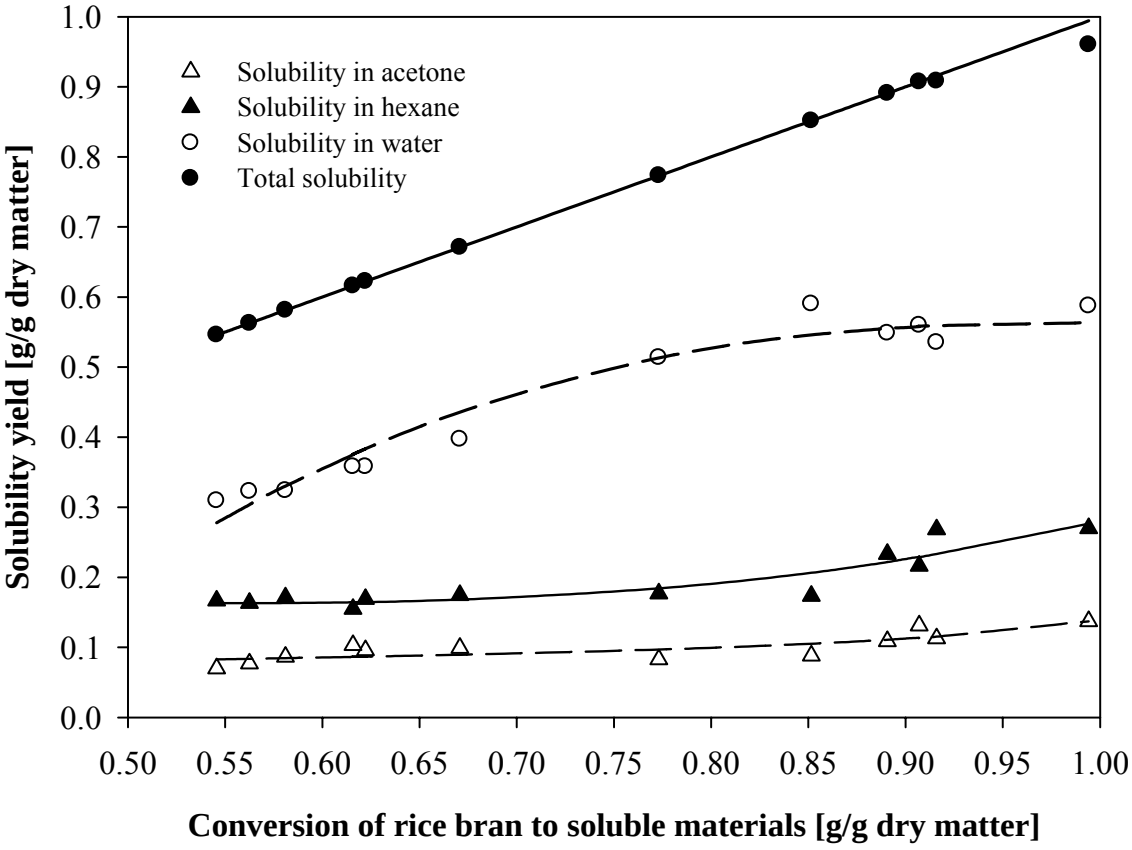


Figure 10. Solubility yields versus rice bran conversion caused by subcritical water treatment.

reaction and hydrolysis in a short reaction time (5 min). The experimental TOC and TN confirmed that protein and cellulosic parts were hydrolyzed and efficiently converted into water-soluble compounds. TOC and TN yield curves showed peaks at around 505 and 553 K, respectively. Subcritical water converted cellulosic parts of rice bran into the water-soluble di- and mono-saccharides. Maximum total yield of sugars produced by the hydrolysis reaction was nearly 20% of initial dry matter. This is a very suitable feed stock for bioethanol production and/or other industrial and food applications. The protein part of rice bran was hydrolyzed to a variety of essential and nonessential amino acids. Totally, more than 14 amino acids were identified in the aqueous phase. Among the obtained amino acids, the most plentiful yields were those of lysine, glutamic acid, alanine, and asparatic acid. Besides amino acids, five organic acids, in considerable amounts, were produced from decomposition of rice bran. Acids may autocatalyze further solubility of rice bran under subcritical water conditions. It was found that amino acid and organic acid yields were functions of subcritical water temperature. The optimum production temperature for most of the amino acids was 400 K, and at temperatures higher than 520 K no amino acid was detected while organic acids production began at temperatures higher than 463 K.

Nomenclature

AS	Acetone-soluble
HS	Hexane-soluble
K	Kelvin
TOC	Total organic carbon
TN	Total nitrogen
WS	Water-soluble

References

Abdelmoez, W., Yoshida, H., Simulation of fast reactions in batch reactors under sub-critical water condition, *AIChE Journal,* 52, 3600-3611, (2006a).

Abdelmoez, W., Yoshida, H., Synthesis of a novel protein-based plastic using sub-critical water technology, *AIChE Journal,* 52, 2607-2616, (2006b).

Bicker, M., Endres, S., Ott, L., Vogel, H., Catalytical conversion of carbohydrates in subcritical water: a new chemical process for lactic acid production, *Journal of Molecular Catalysis A: Chemical,* 239, 151-157, (2005).

Chen, M. H., Bergman, C. J., A rapid procedure for analysing rice bran tocopherol, tocotrienol and γ-oryzanol contents, *Journal of Food Composition and Analysis,* 18, 139-151, (2005).

Danielski, L., Zetzl, C., Hense, H., Brunner, G., A process line for the production of raffinated rice oil from rice bran, *The Journal of Supercritical Fluids,* 34, 133-141, (2005).

Galkin, A. A., Lunin, V. V., Subcritical and supercritical water: a universal medium for chemical reactions, *Russian Chemical Reviews (English Translation),* 74, 21-35, (2005).

Haghighat Khajavi, S., Kimura, Y., Oomori, T., Matsuno, R., Adachi, S., Kinetics on sucrose decomposition in subcritical water, *LWT-Food Science and technology,* 38, 297-302, (2005).

Herrero, M., Cifuentes, A., Ibanez, E., Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae, *Food Chemistry,* 98, 136-148, (2006).

Hodge, J. E., Hofreiter, B. T., Determination of reducing sugars and carbohydrates, *Methods in Carbohydrate Chemistry,* 1, 380-394, (1962).

Holliday, R. L., Jong, Y. M., Kolis, J. W., Organic synthesis in subcritical water: oxidation of alkyl aromatics, *The Journal of Supercritical Fluids,* 12, 255-260, (1998).

Hu, W., Wells, J. H., Shin, T. S., Godber, J. S., Comparison of isopropanol and hexane for extraction of vitamin E and oryzanols from stabilized rice bran, *Journal of the American Oil Chemists' Society,* 73, 1653-1656, (1996).

Kruse, A., Dinjus, E., Hot compressed water as reaction medium and reactant properties and synthesis reactions, *The Journal of Supercritical Fluids,* 39, 362-380, (2007).

Kruse, A., Gawlik, A., Biomass conversion in water at 330-410 °C and 30-40 MPa. Identification of key compounds for indicating different chemical reaction pathways, *Industrial & Engineering Chemistry Research,* 42, 267-279, (2003).

Lamoolphak, W., Goto, M., Sasaki, M., Suphantharika, M., Muangnapoh, C., Prommuag, C., Shotipruk, A., Hydrothermal decomposition of yeast cells for production of proteins and amino acids, *Journal of Hazardous Materials,* B137, 1643-1648, (2006).

Liu, S. X., Mamidipally, P. K., Quality comparison of rice bran oil extracted with d-limonene and hexane, *Cereal Chemistry,* 82, 209-215, (2005).

Luh, B. S., *Rice: Production and Utilization,* AVI Publishing Company, Inc., the USA, (1980).

Mamidipally, P. K., Liu, S. X., First approach on rice bran oil extraction using limonene, *European Journal of Lipid Science and Technology,* 106, 122-125, (2004).

Marshall, W. E., Wadsworth, J. I., *Rice Science and Technology,* Marcel Dekker, Inc., the USA, (1994).

Proctor, A., Bowen, D. J., Ambient-temperature extraction of rice bran oil with hexane and isopropanol, *Journal of the American Oil Chemists' Society,* 73, 811–813, (1996).

Proctor, A., Jackson, V. M., Scott, M., Clark, P. K., Rapid equilibrium extraction of rice barn oil at ambient temperature, *Journal of the American Oil Chemists' Society,* 71, 1295-1296, (1994).

Renuka Devi, R., Arumughan, C., Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment, *Bioresource Technology,* 98, 3037-3043, (2007).

Salak Asghari, F., Yoshida, H., Acid-catalyzed production of 5-hydroxymethyl furfural from *D*-fructose in subcritical water, *Industrial & Engineering Chemistry Research,* 45, 2163-2173, (2006).

Salak Asghari, F., Yoshida, H., Kinetics of the decomposition of fructose catalyzed by hydrochloric acid in subcritical water: formation of 5-hydroxymethylfurfural, levulinic, and

formic acids, *Industrial & Engineering Chemistry Research,* 46, 7703-7710, (2007).

Sasaki, M., Kabyemela, B., Malaluan, R., Hirose, S., Takeda, N., Adschiri, T., Arai, K., Cellulose hydrolysis in subcritical and supercritical water, *The Journal of Supercritical Fluids,* 13, 261-268, (1998).

Sereewatthanawut, I., Prapintip, S., Watchiraruji, K., Goto, M., Sasaki, M., Shotipruk, A., Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis, *Bioresource Technology,* 99, 555–561, (2008).

Tanaka, T., Hoshina, M., Tanabe, S., Sakai, K., Ohtsubo, S., Taniguchi, M., Production of *D*-lactic acid from defatted rice bran by simultaneous saccharification and fermentation, *Bioresource Technology,* 97, 211-217, (2006).

Tavakoli, O., Yoshida, H., Conversion of scallop viscera wastes to valuable compounds using sub-critical water, *Green Chemistry,* 8, 100-106, (2006).

Tavakoli, O., Yoshida, H., Effective recovery of harmful metal ions from squid wastes using subcritical and supercritical water treatments, *Environmental Science and Technology,* 39, 2357-2363, (2005).

Wang, L., Weller, C. L., Recent advances in extraction of nutraceuticals from plants, *Trends in Food Science and Technology,* 17, 300-312, (2006).

Wiboonsirikul, J., Hata, S., Tsuno, T., Kimura, Y., Adachi, S., Production of functional substances from black rice bran by its treatment in subcritical water, *LWT-Food Science and Technology,* 40, 1732-1740, (2007a).

Wiboonsirikul, J., Kimura, Y., Kadota, M., Morita, H., Tsuno, T., Adachi, S., Properties of extracts from defatted rice bran by its subcritical water treatment, *Journal of Agricultural and Food Chemistry,* 55, 8759-8765, (2007b).

Xu, Z., Godber, J. S., Comparison of supercritical fluid and solvent extraction methods in extracting γ-oryzanol from rice bran, *Journal of the American Oil Chemists' Society,* 77, 547-551, (2000).

Yoshida, H., Tavakoli, O., Sub-critical water hydrolysis treatment of squid waste entails and production of organic acid, amino acid, and fatty acids, *Journal of Chemical Engineering of*

Japan, 37, 253-260, (2004).

Yoshida, H., Takahashi, Y., Terashima, M., A simplified reaction model for production of oil, amino acids, and organic acids from fish meat by hydrolysis under sub-critical and supercritical conditions, *Journal of Chemical Engineering of Japan,* 36, 441-448, (2003).

Yoshida, H., Terashima, M., Takahashi, Y., Production of organic acids and amino acids from fish meat by sub-critical water hydrolysis, *Biotechnology Progress,* 15, 1090-1094, (1999).

Zullaikah, S., Lai, C. C., Vali, S. R., Ju, Y. H., A two-step acid-catalyzed process for the production of biodiesel from rice bran oil, *Bioresource Technology,* 96, 1889-1896, (2005).

Chapter 3

Application of Subcritical Water Treatment for Simultaneous Inactivation of Rice Bran Lipase Enzyme and Extraction of Edible Oil

1. Introduction

Rice bran is a major by-product of rice milling process accounting as about 8.0% of milled rice *[Sereewatthanawut et al., 2008]*. In Japan, rice bran is one of the most abundant biomass; about 900 thousand tons are produced per year *[Tanaka et al., 2006]*. This biomass is a natural source of oil, carbohydrates, proteins, vitamins, antioxidants, enzymes, dietary minerals, and dietary fibers *[Luh, 1980; Saunders, 1985-86]*.

Depending on milling procedure, rice bran contains 10.0 to 26.0% oil; therefore, this abundant biomass has considerable potential to world oil supply *[Prabhakar and Venkatesh, 1986]*. Rice bran oil (hereafter called RBO) has vast applications in different industries *[Luh, 1980; Zullaikah et al., 2005]*. Especially, its unique properties make it very appealing oil for food and pharmaceutical companies *[McCaskill and Zhang, 1999; Renuka Devi and Arumughan, 2007]*. However only a small portion (< 10.0%) of RBO is processed into edible oil *[Zullaikah et al., 2005]*; the reason is hydrolysis reaction of its triglyceride into glycerol and free fatty acids (hereafter called FFAs) which occurs soon after rice milling caused by the presence of lipase enzyme as catalyst *[Ju and Vali, 2005; Zullaikah et al., 2005]*. These FFAs produced by the hydrolysis reaction are harmful compounds which make RBO unfit for edible use *[Westphal et al., 2002; Goffman et al., 2003]*. Generally, RBO with an excess of 10.0% FFAs is unfit for human consumption *[Enochian et al., 1981; Tao et al., 1993]*.

The process of hydrolytic rancidity development can be avoided either by rapid oil extraction soon after rice milling process or stabilization of bran *[Ju and Vali, 2005]*. Direct solid-liquid (solvent) and soxhlet extraction techniques are examples of several conventional RBO extraction methods from rice bran. Hu et al. *[1996]* have studied the effects of solvent-to-bran ratio and extraction temperature on direct solid-liquid (hexane and isopropanol) extraction method, and they reported that increasing the solvent-bran ratios and extraction temperature, increased the RBO yield. Mamidipally and Liu *[2004]* extracted RBO using hexane and *d*-limonene at their respective boiling points at various solvent-to-meal ratios; the preliminary data suggested that the optimum solvent-to-meal ratio was 5:1. Recently, biorefinery extraction methods such as supercritical carbon dioxide were used for RBO extraction *[Shen et al., 1996; King and Dunford, 2002; Temelli, 2009]*. Kuk and Dowd *[1998]* investigated supercritical CO_2 temperature effect on extraction yield, and they found

that RBO yield increased with temperature under isobaric conditions. Xu and Godber *[2000]* studied RBO extraction using supercritical CO_2, and they reported that yields of extracts in 20 min extraction time were 90.0 g from 1.0 kg rice bran at 303 K to 130.0 g at 333 K.

As mentioned above, stabilization of rice bran and its oil is another technique for avoiding of RBO deterioration. It depends on temperature, duration of heat treatment, moisture content of treatment medium, pH, and other parameters *[Luh, 1980; Tao et al., 1993]*. Numerous attempts have been made with different degree of success for modification and improvement of the stabilization process of rice bran and its oil. Randall et al. *[1985]* stabilized rice bran by extrusion cooking process at 403 K which showed no significant increase in FFAs content for at least 30-60 days. In another report, lipase enzyme activity could be controlled by lowering the pH from 6.9-6.0 to 4.0 *[Prabhakar and Venkatesh, 1986]*, and increase in FFAs was about 2.0% in a period of 59 days. Rao Lakkakula et al. *[2004]* showed that ohmic heating at 1 and 60 Hz was an effective method for rice bran stabilization with moisture addition. Microwave heating is another treatment method for prevention of rice bran oil rancidity (during rice bran storage) has been studied by Tao et al. *[1993]* at 2450 Hz for 3 min, and they reported that FFAs content in two different kinds of treated samples increased from 4.0% and 4.6% to 4.9% and 6.2%, respectively.

Recently, research has been carried out by subcritical water as an environmentally friendly technique for decomposition *[Yoshida et al., 1999; Yoshida and Tavakoli, 2004; Tavakoli and Yoshida, 2005; Salak Asghari and Yoshida, 2006; Tavakoli and Yoshida, 2006; Salak Asghari and Yoshida, 2007; Wiboonsirikul et al., 2007a and b; Pourali et al., 2009]* and extraction of variety of compounds *[Herrero et al., 2006]*. More recently many studies have been carried out on the biomass conversion by subcritical water; however no studies have been reported on RBO stabilization and extraction using subcritical water.

The main objective of this chapter is to show lipase enzyme inactivation and RBO stabilization by subcritical water would be a feasible process. Moreover, the efficiency of subcritical water for the extraction of RBO from rice bran has been studied.

2. Material and methods

2. 1. Materials

Japonica-type rice (*Oryza sativa*) was used for this research work. All reagents and solvents were purchased from Wako Pure Chemical Industries, Ltd. (Japan). The organic part of the rice bran was identified as 44.9% of carbon, 7.2% of hydrogen, 3.3% of nitrogen, and 1.2% of sulfur. Water content and ash content were 8.8% and 10.0%, respectively.

2. 2. Procedure

The rice samples were milled by a milling system (Satake SKM-5B, Satake Corporation, Japan). The bran was sieved from the milled rice (with a 590 μm-mesh sieve) and then the sieved bran was immediately treated using subcritical water, direct oil extraction methods, and/or it was placed in plastic zipper top bags stored at 298 K for rice bran oil extraction by soxhlet.

The batch reactor used for subcritical water treatment was a stainless steel tube (SUS316, i.d. 16.5 mm × 150.4 mm; inner volume: 32.2 cm^3) with a Swagelok fitting (ready-made, from Swagelok). In a typical experiment, an accurately weighed amount of the bran (about 3.0 g) and about 18.0 cm^3 of distilled water were charged into the reactor. Argon gas was used to force air out of the reactor before the reaction, and it was capped tightly. The reactor was immersed in a preheated oil bath (Thomas Kagaku Co. Ltd., Celsius M type) with temperatures ranging from 393 to 453 K or in a preheated salt bath (Thomas Kagaku Co. Ltd., Celsius 600H) in the temperature range 453 to 513 K for 10 or 20 min. The reactor was then removed from the thermal bath and quickly quenched by soaking in a cold water bath at room temperature. The reaction pressure was estimated from a steam table; the details were explained elsewhere *[Yoshida et al., 1999]*. For conducting this research, 17 reactors were used for each desired operation condition. After subcritical water treatment, all contents in each reactor were removed and washed in a test tube. We took particular care to prevent loss of any of the liquid. The contents were classified into three phases: hexane-soluble (HS) phase, water-soluble (WS) phase, and remained solid phase. The separation procedure was as follows: Hexane was gently added to each tube and allowed to stand for 5 min at 298 K, then they were centrifuged at 2000 g for 10 min and supernatant was separated. In HS phase, yellowish to brownish viscous liquid was obtained and it mainly contained RBO. This procedure was repeated five times. Then aqueous phase and remained solid were separated by

filtration. Hexane was added to the remaining rice bran solid residue and this mixture was shaken for 10 min. After centrifugation, the supernatant was separated and added to the obtained HS phase from WS phase. This procedure was also repeated five times. HS amount was calculated by weight after evaporation of hexane by rotary evaporator (NE-1000, Eyela, Japan). Vacuum condition for evaporation was obtained using an aspirator (Advantec AS-25, Toyo Seisakusho Kaisha Ltd., Japan).

In addition to the research on stabilization of RBO by subcritical water treatment, solvent extraction of RBO was also studied. Conventional rapid solid-liquid extractions of RBO were investigated and compared with the subcritical water extraction. The conventional extraction methods were conducted in three media: hexane-bran, hexane-water-bran, and ethanol-bran. For hexane-bran, 50.0 g fresh bran was mixed with 300.0 g hexane and they were shaken for 240 min at 150 rpm and 298 K. At the second direct method, 50.0 g fresh bran, 100.0 g distilled water, and 200.0 g hexane were mixed and shaken under similar operational conditions to the first option. For rice bran oil extraction by ethanol, 50.0 g fresh bran was mixed with 300.0 g ethanol and this mixture was shaken for 240 min at 150 rpm and 333 K. The obtained dark green phase was ethanol-soluble (ES) phase which contained oil, and other extractive compounds by ethanol. After shaking, ES and HS phases were separated and filtered from other phases and their solvent was evaporated by rotary evaporator to evaluate the quality of oil, and also oil extraction yield.

Lipase activity was determined by measuring the concentration of total FFAs produced by the hydrolysis reaction of oil caused by the lipase. The oils were original rice bran oil (untreated oil), oils extracted by subcritical water, and oils extracted by organic solvents. Soxhlet apparatus was used for oil extraction from untreated sample. Stored milled rice bran (10.0 g), at 298 K, was placed into soxhlet thimble, and extraction of RBO was conducted by hexane as an organic extraction solvent for nearly 240 min. The obtained mixture was concentrated by hexane evaporation to evaluate the quality of oil, and extraction yield.

2. 3. Analysis

The quality of rice bran and its oil is usually determined as total FFAs content in the

oil *[Bhosle and Subramanian, 2005]*. It is expressed as oleic acid percentage in RBO *[Zappe, 1997]*. Another important indicator for RBO quality is acid value (AV) which measures the amount of acid present in oil. AV is the required amount of potassium hydroxide for neutralization of one gram of oil.

Concentration of total FFAs in RBO and AV of RBO were evaluated according to modified alcoholic alkali titration method *[Hoffpauir et al., 1947; Ames and Licata, 1948]*. After extraction and evaporation of hexane or ethanol, 3.0 cm^3 ethanol and a drop of phenolphthalein were added to the specific weight of the extracted oil (nearly 200 mg). This mixture was heated in a water bath at 333 K for 5 min. Then, the heated mixture was titrated by the specific concentration of alcoholic KOH (contains 10.0% mili-Q water) stored in a glass container and protected from atmospheric CO_2. During the titration, this mixture was vigorously shaken until the first permanent pink color appeared.

Total FFAs percentage and AV were calculated from the following equations:

$$\%FFA = \frac{(V_{KOH})\,(N_{KOH})\,(282.5)}{(10)\,(m_{oil})} \tag{1}$$

$$AV = \frac{(V_{KOH})\,(N_{KOH})\,(56.1)}{(m_{oil})} \tag{2}$$

Where %FFA, AV, V_{KOH}, N_{KOH}, m_{oil}, 282.5, and 56.1 were FFA percentage in RBO [g/100 g RBO], acid value of RBO [mg KOH/g RBO], amount of titrant [cm^3], normality of titrant [mmol/cm^3], RBO weight [g], oleic acid molecular weight, and molecular weight of potassium hydroxide, respectively.

A mid-range FTIR spectrophotometer (Shimadzu IRPrestigue-21, Shimadzu Co., Japan) equipped with demountable KBr window cells (Shimadzu 202-32000-20, Shimadzu Co., Japan) was used for obtaining absorption spectra.

3. Results and discussion

3. 1. FFAs formation in rice bran

FFAs are formed as result of hydrolysis reaction of triglyceride in the RBO, and then are produced in the course of rice bran storage. Lipase enzyme catalyzes this reaction to

proceed rapidly. For better understanding of FFAs formation in the rice bran, their concentration were quantified during 12 weeks of storage (see Figure 1). This figure indicates that total FFAs concentration increased in the course of rice bran storage. However, the concentration profile has three major time intervals: during the first three weeks total FFAs content increased sharply from 5.6% to 25.6% which demonstrates that most of the hydrolysis reaction occurred in this interval, and this phenomenon can be attributed to the high concentration of reactant (triglycerides); in the second time interval (from third to eighth weeks of storage) total FFAs concentration rose moderately, and finally at third interval it increased gradually to level-off at concentration of about 36.0%. It seems that there was no triglyceride to convert into FFAs; therefore as result, the FFAs formation profile levels-off in the third interval. Thus, as main conclusion, untreated RBO cannot be used for edible oil production even after one week from the date of rice milling; in order to overcome this problem and to obtain the stabilized RBO, lipase enzyme must be necessarily inactivated.

3. 2. Stabilization of RBO

3. 2. 1. Stabilization by subcritical water

Subcritical water treatment as a green technique has unique physicochemical properties which can influence and control different parameters of chemical reactions and processes *[Kruse and Dinjus, 2007]*. Because of these unique properties subcritical water has vast applicants like hydrolysis and decomposition processes *[Galkin and Lunin, 2005]*. Yoshida et al. *[1999]* have shown that proteins were decomposed to small soluble proteins, peptides, amino acids, and organic acids. Also Yoshida et al. *[2007]* have shown that even prion could be decomposed in conjunction with dangerous parts of cow become harmless.

Since enzymes are protein, they may breakdown and denature in subcritical water. In order to eliminate the problematic enzyme, a series of subcritical water treatments were carried out on rice bran in the temperature range between 393 to 513 K and reaction times of 10 and/or 20 min. Figure 2 shows the generation of total FFAs concentration of the RBO as a function of time after subcritical water treatment at different temperatures. No changes in FFAs concentrations occurred in the sample treated by subcritical water even after 12 weeks.

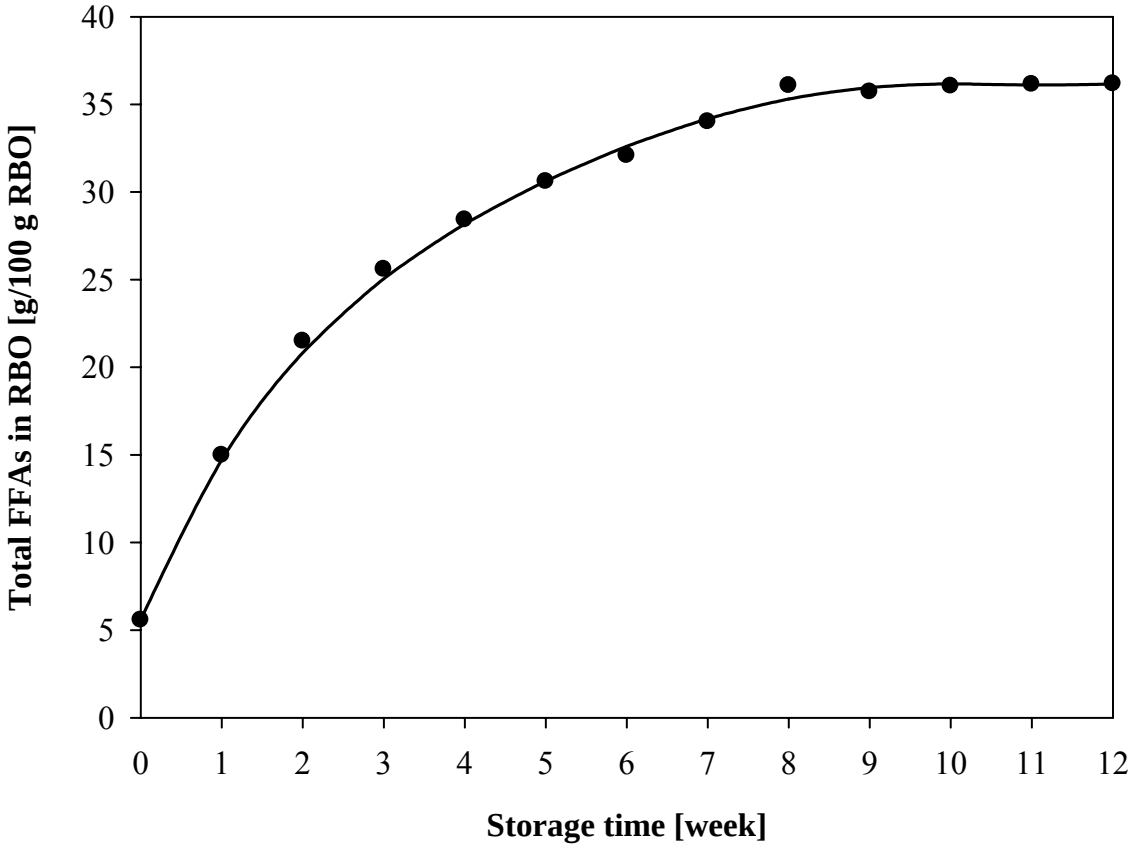


Figure 1. Total FFAs concentration (as oleic acid percentage in RBO) in untreated rice bran during storage at 298 K.

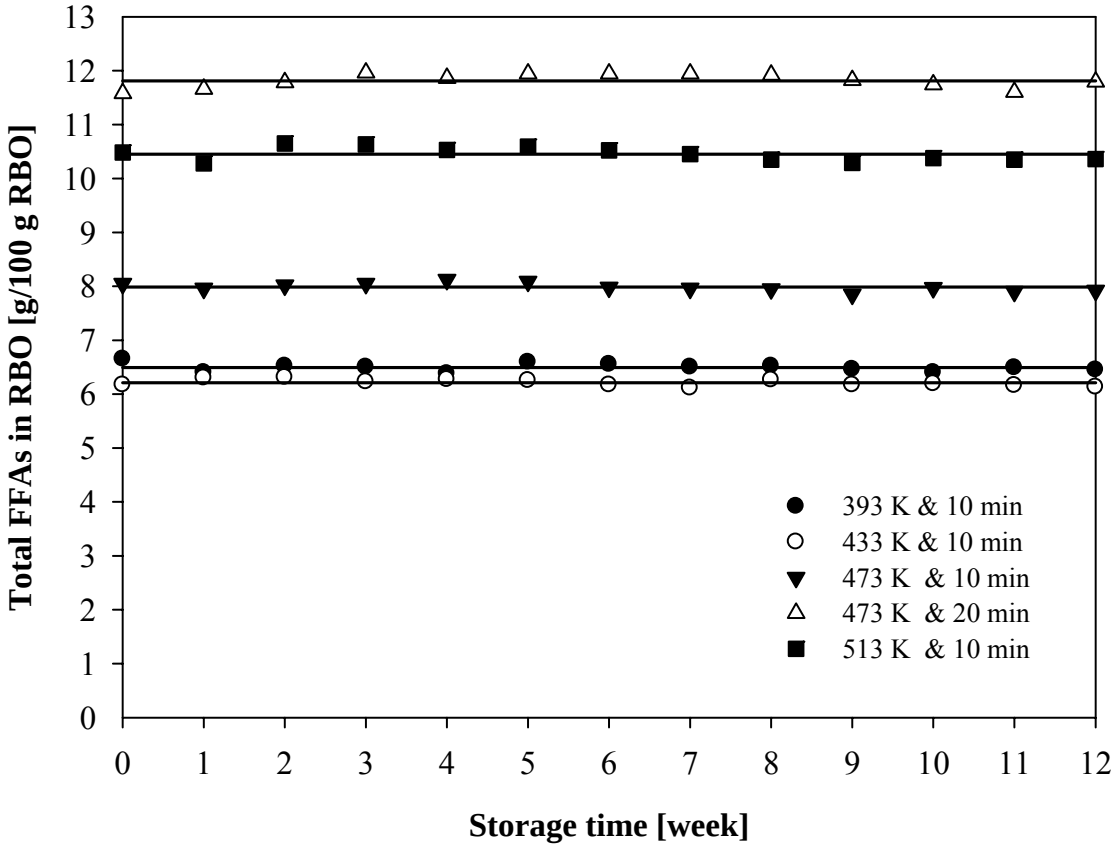


Figure 2. Effect of subcritical water treatment on FFAs level in the stored treated RBO; total initial FFAs concentration before reaction (and just after rice milling) was 5.6%.

Inactivation and decomposition of lipase enzyme is the most probably reason for this. The lowest and highest concentrations of FFAs were obtained at 433 K for 10 min and 473 K for 20 min, respectively. It was found that FFAs concentration generally increased by raising the subcritical reaction temperature. Also, it was found that the reaction time influenced the concentration of total FFAs in the treated oils.

These results also revealed that the lipase inactivation by subcritical water was an irreversible process (see Figure 2) which leads to the conclusion that lipase enzyme completely decomposed in subcritical water medium. In fact, the combined effects of temperature and reaction time in subcritical water medium can provide very suitable conditions for inactivation of lipase enzyme.

In this research work, generally AV attributes to the total FFAs amount in RBO. On the other hand, since subcritical water inactivates completely the lipase enzyme activity; therefore, any increasing amount of AV might be attributed to the hydrolysis of RBO triglyceride into FFAs during subcritical water treatment. Figure 3 shows the relationship between AV of the RBO and subcritical water temperature (reaction time: 10 min). AV at low temperatures (up to 410 K) was nearly constant and then it increased with increase of processing. From Figures 2, 3, and above assumption it can be concluded that subcritical water temperature has major effect on the formation of FFAs in the RBO; when the temperature is lower than 433 K, AV is almost the same as the initial value, and generally the higher subcritical water temperature is, the more RBO triglyceride is hydrolyzed. It should be noted that AV of RBO for cooking must be low as much as possible (i.e. based on Japanese food regulation it must be lower than 1 mg KOH/g RBO *[Kubo et al., 1987]*); however, the AV of the obtained RBO was higher than this limitation even just after milling rice sample. AV amount must be decreased into reasonable values in order to use as edible oil by contacting NaOH aqueous solution to remove FFAs from RBO.

3. 2. 1. 1. FTIR studies

FTIR analytical technique was used for evaluation of FFAs in the RBOs. Figures 4a-e show the FTIR spectra for FFAs standard mixture (palmitic, stearic, oleic, and linolenic acids), fresh control, stored control, fresh treated, and stored treated samples, respectively. Standard

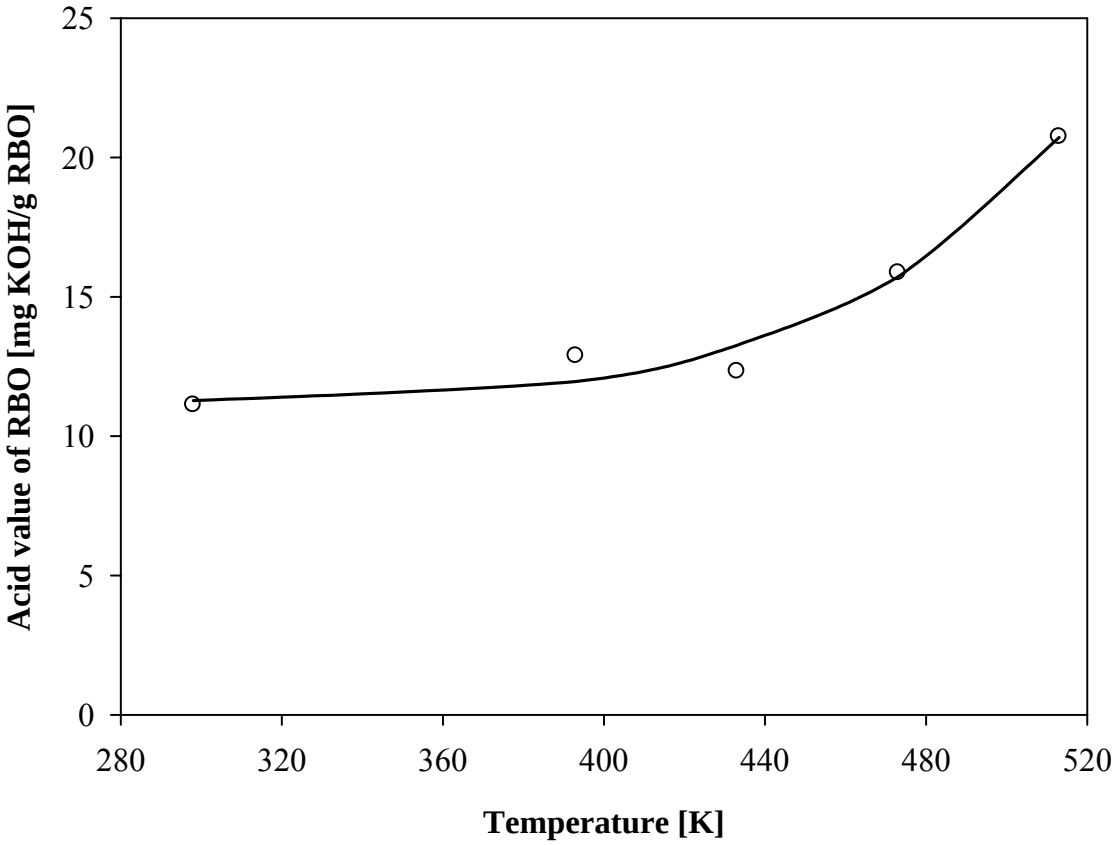


Figure 3. Effect of subcritical water temperature on AV in the stabilized RBO; reaction time: 10 min.

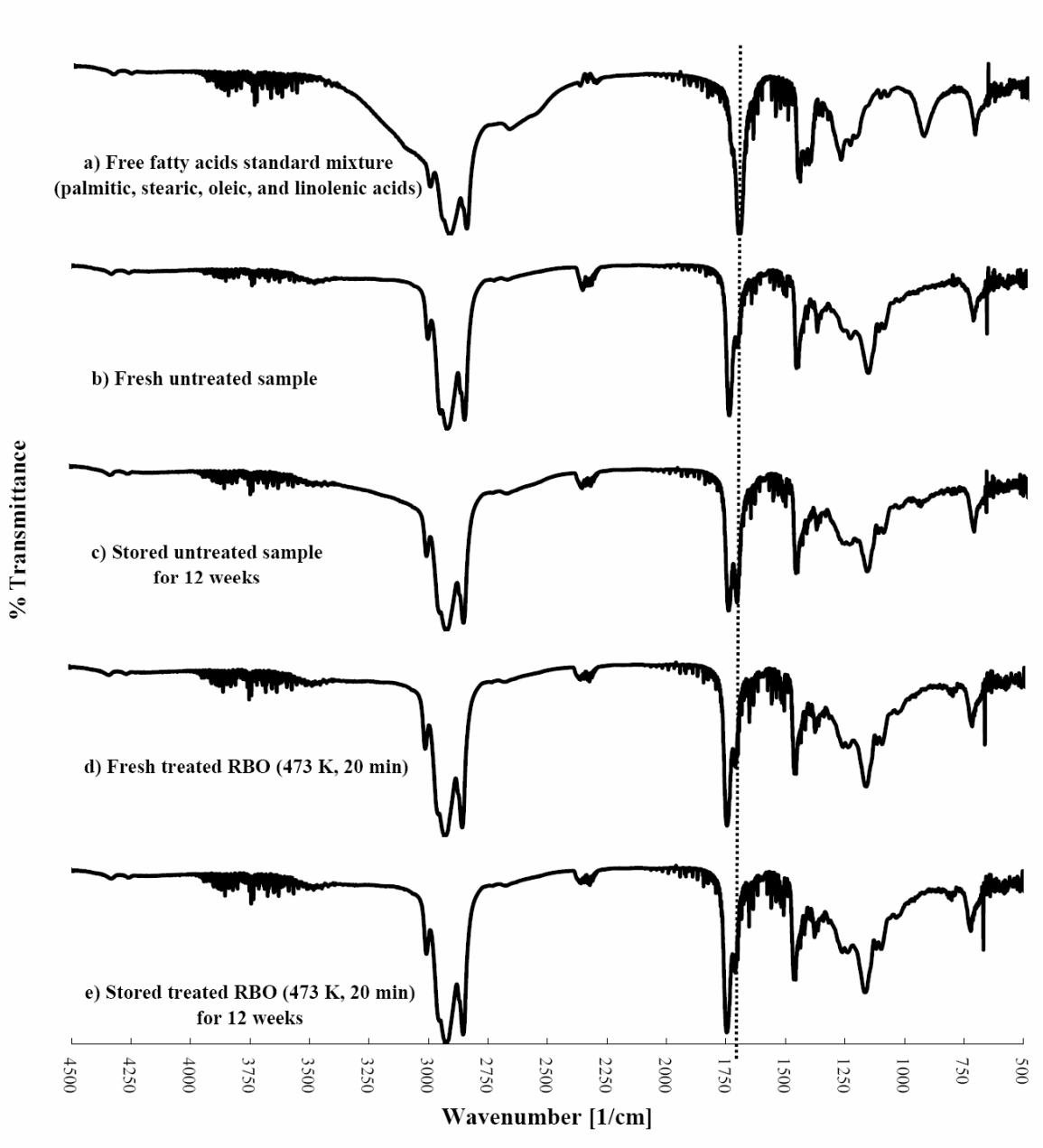


Figure 4. Comparison of FTIR spectra in different RBOs, including standard FFAs mixture.

mixture showed a sharp peak at 1720 cm-1. At the same wavenumber, relatively very smaller peak was observed for fresh untreated sample (Figure 4b). This peak increased in the untreated sample which was stored for 12 weeks (see Figure 4c). It is due to total FFAs level increasing during storage period in the untreated samples. In Figure 4d the spectrum of fresh RBO treated by subcritical water showed the same peak at 1720 cm^{-1} of which intensity is relatively higher than the fresh untreated sample one. It is realized that total FFAs amount in the treated sample by subcritical water is somewhat higher than those obtained by fresh untreated sample. Taking into account that lipase enzyme can be completely inactivated under subcritical water condition; the difference between amount of FFAs of fresh control and fresh treated samples again proves that FFAs may be produced during subcritical water treatment from hydrolysis of RBO triglycerides.

The spectrum of the stored RBO after treating by subcritical water was almost the same as the spectrum of the fresh treated sample (see Figures 4d and e). As one of the very important findings, it can be concluded that in subcritical water treated RBOs, there is no definitely change in the concentration of FFAs by storage due to decomposition and/or irreversible inactivation of lipase enzyme.

The competitive FTIR study implies that subcritical water poses a great potential to completely inactivate the lipase enzyme in order to obtain the RBO with edible quality. Without formation of extra FFAs during storage of the treated RBO, it was concluded that lipase enzyme, and also stabilization of this edible oil by subcritical water was an irreversible process. This stabilized edible oil can be used even after long time storage.

3. 2. 2. Comparison between subcritical water and conventional methods in the stabilization (treatment) of RBO

Direct oil extraction methods using organic solvents are conventional techniques. Stabilization and extraction efficiency of the conventional methods were studied and compared with subcritical water technique. Two common solvents, hexane and ethanol are used in the conventional techniques *[Japan Oil Plant Association (JOPA), online; Johnson and Lusas, 1983]* were utilized in this study.

Figure 5 shows time courses of total FFAs concentration in the treated RBOs by using

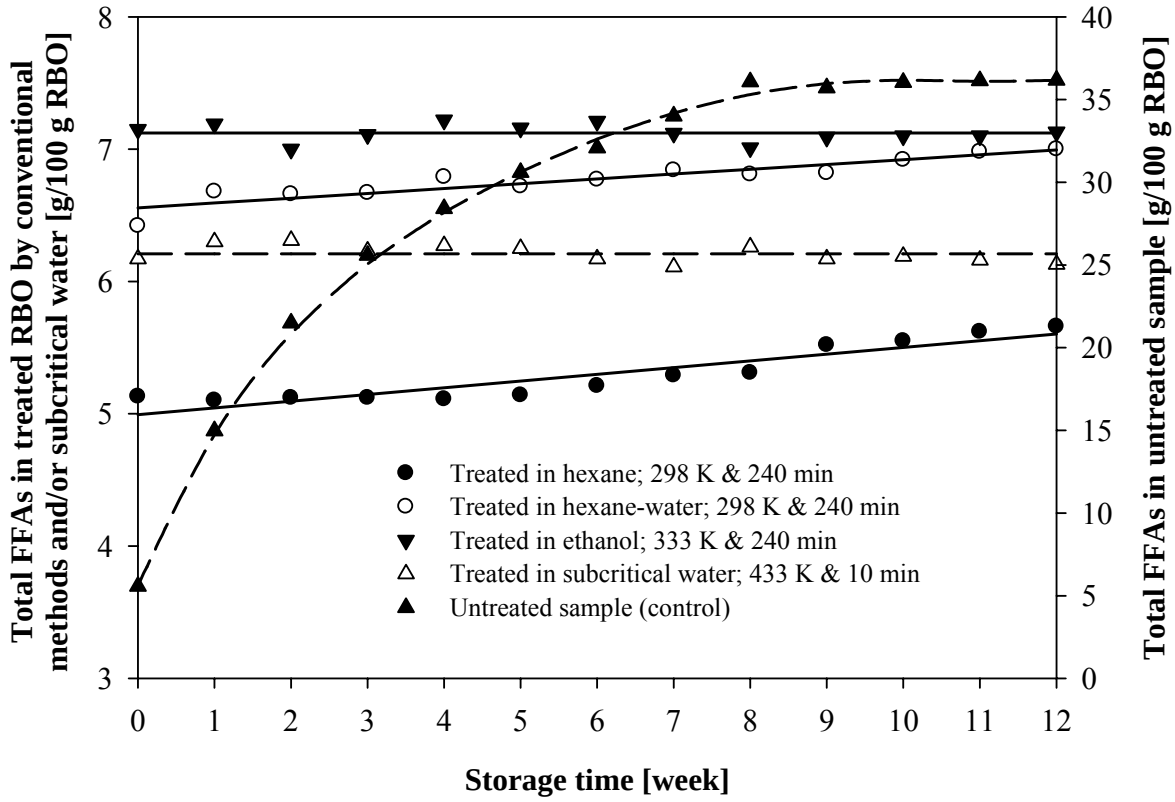


Figure 5. Time course of total FFAs concentration in control, and the treated oils extracted by conventional methods and subcritical water.

the organic solvents and subcritical water. The total concentration of FFAs significantly increased with time (from 5.6% to 36.0%) in control. In the extraction using hexane at 298 K, total FFAs concentration in the oil gradually increased from 5.0% to 5.6% after 12 weeks. Using hexane-water (67:33) mixture for the extraction as the same condition as above, total FFAs concentration increased as well; its level increased steadily from 6.5% to 7.0%. FFAs level in the oil treated by the hexane-water was higher than that of treated by pure hexane. This is because lipase enzyme activity in hexane-water solvent is higher than in pure hexane; lipase enzyme needs water to be active *[Gubicza et al., 2000]*. Increase in the total FFAs reveals that these two methods cannot completely inactivate the lipase in RBO. The conventional experiments by hexane were performed at room temperature (298 K); in ethanol, it was possible to increase the temperature up to 333 K; in this case total FFAs concentration was almost constant with time (see Figure 5). This may be caused by the higher temperature than hexane method. The total FFAs profile of the control and treated sample by subcritical water (433 K & 10 min) are also shown in Figure 5; Total FFAs concentration in this treated sample by subcritical water was constant (6.2%) and no increase was observed during oil storage period.

It is worth to note that subcritical water is performed by utilization of water as treatment medium which is green, abundant, cheap, and safe while in the conventional methods, organic solvents are used which are usually toxic, expensive, and flammable. Subcritical water could completely stabilize oil in a very short reaction time compared to the conventional methods. In addition, it was proved when using subcritical water treatment was appropriate for inactivation of lipase enzyme.

3. 3. Simultaneous RBO extraction and stabilization

The above results show that subcritical water could extract RBO and inactivate lipase enzyme simultaneously. Extraction efficiency was compared to those of the conventional direct solid-solvent and soxhlet extraction methods; results are summarized in Table 1.

The highest extraction yield was achieved by utilization of soxhlet extraction using hexane. Conventional direct contacting of rice bran with hexane, gave relatively lower yield of RBO extraction than the soxhlet extraction. Direct extraction of RBO by hexane-water

Table 1. RBO yield by utilization of various extraction methods.

Extraction method	Solvent	Temperature [K]	Extraction time [min]	RBO yield [mg/g dry matter]
Soxhlet	Hexane	345	240	266
Conventional (direct)	Hexane	298	240	218
Conventional (direct)	Hexane-water	298	240	141
Conventional (direct)	Ethanol	333	240	200
Subcritical water	Pure water	393	10	141
Subcritical water	Pure water	433	10	174
Subcritical water	Pure water	473	10	196
Subcritical water	Pure water	473	20	209
Subcritical water	Pure water	513	10	249

mixture had the lowest yield among the conventional methods; it is obvious that water (in hexane-water mixture) prevented hexane efficiently contacted with bran which caused RBO yield became less than that of extraction by pure hexane.

Extraction yield of RBO by subcritical water was enhanced from 141 to 249 (mg/g dry matter) by raising the temperature from 393 to 513 K. Reaction time was another important factor which influenced the yield of RBO; the longer the reaction time was, the more RBO was extracted (see Table 1). It was also observed RBO color was a function of extraction time and subcritical water temperature. It became darker by increasing the extraction time and temperature, especially at higher than 473 K (see Figure 6). This phenomenon might be due to the formation of oxidative materials including polymers and other oil-soluble products undergoing the Millard reactions *[Liu and Mamidipally, 2005]* and pyrolysis reaction. These impurities can be removed by a series of processes, called oil refining. The enhancement of RBO (and also other extractive materials) extraction yield by subcritical water temperature is due to the variation of water dielectric constant with temperature *[Yoshida and Tavakoli, 2004; Herrero et al., 2006]*. A decrease in the dielectric constant which as it approaches the critical temperature can solubilize organic compounds *[Yoshida et al., 1999; King, 2000; Galkin and Lunin, 2005]*.

These results demonstrate that extraction by soxhlet (at low temperature of 345 K) had always higher yield compare to subcritical water which was performed at relatively higher temperatures of 393 to 513 K. However, from temperature point of view, at relatively lower subcritical water temperatures RBO yield was less than the conventional direct extraction methods (at 298 or 333 K) while RBO yield at above moderately high temperatures of subcritical water exceeded the yield of the conventional direct extraction methods (see Table 1). In contrast, direct extraction and soxhlet extraction need long time and they are cost consuming methods while subcritical water could effectively extract RBO in a very short reaction time. Considering the reaction time and extraction efficiency relative to different extraction methods, it can be concluded that subcritical water is competitive and alternative method with respect to the other extraction methods.

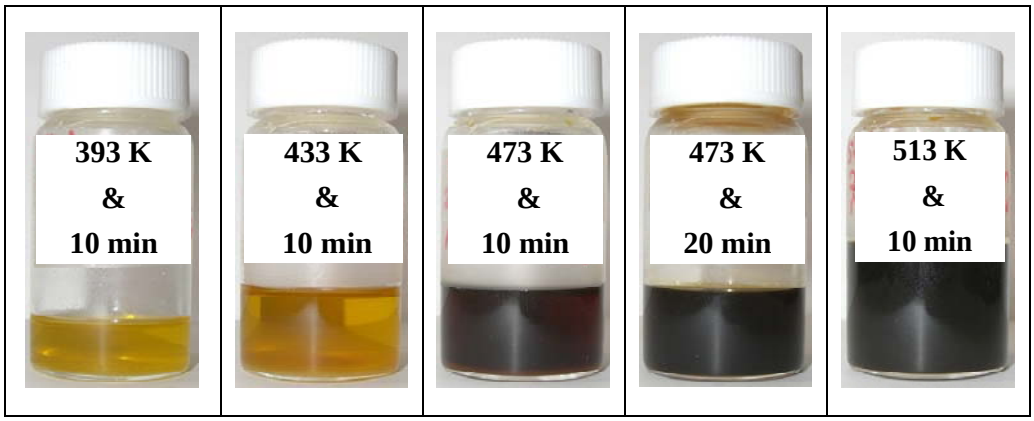


Figure 6. Typical photographs of the extracted oils by subcritical water.

3. 4. Kinetics of free fatty acids formation in the stored rice bran

Oil stabilization and extraction must be performed as soon as possible after rice milling process to obtain high quality edible oil. This is not feasible in most developing countries because of the lack of integrated facilities *[Randall et al., 1985]*. Due to these and other problems, there is usually a time lag between rice bran production, oil stabilization, and extraction which facilitates the deterioration of RBO. In this way, FFAs formation plays an important role in the quality of RBO; therefore, determination of RBO rancidity rate by an accurate kinetic model is very useful tool for food industries.

The kinetic of FFAs formation was only investigated for control (untreated) sample. Due to not variation in the concentration of total FFAs, treated samples were not studied kinetically.

For enzyme-catalyzed reaction like formation of FFAs in RBO (see equation (3)), reaction kinetic was described by the following model:

$$\text{Triglycerides} \xrightarrow[k \text{ (includes } k_1 \text{ and } k_2)]{\text{Lipase enzyme}} \text{FFAs} \qquad (3)$$

$$r_{FFA} = \frac{dC}{dt} = \frac{k_1 C}{k_2 C - 1} \qquad (4)$$

where C is total FFAs concentration [g/100 g RBO], t is time [week], and k_1 [$week^{-1}$] and k_2 [100 g RBO/g FFAs] are kinetic parameters of FFAs formation. This equation is "shifting order" kinetic model which indicates reaction order in the stored rice bran at high concentration and low concentration of FFAs was found to be different *[Levenspiel, 1999]* (see Figure 1); k_1 and k_1/k_2 are reaction rate constants for the first-order and zero-order kinetics, respectively. Integration of equation (4) under the initial condition of $C = C_0$ at $t = 0$ gives the following equation:

$$\frac{\ln(A+1)}{t} = \frac{k_2 C_0 A}{t} - k_1 \qquad (5)$$

where A is defined as:

$$A = \frac{C - C_0}{C_0} \qquad (6)$$

By plotting of $(\ln(A+1))t^{-1}$ versus At^{-1}, kinetic parameters (k_1 and k_2) can be

determined for the FFAs formation reaction in the stored rice bran at 298 K, as demonstrated in Figure 7.

The solid line in Figure 7 is the regression line obtained by kinetics data. The slope determined the k_2 value (k_2 = 0.1126 [100 g RBO/g FFAs]), and k_1 value was obtained by intercept (k_1 = 0.1831 [$week^{-1}$]). From this kinetic model it was concluded that at high concentration of FFAs (or $k_2C >> 1$) the reaction is zero order with rate constant of k_1/k_2. Considering the obtained kinetic parameters, FFAs formation in untreated RBO can be calculated. Figure 8 shows the experimental and theoretical total FFAs concentration versus storage time. Figures 7 and 8 reveal that there is a good agreement between the experimental and kinetics data, and that the kinetic model explained fairly well the formation of FFAs. These figures also demonstrate that there are some deviations between the experimental results and calculated line especially at the first and last weeks of rice bran storage (the highest and lowest values of At^{-1}).

Finally, it has been shown that the simplified kinetics model can successfully describe and predict the FFAs formation in the course of rice bran storage which can be used by food industries to prepare an accurate manufacturing plan for processing the obtained bran into edible oil prior to its deterioration.

4. Conclusions

Subcritical water as an environmentally friendly technique was applied to provide a green, simple and non-flammable medium for lipase enzyme inactivation and consequently stabilization of RBO, along with extraction of RBO from rice bran.

Due to the enzyme activity, it was realized that total FFAs concentration (as criterion of oil quality) in untreated sample increased from 5.6% to 36.0% during 12 weeks. On the other words, in less than one week from the date of rice milling, the RBO deteriorated and became inedible. This enzymatic problem effectively overcame by application of subcritical water treatment. Lipase enzyme was denatured and as a result, no increase was observed in total FFAs concentration in the treated RBO by time.

It was also understood that the conventional solvent extraction methods, such as using hexane at ambient temperature, could not completely stabilize RBO and results showed that

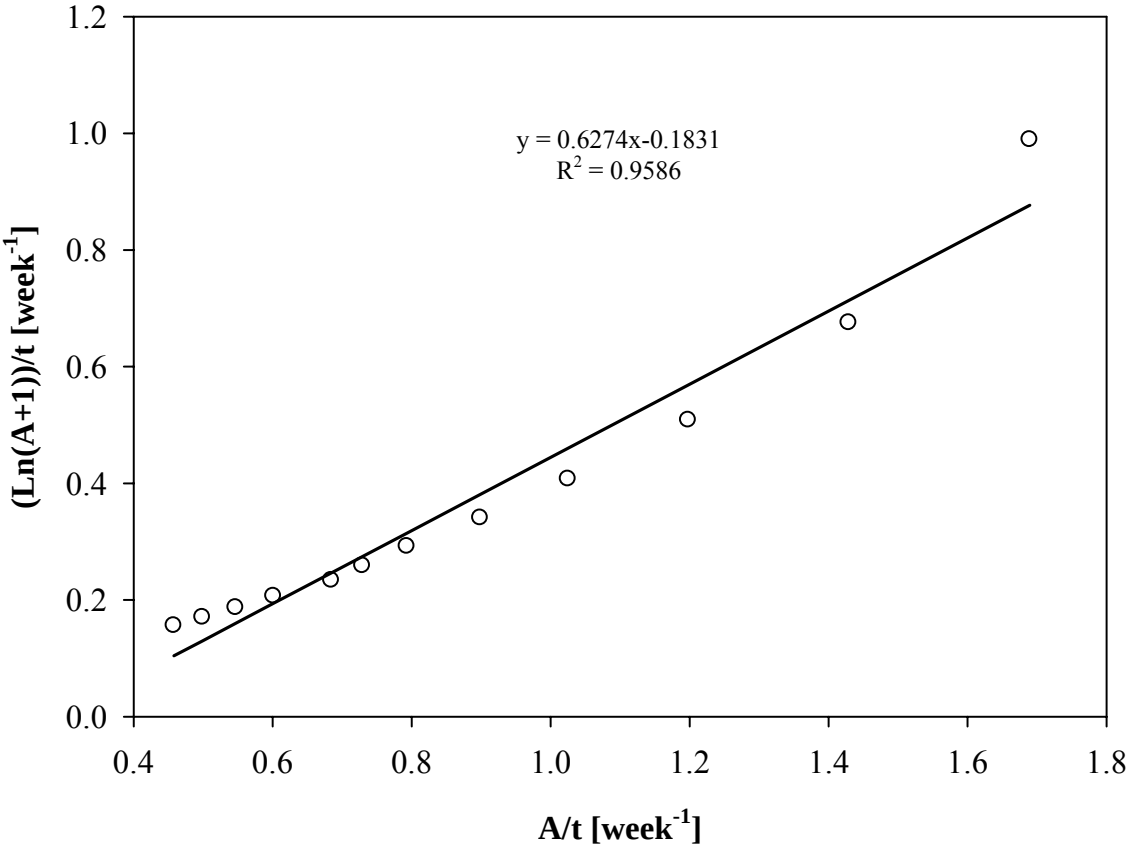


Figure 7. Plot of $(\ln(A+1))t^{-1}$ versus At^{-1} at the storage temperature (298 K) of control.

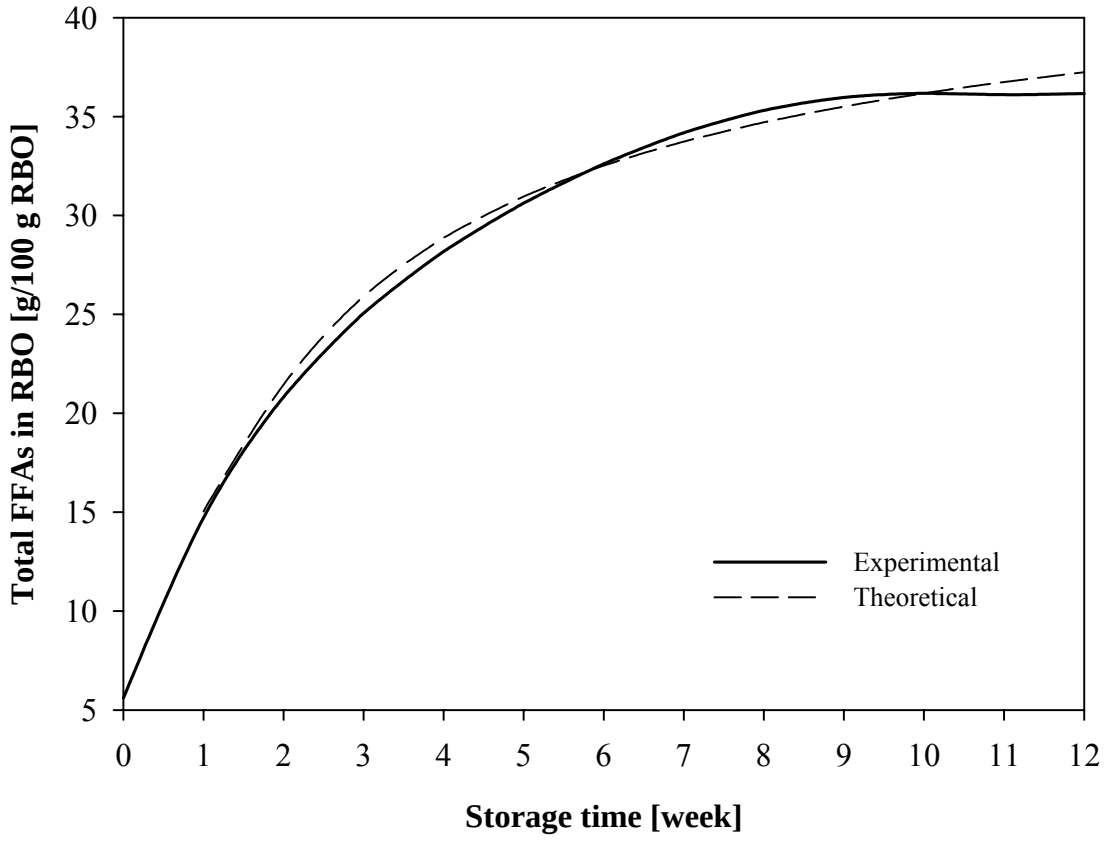


Figure 8. Comparison of experimental data and kinetic model of FFAs formation in untreated RBO at the storage temperature of 298 K.

total FFAs concentration increased in the course of RBO storage.

This study revealed that subcritical water could also be used as an effective green medium for RBO extraction in shorter reaction times. Temperature also influenced the RBO extraction. The higher temperature, the greater was the amount of the extracted oil. Maximum extracted RBO was 249 mg/g dry matter which is approximately 94% of total oil of rice bran.

Acid value, color and appearance of the extracted and stabilized oils by subcritical water can be modified by the refining processes.

The described kinetic model of FFAs formation can be used for determination of FFAs concentration in rice bran which can be utilized in food industries to process the oil from bran prior to its deterioration.

Nomenclature

AV	Acid value of rice bran oil
ES	Ethanol-soluble
FFAs	Free fatty acids
HS	Hexane-soluble
K	Kelvin
k_1	Reaction rate constant
k_2	Reaction rate constant
RBO	Rice bran oil
WS	Water-soluble

References

Ames, S. R., Licata, S. B., Colorimetric and potentiometric determination of acid numbers of vegetable and marine oils, *Journal of the American Oil Chemists' Society,* June, 203-206, (1948).

Bhosle, B. M., Subramanian, R., New approaches in deacidification of edible oils: a review, *Journal of Food Engineering,* 69, 481-494, (2005).

Enochian, R. V., Saunders, R. M., Schultz, W. G., Beagle, E. C., Crowley, P. R., *Stabilization of rice bran with extruder cookers and recovery of edible oil: a preliminary analysis of operational and financial feasibility,* Marketing Research Report, USDA No. 1120, (1981).

Galkin, A. A., Lunin, V. V., Subcritical and supercritical water: a universal medium for chemical reactions, *Russian Chemical Reviews (English Translation),* 74, 21-35, (2005).

Goffman, F. D., Pinson, S., Bergman, C., Genetic diversity for lipid content and fatty acid profile in rice bran, *Journal of the American Oil Chemists' Society,* 80, 485-490, (2003).

Gubicza, L., Szekely, K., Ulbert, O., Belafi-Bako, K., Enhancement of the thermostability of *candida cylindracea lipase* by medium engineering, *Chemical Papers,* 54, 351-354, (2000).

Herrero, M., Cifuentes, A., Ibanez, E., Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae: a review, *Food Chemistry,* 98, 136-148, (2006).

Hoffpauir, C. L., Petty, D. H., Guther, J. D., Germination and free fatty acid in individual cotton seeds, *Science,* October, 344-345, (1947).

Hu, W., Wells, J. H., Shin, T. S., Godber, J. S., Comparison of isopropanol and hexane for extraction of vitamin E and oryzanols from stabilized rice bran, *Journal of the American Oil Chemists' Society,* 73, 1653-1656, (1996).

Japan Oil Plant Association (JOPA), *Extraction solvent (in Japanese)*, Available from www.oil.or.jp/yougo/index.html.

Johnson, L. A., Lusas, E. W., Comparison of alternative solvents for oils extraction, *Journal of the American Oil Chemists' Society,* 60, 229-242, (1983).

Ju, Y. H., Vali, S. R., Rice bran oil as a potential resource for biodiesel: a review, *Journal of Scientific & Industrial Research,* 64, 866-882, (2005).

King, J. W., Advances in critical fluid technology for food processing: review, *Food Science and Technology Today,* 14, 186-191, (2000).

King, J. W., Dunford, N. T., Phytosterol-enriched triglyceride fractions from vegetable oil deodorizer distillates utilizing supercritical fluid fractionation technology, *Separation Science and Technology,* 37, 451-462, (2002).

Kruse, A., Dinjus, E., Hot compressed water as reaction medium and reactant properties and synthesis reactions, *The Journal of Supercritical Fluids,* 39, 362-380, (2007).

Kubo, R., Nagakura, S., Inokuchi, H., Ezawa, H., *Iwanami Rikagaku Jiten [Iwanami Dictionary of Physics and Chemistry] (in Japanese),* Fourth edition, Iwanami Shoten Publishers, Japan, (1987).

Kuk, M. S., Dowd, M. K., Supercritical CO_2 extraction of rice bran, *Journal of the American Oil Chemists' Society,* 75, 623-628, (1998).

Levenspiel, O., *Chemical Reaction Engineering,* third edition, John Wiley & Sons, the USA, (1999).

Liu, S. X., Mamidipally, P. K., Quality comparison of rice bran oil extracted with d-limonene and hexane, *Cereal Chemistry,* 82, 209-215, (2005).

Luh, B. S., *Rice: Production and Utilization,* AVI Publishing Company, Inc., the USA, (1980).

Mamidipally, P. K., Liu, S. X., First approach on rice bran oil extraction using limonene, *European Journal of Lipid Science and Technology,* 106, 122-125, (2004).

McCaskill, D. R., Zhang, F., Use of rice bran oil in foods, *Food Technology,* 53, 50-53, (1999).

Pourali, O., Salak Asghari, F., Yoshida, H., Sub-critical water treatment of rice bran to produce valuable materials, *Food Chemistry,* 115, 1-7, (2009).

Prabhakar, J. V., Venkatesh, K. V. L., A simple chemical method for stabilization of rice bran, *Journal of the American Oil Chemists' Society,* 63, 644-646, (1986).

Randall, J. M., Sayre, R. N., Schultz, W. G., Fong, R. Y., Mossman, A. P., Tribelhorn, R. E.,

Saunders, R. M., Rice bran stabilization by extrusion cooking for extraction of edible oil, *Journal of Food Science,* 50, 361-364, (1985).

Rao Lakkakula, N., Lima, M., Walker, T., Rice bran stabilization and rice bran oil extraction using ohmic heating, *Bioresource Technology,* 92, 157-161, (2004).

Renuka Devi R., Arumughan C., Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment, *Bioresource Technology,* 98, 3037-3043, (2007).

Salak Asghari, F., Yoshida, H., Acid-catalyzed production of 5-hydroxymethyl furfural from *D*-fructose in subcritical water, *Industrial & Engineering Chemistry Research,* 45, 2163-2173, (2006).

Salak Asghari, F., Yoshida, H., Kinetics of the decomposition of fructose catalyzed by hydrochloric acid in subcritical water: formation of 5-hydroxymethylfurfural, levulinic, and formic acids, *Industrial & Engineering Chemistry Research,* 46, 7703-7710, (2007).

Saunders, R. M., Rice bran: composition and potential food uses, *Food Reviews International,* 1, 465-495, (1985-86).

Sereewatthanawut, I., Prapintip, S., Watchiraruji, K., Goto, M., Sasaki, M., Shotipruk, A., Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis, *Bioresource Technology,* 99, 555–561, (2008).

Shen, Z., Palmer, M. V., Ting, S. S. T., Fairclough, R. J., Pilot scale extraction of rice bran oil with dense carbon dioxide, *Journal of Agricultural and Food Chemistry,* 44, 3033-3039, (1996).

Tanaka, T., Hoshina, M., Tanabe, S., Sakai, K., Ohtsubo, S., Taniguchi, M., Production of *D*-lactic acid from defatted rice bran by simultaneous saccharification and fermentation, *Bioresource Technology,* 97, 211-217, (2006).

Tao, J., Rao, R., Liuzzo, J., Microwave heating for rice bran stabilization, *Journal of Microwave Power and Electromagnetic Energy,* 28, 156-164, (1993).

Tavakoli, O., Yoshida, H., Conversion of scallop viscera wastes to valuable compounds using sub-critical water, *Green Chemistry,* 8, 100-106, (2006).

Tavakoli, O., Yoshida, H., Effective recovery of harmful metal ions from squid wastes using subcritical and supercritical water treatments, *Environmental Science & Technology,* 39, 2357-2363, (2005).

Temelli, F., Perspectives on supercritical fluid processing of fats and oils: review, *The Journal of Supercritical Fluids,* 47, 583-590, (2009).

Westphal, S., Gekeler, G. H., Dierkes, J., Wieland, H., Luley, C., A free fatty acid tolerance test identifies patients with coronary artery disease among individuals with a low conventional coronary risk profile, *Heart Vessels,* 16, 79-85, (2002).

Wiboonsirikul, J., Hata, S., Tsuno, T., Kimura, Y., Adachi, S., Production of functional substances from black rice bran by its treatment in subcritical water, *LWT-Food Science and Technology,* 40, 1732-1740, (2007a).

Wiboonsirikul, J., Kimura, Y., Kadota, M., Morita, H., Tsuno, T., Adachi, S., Properties of extracts from defatted rice bran by its subcritical water treatment, *Journal of Agricultural and Food Chemistry,* 55, 8759-8765, (2007b).

Xu, Z., Godber, J. S., Comparison of supercritical fluid and solvent extraction methods in extracting γ-oryzanol from rice bran, *Journal of the American Oil Chemists' Society,* 77, 547-551, (2000).

Yoshida, H., Tavakoli, O., Sub-critical water hydrolysis treatment of squid waste entails and production of organic acid, amino acid, and fatty acids, *Journal of Chemical Engineering of Japan,* 37, 253-260, (2004).

Yoshida, H., Terashima, M., Takahashi, Y., Production of organic acids and amino acids from fish meat by sub-critical water hydrolysis, *Biotechnology Progress,* 15, 1090-1094, (1999).

Yoshida, H., Yoshioka, M., Murayama, Y., Takata, M., Yokoyama, T., Prion decomposition and inactivation by sub-critical water hydrolysis, In: *Proceeding of the International Symposium on Eco Topia Science,* Japan, (2007).

Zappe, R. J., Automated method and test kit for free fatty acids in cooking fats and oils, *US Patent,* 5620897, (1997).

Zullaikah, S., Lai, C. C., Vali, S. R., Ju, Y. H., A two-step acid-catalyzed process for the

production of biodiesel from rice bran oil, *Bioresource Technology,* 96, 1889-1896, (2005).

Chapter 4

Production of Phenolic Compounds from Rice Bran in Subcritical Water Medium

1. Introduction

Rice bran, a brown layer between rice and the outer husk of the paddy *[Lin et al., 2009]*, is well known to be rich in various phenolic compounds *[Iqbal et al., 2005]*. It is a low-cost and abundant biomass; about 50-60 million tons of rice bran is annually produced in the world *[Renuka and Arumughan, 2007]*.

On the other hand, there has been a considerable increasing demand for natural phenolic compounds in recent years *[Velioglu et al., 1998]*. Natural phenolic compounds are not uniformly distributed in plants: some of them linked with cell walls, while others exist without any chemical bonds within the plant cell vacuoles *[Naczk and Shahidi, 2004]*. Phenolic compounds are important due to their antioxidant activities. They possess aromatic structure along with hydroxyl substituents which enable them to protect the compounds or human tissues from damages caused by oxygen or free radicals *[Villano et al., 2007]*, and consequently reduce the risk of different diseases, and offer beneficial effects against cancers, cardiovascular disease, diabetes, and Alzheimer's disease *[Zhao and Moghadasian, 2008]*. For instance, ferulic acid (3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid) is one of the major phenolic compounds that owing to its high antioxidant properties, shows large potential applications in food industries as well as in the health and cosmetic markets *[Barberousse et al., 2008]*.

Rice bran as a natural source of phenolic compounds is currently underutilized and a large quantity of rice bran remains unused as agricultural waste or use as animal feed and boiler fuel *[Zullaikah et al., 2005; Parrado et al., 2006]*. As mentioned in Chapter 1, in Japan, nearly 30% of the produced rice bran goes to waste *[Pourali et al., 2009b]*.

So far, numerous attempts have been conducted for recovery and extraction of phenolic compounds from rice bran using conventional techniques. For this purpose, application of organic solvents such as methanol, ethanol, propanol, acetone, ethyl acetate, dimethylformamide and/or their combinations have been reported *[Naczk and Shahidi, 2006]*. For example, Renuka and Arumughan *[2007]* have studied the extraction of phenolic compounds from rice bran by using organic solvents and utilization of soxhlet technique. Chotimarkorn et al. *[2008]* and Iqbal et al. *[2005]* extracted phenolic compounds with methanol from various rice brans by application of direct solvent-solid extraction method.

Taniguchi et al. *[1994]* have patented a method for hydrolyzing rice bran oil waste at 373 K, pH of 10, and reaction time from 8 to 10 hours. The produced ferulic acid was extracted by hexane solvent.

Conventional extraction methods have several drawbacks; e.g. time-consuming, low selectivity, low extraction yield, and consumption of large amount of expensive, explosive, and sometimes toxic organic solvents *[Wang and Weller, 2006]*. Furthermore, phenolic compounds in rice bran are extensively bonded to carbohydrate and lignin in the cell wall, and their solubility in common organic solvent is low, unless rice bran is treated at high temperature and/or under acidic and basic conditions *[Wiboonsirikul et al., 2007b]*. Therefore, utilization of supercritical carbon dioxide and particularly subcritical water methods has been widely reported recently to eliminate or reduce the above drawbacks *[Hasbay Adil et al., 2007]*.

Generally, subcritical water has been utilized in various fields of green engineering and material cycling *[Yoshida et al., 1999; Galkin and Lunin, 2005; Tavakoli and Yoshida, 2005; Herrero et al., 2006; Salak Asghari and Yoshida, 2006; Tavakoli and Yoshida, 2006; Kruse and Dinjus, 2007; Salak Asghari and Yoshida, 2007; Pourali et al., 2009a and b]*. In fact, its applications are due to the easy manipulation of dielectric constant, and higher concentration of hydrogen and hydroxide ions with temperature. For instance, water dielectric constant decreases from 80 (at room temperature) to 27 (at 523 K) almost equaling to that of ethanol at ambient temperature *[Luque de Castro et al., 1999]*. The increasing of hydrogen and hydroxide ions production of subcritical water *[Hata et al., 2008]* along with the decreasing of its dielectric constant, make it very suitable medium and technique for extraction and hydrolysis of natural compounds.

However, academic and application reports on subcritical water for production of valuable materials from rice bran have been limited. Wiboonsirikul et al. *[2007b and 2008]* produced phenolic compounds from defatted rice bran using subcritical water at 323 to 523 K and 293 to 533 K for 5 min, and also at 473 and 533 K for 5 to 120 min; they investigated total phenolic content (TPC) yield and antioxidant activity of the obtained solution. In another report *[Hata et al., 2008]* antioxidant activity and total soluble sugars yield were evaluated after subcritical water treatment of the defatted rice bran at the limited temperature range of 453 to 553 K for 5 min.

To the best of our knowledge, there is no comprehensive report on the study of rice bran hydrolysis into phenolic compounds over the whole temperature range of subcritical water. The objective of this chapter is to investigate the possibility of phenolic compounds production by decomposition of rice bran under subcritical water conditions as green and environmentally friendly treatment technique. The influences of whole subcritical water temperature and reaction time as main parameters are studied in detail. Meanwhile, several products after subcritical water treatment have been investigated.

2. Materials and methods

2. 1. Materials

Japonica-type rice (*Oryza sativa*) was used in this study. Gallic acid (3, 4, 5-trihydroxybenzoic acid) was purchased from Tokyo Chemical Industry Co. Ltd. (Japan). Sodium bicarbonate (sodium hydrogen carbonate) and phenol (hydroxybenzene) were obtained from Nacalai Tesque, Inc. (Japan). Folin-ciocalteu phenol reagent, gentisic acid (2,5-dihydroxybenzoic acid), p-coumaric acid (3-(4-hydroxyphenyl)-2-propenoic acid), sinapic acid (3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid), syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid), vanillic acid (4-hydroxy-3-methoxybenzoic acid), and vitamin C (2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol) were obtained from Sigma-Aldrich, Inc. (USA). All other reagents and solvents were purchased from Wako Pure Chemical Industries, Ltd. (Japan).

2. 2. Procedure

The rice sample containing bran was milled by a milling machine (Satake SKM-5B, Satake Corporation, Japan). The obtained bran was sieved with a 590 μm-mesh sieve, and then the sieved bran was immediately treated by subcritical water.

Defatted rice bran was obtained by soxhlation of milled rice bran. It was placed into soxhlet thimble, and extraction of rice bran oil was conducted by hexane at the reaction time of 240 min.

A batch reactor used for subcritical water treatment was a stainless steel tube (SUS316, i.d. 16.5 mm × 150.4 mm) with a Swagelok fitting (ready-made, from Swagelok). In a typical experiment, an accurately weighed amount of rice bran and/or defatted rice bran (about 3.0 g) and about 18.0 cm^3 of distilled water were charged into the reactor. Argon gas was used to force air out of the reactor before the reaction, and it was capped tightly. The reactor was immersed in a preheated oil bath (Thomas Kagaku Co. Ltd., Celsius M type) with temperatures ranging from 373 to 453 K for 10 min or in a preheated salt bath (Thomas Kagaku Co. Ltd., Celsius 600H) in the temperature range 453 to 633 K for 10 min, and at 493 K for 2 to 30 min. The reactor was then removed from the thermal bath and quickly quenched by soaking in a cold water bath at room temperature. The reaction pressure was estimated from a steam table *[Yoshida et al., 1999]*.

After subcritical water treatment, reactor contents were transferred to a 50 cm^3 test tube, taking particular care to prevent loss of any of the liquid and/or remained solid. Figures 1 and 2 show the photographs of rice bran after subcritical water treatment at 373 to 633 K for 10 min, and at 493 K for 2 to 30 min, respectively.

The contents were isolated and classified into three phases: aqueous solution, ethanolic solution, and remained solid. Phase isolation procedure was as follows: each tube was centrifuged at 1500 g for 10 min, and then aqueous solution and remained solid were separated with taking out and transferring of supernatant (aqueous solution) to a volumetric flask by Pasteur pipette. The supernatant was made up to the final volume of 20 cm^3 with mili-Q water, and transferred to the new test tube, and then its pH, conductivity, and total soluble sugars were measured according to section 2. 3. Then 9 cm^3 of ethanol (95%) was added to the above remained solid to dissolve the obtained phenolic compounds which are insoluble in water at room temperature *[Naczk and Shahidi, 2004; Buranov and Mazza, 2009]*. It was shaken for 1 min, and then centrifuged at 1500 g for 5 min. The supernatant (ethanol soluble phase) was isolated by Pasteur pipette and added to the aqueous solution (this mixture hereafter called ethanolic solution). Mixing of this 9 cm^3 of ethanol and the aqueous solution allowed phenolic compounds to be soluble even in higher amounts at room temperature while wax, hemicelluloses and other undesired materials were precipitated *[Buranov and Mazza, 2009]*. This procedure was repeated three times to extract ethanol soluble compounds completely. The precipitate was separated from the ethanolic solution by centrifuging at

Control	373 K	393 K	413 K	433 K
				
453 K	**473 K**	**493 K**	**513 K**	**533 K**
				
553 K	**573 K**	**593 K**	**613 K**	**633 K**
				

Figure 1. Typical photographs of subcritical water treatment of rice bran as function of temperature at reaction time of 10 min.

Control	2 min	5 min	10 min	15 min	20 min	25 min	30 min
							

Figure 2. Typical photographs of subcritical water treatment of rice bran as function of reaction time at 493 K.

2000 g for 5 min. Supernatant was taken out and made up to the final volume of 50 cm^3 with ethanol (95%), and filtered with a 0.2 μm filter. The filtrated ethanolic solution was analyzed by UV-visible and HPLC according to section 2. 3. Remained solid was placed in an oven at 333 K to dry to constant weight.

2. 3. Analysis

Total soluble sugars of aqueous solution was analyzed by a photometric method *[Hodge and Hofreiter, 1962]*. Briefly, 0.4 cm^3 of aqueous solution or standard was mixed with 0.4 cm^3 aqueous phenol solution (5% w/v), and this mixture was vigorously shaken at ambient temperature for 5 min. Then, 2 cm^3 of sulfuric acid (98%) was added to the mixture. The mixture was vigorously shaken and kept at ambient temperature for 10 min to complete the reaction. Finally, this mixture was shaken again, and its total soluble sugars concentration was evaluated by a UV-visible spectrophotometer at 490 nm. Glucose ((3R,4S,5R,6R)-6-(hydroxymethyl)oxane-2,3,4,5-tetrol) was used as the standard, and total soluble sugars concentration in the obtained results was expressed as "glucose equivalents mg/g dry matter".

The pH of all aqueous solutions was measured using a glass pH electrode attached to a Horiba pH meter F-52 (Horiba Co., Japan). Also the conductivity of the aqueous solution was determined by a glass conductivity electrode attached to a Horiba conductivity meter DS-51 (Horiba Co., Japan).

TPC (total phenolic content) in ethanolic solution was determined using folin-ciocalteu phenol reagent *[Abdul-Hamid et al., 2007]*. Briefly, 1 cm^3 of ethanolic solution or standard was mixed with 1 cm^3 folin-ciocalteu reagent (previously diluted 10-fold with distilled water), and this mixture was vigorously shaken and allowed to stand at ambient temperature for 5 min. Then, 1 cm^3 of sodium hydrogen carbonate solution (60 g/l) was added to the mixture. This mixture was vigorously shaken, covered with aluminum foil, and kept in the dark at ambient temperature for 90 min to complete the reaction. Finally, this mixture was shaken again, and its TPC concentration was evaluated by a UV-visible spectrophotometer (Shimadzu UV-160, Shimadzu Co., Japan) at 725 nm. Ferulic acid was used as the standard, and TPC concentration in the obtained results was expressed as "ferulic acid equivalents mg/g dry matter".

Antioxidant activity of ethanolic solution was assayed according to the modified methods of McCue and Shetty *[2004]* and Wiboonsirikul et al. *[2007b and 2008]*. For this propose, 1 cm^3 of the prepared 1,1-diphenyl-2-picrylhydrazyl (diphenyl-(2,4,6-trinitrophenyl)-iminoazanium) solution (0.5 mM 1,1-diphenyl-2-picrylhydrazyl in 95% ethanol) was added to 3 cm^3 of ethanolic solution or standard, and then was well shaken and covered with aluminum foil, and placed in the dark at ambient temperature for 30 min to complete the reaction. Thereafter, the antioxidant activity was determined by a UV-visible spectrophotometer at 517 nm. Vitamin C was used as the standard, and antioxidant activity was expressed as "vitamin C equivalents mg/g dry matter".

A CSPAK narrow-bore column C18 (2.0 mm × 150 mm) from Chromato Science Co. Ltd. (Japan) in a HPLC using two Varian ProStar210 (Varian Inc., USA) solvent-delivery modules coupled with PDA (photodiode array) detector (Varian PDA 330 Detector, Varian Inc., USA) was used for quantitative analysis of products (in ethanolic solution). PDA collected data between 250 nm and 330 nm and absorbance was monitored at 270 nm. Column temperature was kept at 298 K. Gradient elution program at 0.2 cm^3/min flow rate was used as mentioned in Table 1:

Table 1. Gradient elution program.

Time [min]	% of Mobile phase A *(1.0% Acetic acid solution)*	% of Mobile phase B *(Methanol)*
0	100	0
5	100	0
110	0	100
140	0	100
142	100	0
150	100	0

3. Results and discussion

3. 1. TPC (total phenolic content) yield and antioxidant activity of ethanolic solution

In order to realize the application of subcritical water for production of phenolic compounds from rice bran and/or defatted rice barn, a series of experiments were performed over a temperature range of 373 to 633 K at reaction time of 10 min. Figure 3 shows the effect of reaction temperature on the yield of TPC obtained from rice bran and defatted rice bran. Based on previous reports, there are two possibilities for formation of TPC: from decomposition of bonds between lignin, cellulose, and hemicellulose *[Wiboonsirikul et al., 2007b and 2008]*, and/or production from oil part of the rice bran *[Taniguchi et al., 1994]*.

For rice bran, TPC yield sharply increased from 5 to 42 mg/g dry matter (ferulic acid equivalents) when temperature increased from 423 to 493 K. This increase was attributed to higher bond cleavage rate of lignin/phenolic-carbohydrate complexes of rice bran, and also to the more solubility and consequently extraction of TPC in water with relating lower polarity of subcritical water. Figure 3 also demonstrates that TPC yield remained constant at temperatures higher than 493 K. This may be caused by extracting all TPC from the rice bran in this temperature range.

As mentioned before, a series of experiments were used to evaluate the share of rice bran oil on the TPC production. Therefore, the defatted rice bran was utilized under subcritical water conditions and at the same conditions as rice bran. Results showed that the TPC curve of rice bran and defatted rice bran were extremely similar. Therefore, it concluded that majority of phenolic compounds were produced from decomposition of lignin/phenolics-carbohydrate complex part of rice bran and not from its oil.

Generally, phenolic compounds have antioxidant activity; however, it was probable that besides phenolic compounds, other nonphenolic compounds with antioxidant activity were also produced and/or extracted from rice bran in subcritical water medium. Therefore, antioxidant activity as a criterion of total produced antioxidants was also investigated. Figure 3 shows the activity of the antioxidants in the ethanolic solution versus subcritical water temperature. Results indicate that the shape of this profile is quite similar to the TPC yield profile; hence, it can be concluded that most of the produced antioxidants under subcritical

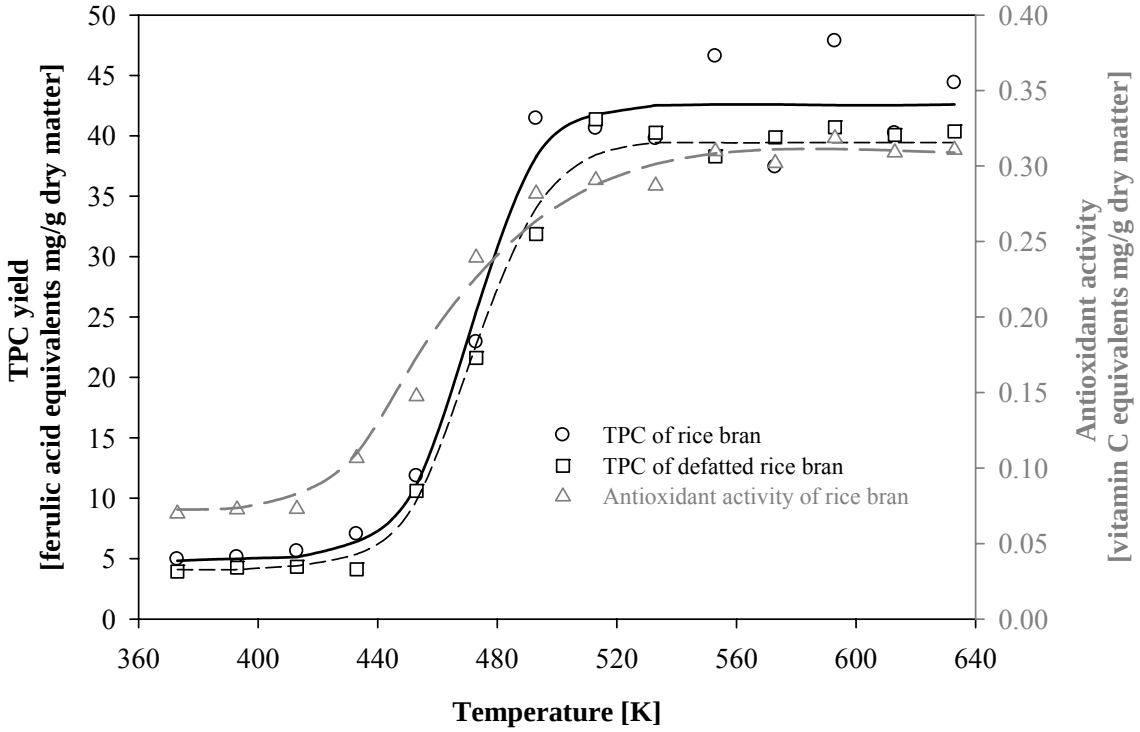


Figure 3. Effect of subcritical water temperature on TPC yield and antioxidant activity at reaction time of 10 min.

water conditions corresponded to the phenolic compounds.

Figure 4 shows the influence of subcritical water reaction time on the yield of TPC and antioxidant activity at 493 K. Obviously the production of phenolic compounds was also a function of reaction time *[Naczk and Shahidi, 2006]*. Both TPC yield and antioxidant activity showed peak at around 15 min, and then decreased somewhat by increasing reaction time. After 15 min, produced TPC may be decomposed by subcritical water. Figure 4 also demonstrates that the shape of antioxidant activity profile is similar to TPC curve which suggested again that antioxidant activity corresponded mainly to the produced phenolic compounds.

Results indicate that subcritical water technique could successfully hydrolyze rice bran to obtain phenolic compounds. It has been reported that phenolic compounds exist in the insoluble-bound forms with lignin and carbohydrates (hemicellulose and cellulose) in rice bran cell wall (see Figure 5) *[Hung and Morita, 2008; Wiboonsirikul et al., 2008]*; lignin, cellulose, and hemicellulose contents in commercial rice bran ranged from 7.7 to 13.1%, 9.6 to 12.8%, and 8.7 to 11.4%, respectively *[Saunders, 1985-86]*. It was understood that the existing bonds (ester and/or ether bonds) between these materials can be effectively hydrolyzed by subcritical water and consequently phenolic compounds, lignin, and carbohydrate are released. In addition, the liberated lignin and carbohydrate parts can be decomposed to the other smaller components by subcritical water hydrolysis reactions (i.e. phenolic compounds and soluble sugars, respectively) *[Sasaki et al., 1998; Otles, 2005; Pourali et al., 2009b]* in subcritical water medium.

3. 2. Identified phenolic compounds in ethanolic solution

Some of phenolic compounds obtained from decomposition of rice bran under subcritical water conditions were identified and qualified for first time in this work. Up to eleven phenolic compounds were identified from decomposition of rice bran: caffeic ((E)-3-(3,4-dihydroxyphenyl)-2-propenoic acid), ferulic, gallic, gentisic, p-coumaric, p-hydroxybenzoic (4-hydroxybenzoic acid), protocatechuic (3,4-dihydroxybenzoic acid), sinapic, syringic, vanillic acids, and vanillin (4-hydroxy-3-methoxybenzaldehyde). The phenolic compounds (except gentisic and sinapic acids) were quantified in this research work.

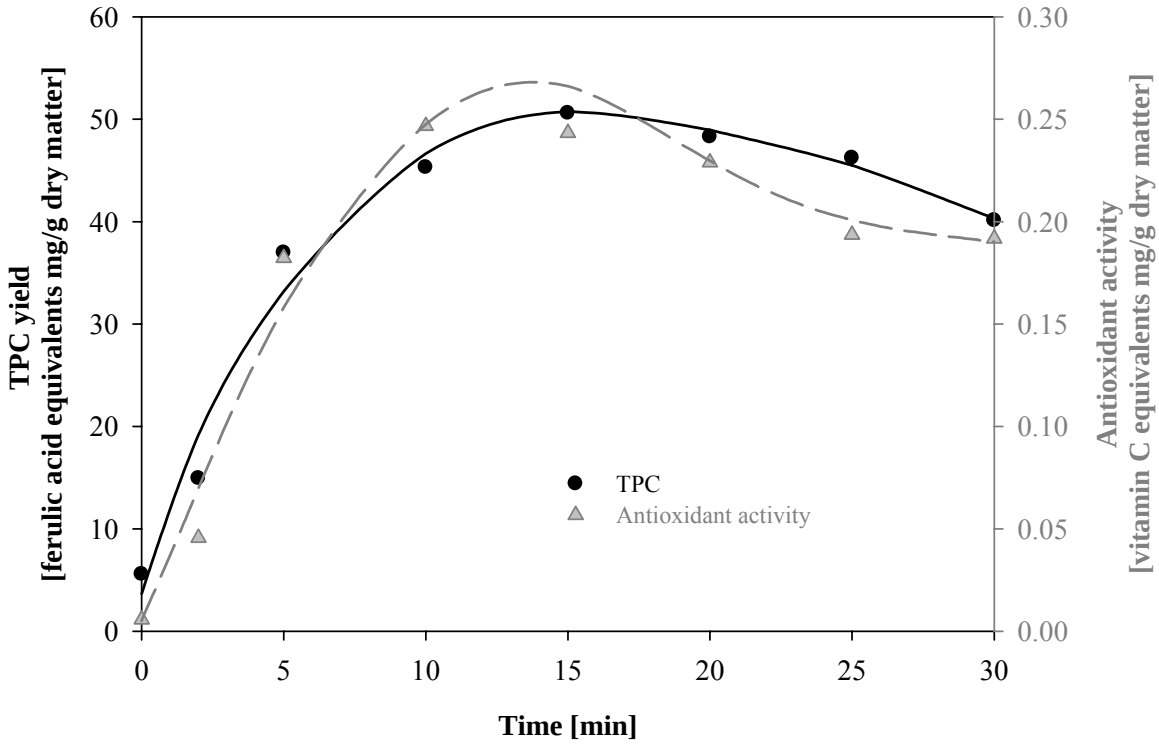


Figure 4. Effect of subcritical water reaction time on TPC yield and antioxidant activity at 493 K.

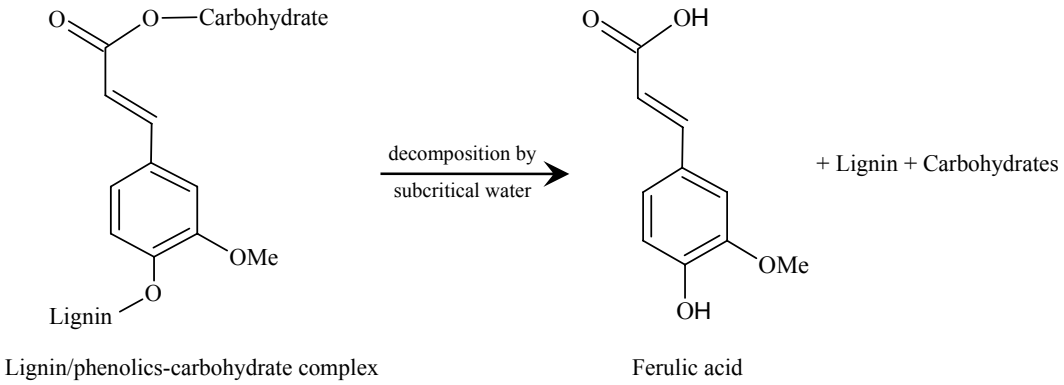


Figure 5. Hydrothermal degradation of a typical lignin/phenolics-carbohydrate complex under subcritical water conditions *[Buranov and Mazza, 2009]*.

Figure 6 shows the effect of reaction temperature on the production yields of individual phenolic compounds at reaction time of 10 min. Protocatechuic and vanillic acids showed the highest yields among the others. They were considered as major products obtained from rice bran. Protocatechuic and vanillic acid showed peaks at temperatures of 503 and 568 K, respectively. Due to the decomposition reactions *[Shopova and Milkova, 1998; Rangsriwong et al., 2009]*, their yield decreased at high temperatures (see Figure 6). Vanillin and p-coumaric acid showed peaks at low temperature region while the other ones generally showed peaks at temperatures higher than 520 K. Mass balance difference between TPC yield and sum of concentration of individual phenolic compounds confirmed the presence of still other unknown phenolic compounds from decomposition of rice bran in subcritical water medium which could not be identified here.

Time dependence of production of identified phenolic compounds at 493 K is shown in Figure 7. In general, most of the peaks appeared in the range of 10 to 20 min. Protocatechuic and vanillic acids showed peaks in 15 and 23 min, respectively. It was understood that longer reaction times as well as higher temperatures had destructive effects on the phenolic compounds yield; further decomposition reactions may occur under subcritical water conditions.

3. 3. Decomposition of carbohydrate part of rice bran

Subcritical water treatment of lignin/phenolics-carbohydrate complexes of rice bran not only produces phenolic compounds but also may hydrolyze carbohydrates and lignin. Carbohydrates can be depolymerized and decomposed into smaller sugars depending on the subcritical water conditions *[Sasaki et al., 1998]*. Figure 8 shows the influence of subcritical water temperature on the yield of total produced sugars in the aqueous phase. It demonstrates that total soluble sugars yield increased with temperature increasing to reach a peak at 463 K, and then decreased drastically to zero at temperatures above 573 K. The results proved that water insoluble carbohydrate part of rice bran could be effectively hydrolyzed into water-soluble oligomers and monomers by subcritical water treatment. In addition, yield decreasing at high temperatures could be interpreted as a sign of the conversion of soluble sugars into other constituents, mainly to HMF (5-hydroxymethyl-2-furfural) and soluble

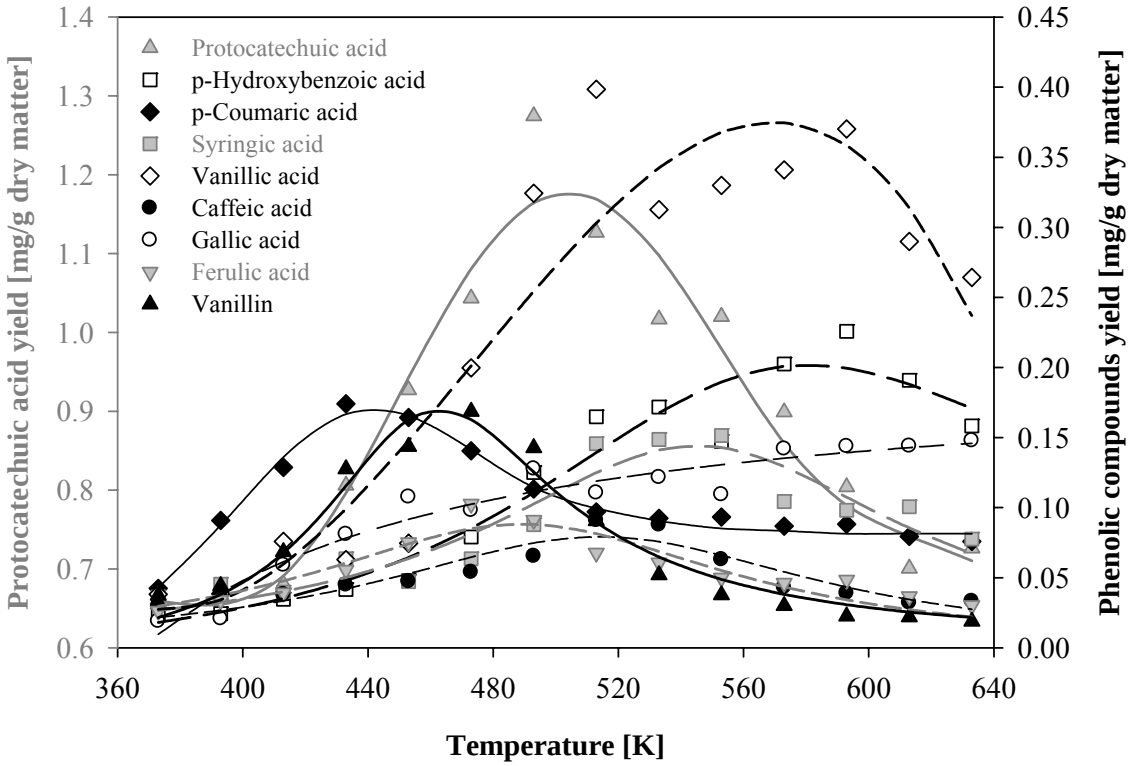


Figure 6. Effect of subcritical water temperature on the production yield of identified phenolic compounds at reaction time of 10 min.

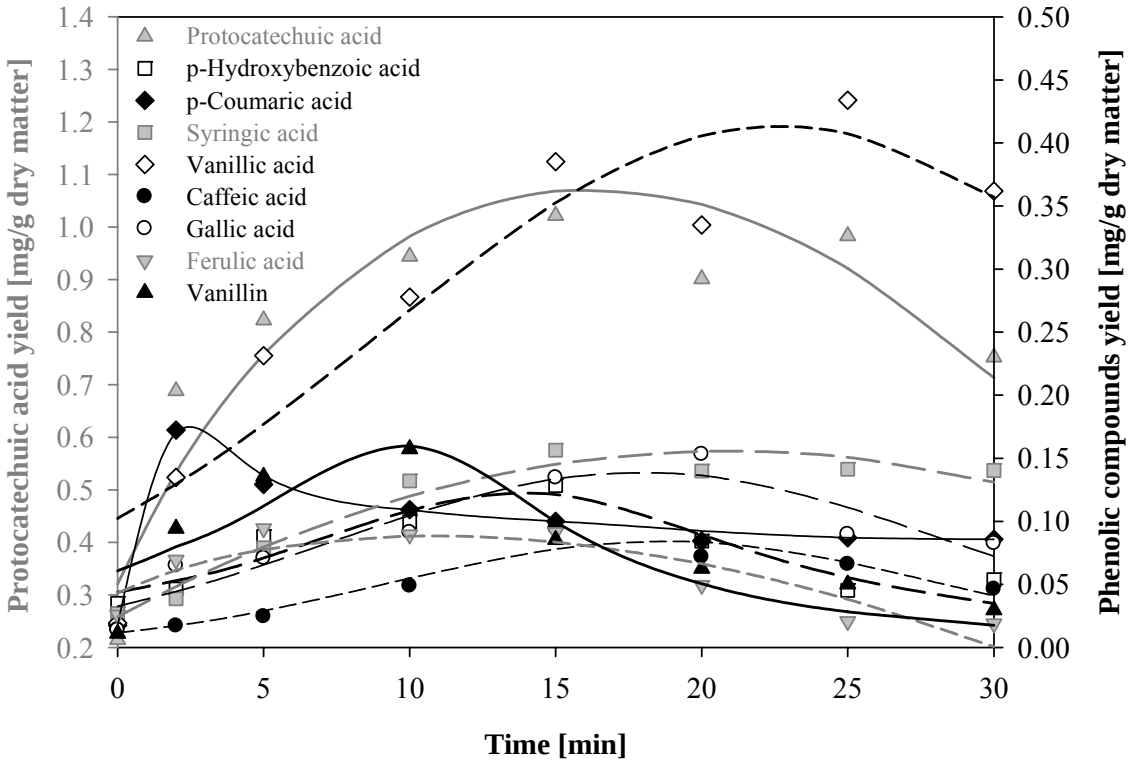


Figure 7. Effect of reaction time on the production yield of identified phenolic compounds at subcritical water temperature of 493 K.

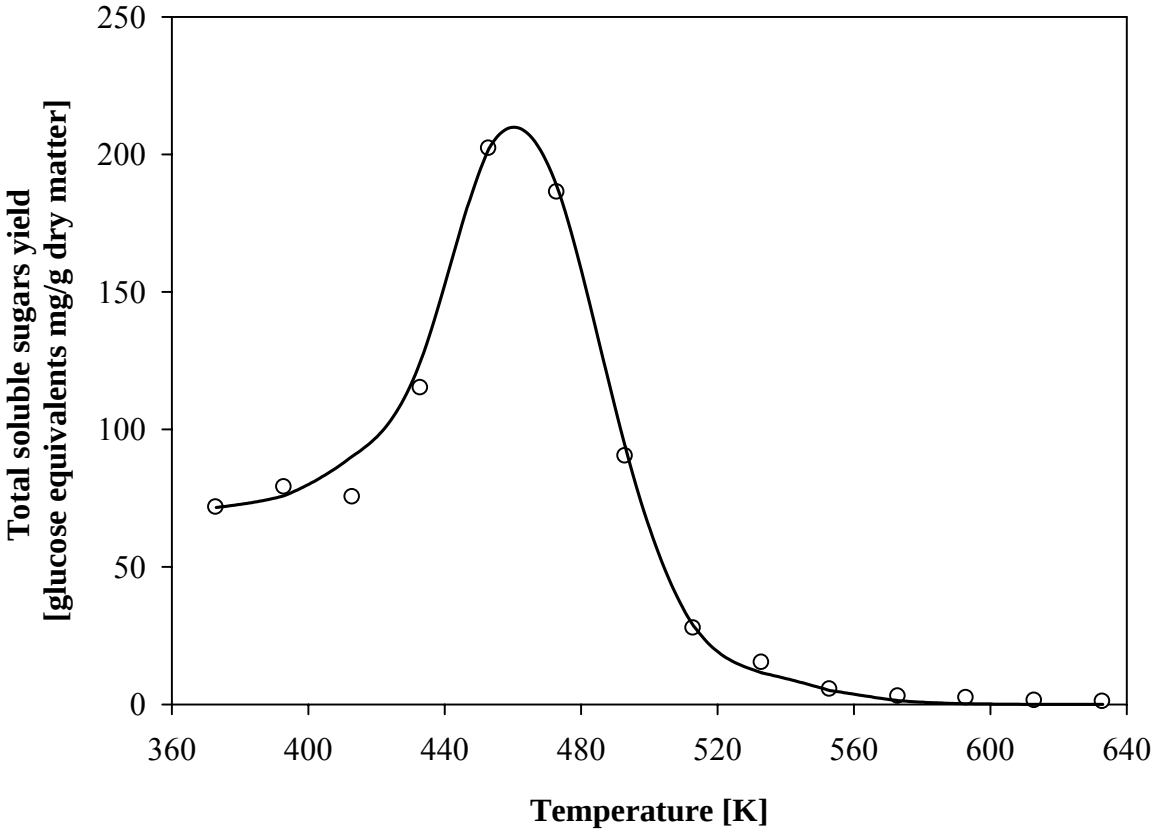


Figure 8. Effect of subcritical water temperature on the yield of total soluble sugars at reaction time of 10 min.

polymers *[Salak Asghari and Yoshida, 2006; Hata et al., 2008]*.

Figure 9 shows the effect of subcritical water reaction time on the yield of total produced sugars at temperature of 493 K. The yield profile showed a peak at around 2 min, and then it decreased steeply with reaction time.

It was observed that the color of aqueous solution after the reaction became darker by increasing the temperature and reaction time (see Figures 1 and 2). This phenomenon might be due to the formation of HMF and soluble polymers from decomposition of the produced soluble sugars (from carbohydrate part of rice bran) in subcritical water medium *[Salak Asghari and Yoshida, 2006]*. It is also attributed to the formation of undesired materials undergoing the Millard browning reaction *[Wiboonsirikul et al., 2007a]*.

3. 4. pH and conductivity of the aqueous solution

Figure 10 shows that pH of aqueous solution measured after subcritical water reaction. It decreased as temperature increased and reached a minimum. The minimum pH was 4.4 at around 493 K and gradually increased with temperature to a constant value about 5.0 at temperatures above 613 K. Obviously, pH decrease indicates that aqueous solution contains acidic materials, such as phenolic compounds and organic acids. As shown in Chapter 2, other compounds with acidic function such as organic acids and amino acids were produced by decomposition of rice bran *[Salak Asghari and Yoshida, 2006; Pourali et al., 2009b]* which change the pH of aqueous solution.

In fact, pH has destructive effect on the existing (ester and/or ether) bonds of lignin/phenolics-carbohydrates complex of biomass *[Bobleter 1994]*; therefore, production of acidic materials and consequently decreasing of pH led to conclusion that autocatalysis may occur during subcritical water treatment of rice bran.

In addition, the observed increase in the pH of the solution at temperatures above 493 K may be attributed to the decomposition of the acidic compounds to the other substances.

Figure 10 also shows the electrical conductivity of aqueous solution, measured after subcritical water reaction, as a function of subcritical water temperature. Electrical conductivity of aqueous solution is mainly a function of ions amount within the solution *[Prikopsky et al., 2007]*. It steadily rose by temperature up to 513 K. This increase may be

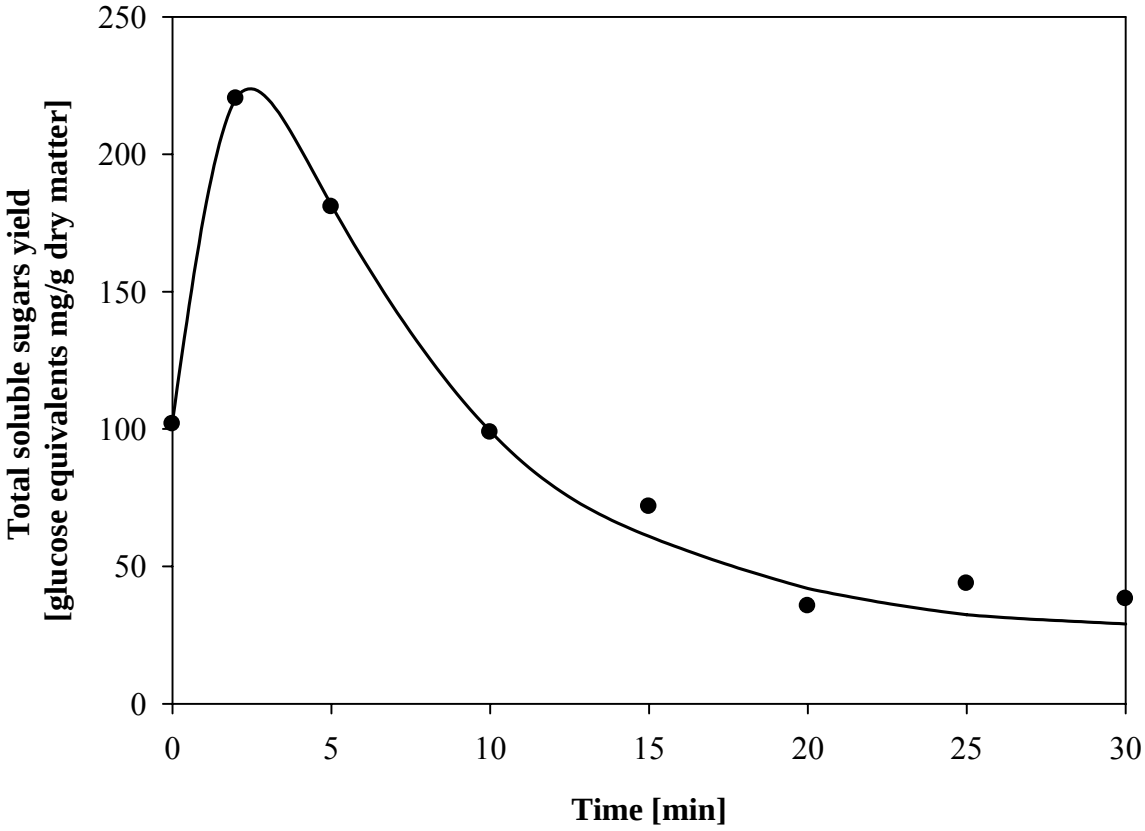


Figure 9. Effect of reaction time on the yield of total soluble sugars at subcritical water temperature of 493 K.

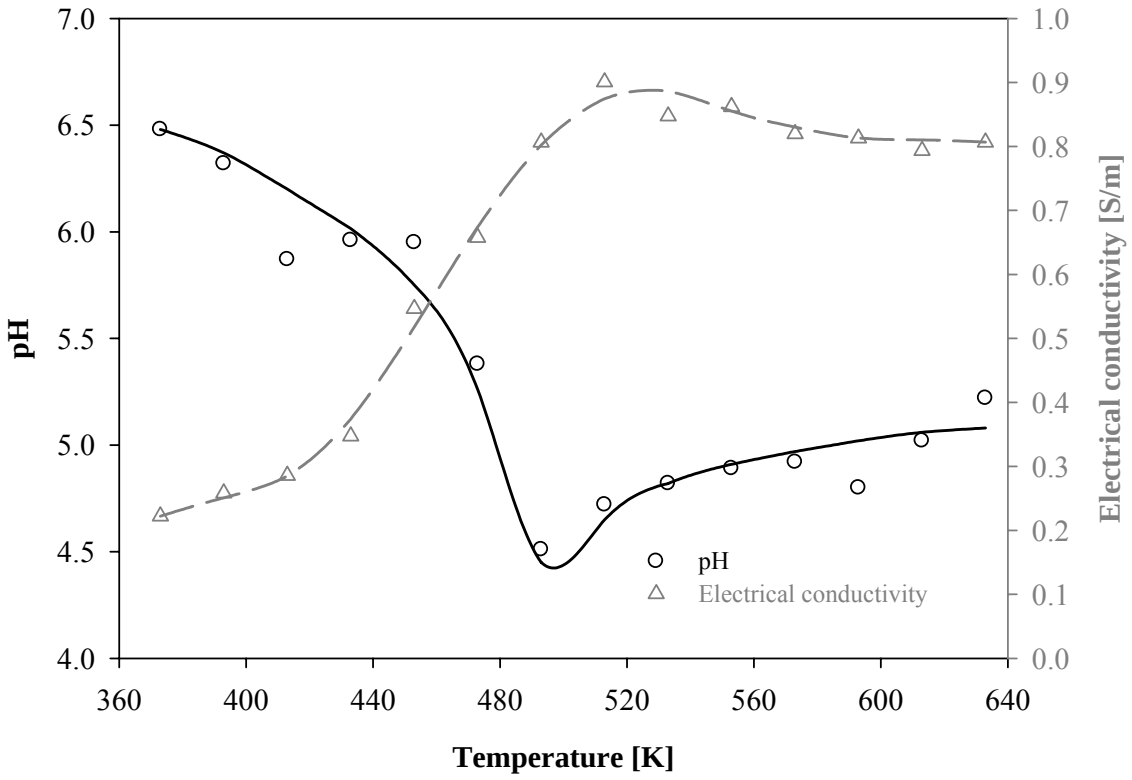


Figure 10. Effect of subcritical water temperature on the pH and electrical conductivity of the aqueous solution at reaction time of 10 min.

attributed to the pH lowering, dissolution of rice bran minerals, and production of other ions and organic acids over the treatment process. Change in the electrical conductivity as well as pH shows the promising results for decomposition of rice bran in water. Figure 10 demonstrates that the conductivity decreased somewhat when temperature increased above 513 K. It was owing to the decomposition of some organic acids to neutral organics.

Figure 11 shows the effect of reaction time on pH and electrical conductivity of aqueous solution measured after subcritical water reaction. The pH of aqueous solution decreased sharply up to 15 min by reaction time and then leveled off. Figure 11 also proved that the electrical conductivity of solution was influenced by reaction time. It continuously rose with reaction time prolonging.

3. 5. Remained solid after treatment of rice bran

The amount of remained solid after subcritical water treatment was also evaluated. This residue mainly consisted of un-reacted rice bran, carbonized rice bran, hydrolyzed but still insoluble parts of rice bran as well as insoluble inorganic compounds. Their amount after treatment in temperature range of 373 to 633 K for 10 min is shown in Figure 12. Amount of remained solid slightly decreased from 373 to 413 K, and then sharply decreased to a minimum of 8% at 633 K. This sharp decrease proved that subcritical water could effectively decompose insoluble macromolecules of rice bran into smaller soluble compounds in a short reaction time. The composition of remained solid was not investigated in this research work.

Figure 13 shows the effect of reaction time on the amount of remained solid at 493 K. It decreased drastically by reaction time increasing and then stayed constant (about 40%) in the reaction times longer than 15 min.

There was a considerable difference between the final minimum amounts of remained solid obtained from temperature and time effect studies (8% and 40%, respectively) which proves clearly that subcritical temperature is more effective than reaction time on the dissolution and decomposition of rice bran (see Figures 12 and 13).

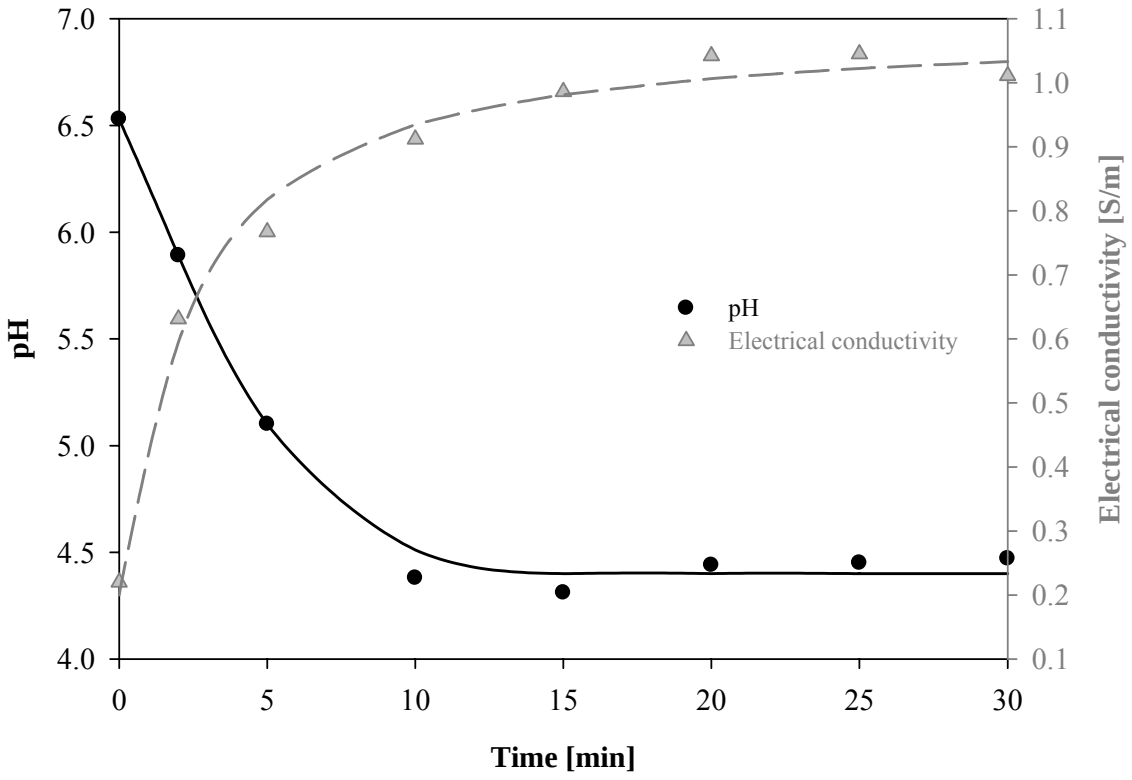


Figure 11. Effect of reaction time on the pH and electrical conductivity of the aqueous solution at subcritical water temperature of 493 K.

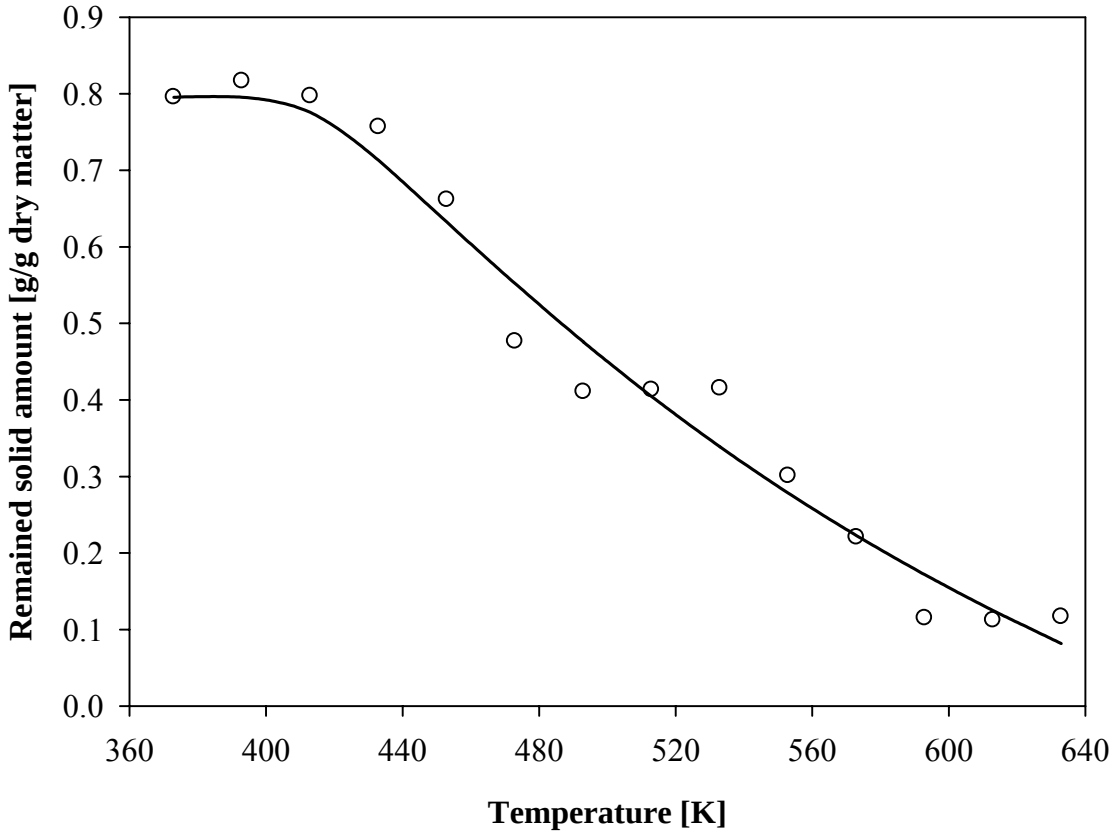


Figure 12. Effect of subcritical water temperature on the amount of remained solid at reaction time of 10 min.

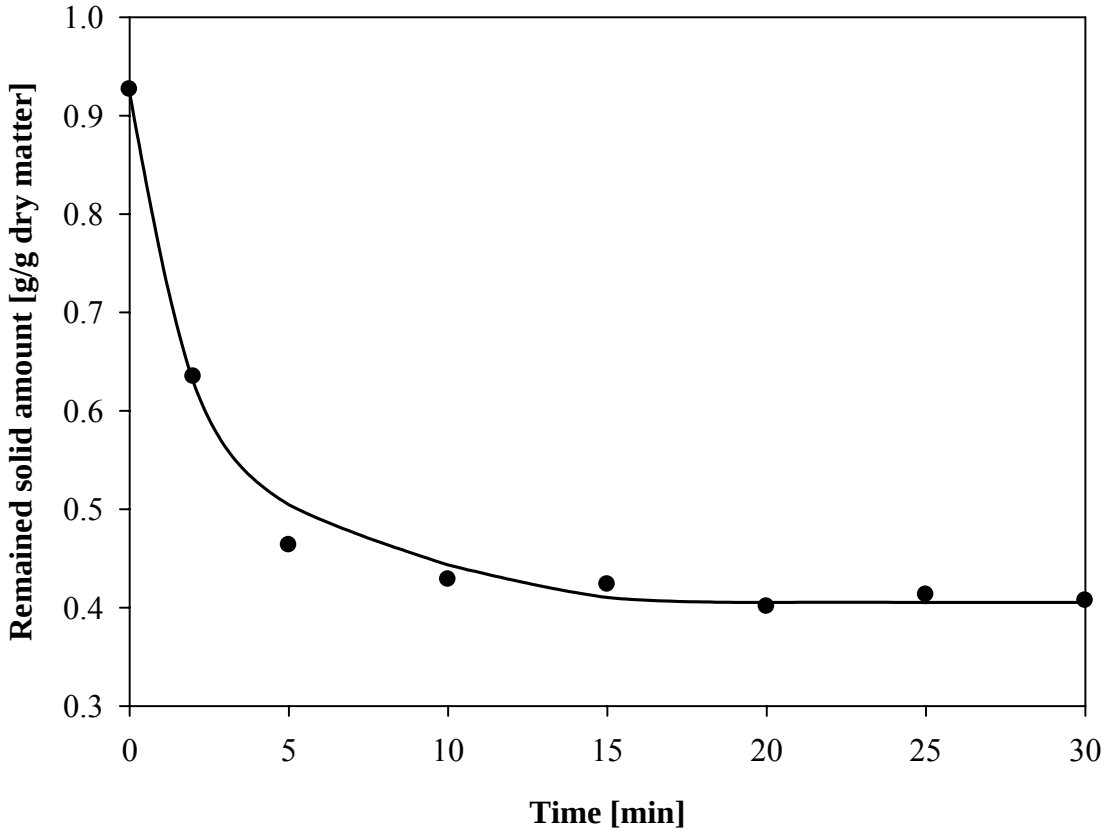


Figure 13. Effect of reaction time on the amount of remained solid at subcritical water temperature of 493 K.

4. Conclusions

Decomposition and conversion of rice bran into valuable chemical compounds were successfully conducted using subcritical water. Degradation of the lignin/phenolics-carbohydrates complexes of rice bran were achieved (up to 92% of rice bran) in the water without using organic solvent, acid, base, and/or enzyme. Decomposition of rice bran and defatted rice bran have resulted almost the same amount of phenolic compounds; it was understood that phenolic compounds were mainly produced from decomposition of bonds between lignin, carbohydrate and phenolic compounds, and a little from rice bran oil. Some of phenolic compounds were identified and quantified for the first time in this work. Protocatechuic and vanillic acids were the major ones among identified phenolic compounds.

Subcritical water temperature and reaction time were two studied parameters which influenced the decomposition of rice bran and production of phenolic compounds. It was found that phenolic compounds could be selectively produced by temperature variations. From reaction time point of view, production of phenolic compounds could be efficiently achieved in a very short time which was much less than those reported in conventional methods that increases economic feasibility of this method.

As phenolic compounds had antioxidant activity, they have promising potential for preventing and treatment of diseases which can be utilized by pharmaceutical industries as natural and appealing feed stock.

Also subcritical water could efficiently degraded carbohydrate macromolecules of rice bran into water-soluble sugars. Significant amount of the produced soluble sugars can be used as a low-cost feed stock for ethanol production with vast food and industrial applications.

The pH studies and the nature of the identified products proved that without utilization of any acids, autocatalysis decomposition reaction may occur under subcritical water conditions.

Finally, production of phenolic compounds and sugars from decomposition of rice bran using subcritical water as green, simple, and non-flammable medium can be scaled up to the industrial level to treat underutilized rice bran before discarding which may be practical and cost-effective.

Nomenclature

HMF	5-Hydroxymethyl-2-furfural
TPC	Total phenolic content

References

Abdul-Hamid, A., Raja Sulaiman, R. R., Osman, A., Saari, N., Preliminary study of the chemical composition of rice milling fractions stabilized by microwave heating, *Journal of Food Composition and Analysis,* 20, 627-637, (2007).

Barberousse, H., Roiseux, O., Robert, C., Paquot, M., Deroanne, C., Blecker, C., Analytical methodologies for quantification of ferulic acid and its oligomers, *Journal of the Science of Food and Agriculture,* 88, 1494-1511, (2008).

Bobleter, O., Hydrothermal degradation of polymers derived from plants, *Progress in Polymer Science,* 19, 797-841, (1994).

Buranov, A. U., Mazza, G., Extraction and purification of ferulic acid from flax shives, wheat and corn bran by alkaline hydrolysis and pressurized solvents, *Food Chemistry,* 115, 1542-1548, (2009).

Chotimarkorn, C., Benjakul, S., Silalai, N., Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand, *Food Chemistry,* 111, 636-641, (2008).

Galkin, A. A., Lunin, V. V., Subcritical and supercritical water: a universal medium for chemical reactions, *Russian Chemical Reviews (English Translation),* 74, 21-35, (2005).

Hasbay Adil, I., Cetin, H. I., Yener, M. E., Bayindirli, A., Subcritical (carbon dioxide + ethanol) extraction of polyphenols from apple and peach pomaces, and determination of the antioxidant activities of the extracts, *The Journal of Supercritical Fluids,* 43, 55-63, (2007).

Hata, S., Wiboonsirikul, J., Maeda, A., Kimura, Y., Adachi, S., Extraction of defatted rice bran by subcritical water treatment, *Biochemical Engineering Journal,* 40, 44-53, (2008).

Herrero, M., Cifuentes, A., Ibanez, E., Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae: a review, *Food Chemistry,* 98, 136-148, (2006).

Hodge, J. E., Hofreiter, B. T., Determination of reducing sugars and carbohydrates, *Methods in Carbohydrates Chemistry,* 1, 380-394, (1962).

Hung, P. V., Morita, N., Distribution of phenolic compounds in the graded flours milled from whole buckwheat grains and their antioxidant capacities, *Food Chemistry,* 109, 325-331, (2008).

Iqbal, S., Bhanger, M. I., Anwar, F., Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan, *Food Chemistry,* 93, 265-272, (2005).

Kruse, A., Dinjus, E., Hot compressed water as reaction medium and reactant properties and synthesis reactions, *The Journal of Supercritical Fluids,* 39, 362-380, (2007).

Lin, L., Ying, D., Chaitep, S., Vittayapadung, S., Biodiesel production from crude rice bran oil and properties as fuel, *Applied Energy,* 86, 681-688, (2009).

Luque de Castro, M. D., Jimenez-Carmona, M. M., Fernandez-Perez, V., Towards more rational technique for the isolation of valuable essential oils from plants, *Trends in Analytical Chemistry,* 18, 708-716, (1999).

McCue, P. P., Shetty, K., A role for amylase peroxidase-linked polymerization in phenolic antioxidant mobilization in dark-germinated soybean and implications for health, *Process Biochemistry,* 39, 1785-1791, (2004).

Naczk, M., Shahidi, F., Extraction and analysis of phenolics in food: a review, *Journal of Chromatography A,* 1054, 95-111, (2004).

Naczk, M., Shahidi, F., Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis: a review, *Journal of Pharmaceutical and Biomedical Analysis,* 41, 1523-1542, (2006).

Otles, S., *Methods of Analysis of Food Components and Additives,* CRC Press, the USA, (2005).

Parrado, J., Miramontes, E., Jover, M., Gutierrez, J. F., Teran, L. C. D., Bautista, J., Preparation of a rice bran enzymatic extract with potential use as functional food, *Food Chemistry,* 98, 742-748, (2006).

Pourali, O., Salak Asghari, F., Yoshida, H., Simultaneous rice bran oil stabilization and extraction using sub-critical water medium, *Journal of Food Engineering,* 95, 510-516,

(2009a).

Pourali, O., Salak Asghari, F., Yoshida, H., Sub-critical water treatment of rice bran to produce valuable materials, *Food Chemistry,* 115, 1-7, (2009b).

Prinkopsky, K., Wellig, B., Rudolf von Rohr, P., SCWO of salt containing artificial wastewater using a transpiring-wall reactor: experimental results, *The Journal of Supercritical Fluids,* 40, 246-257, (2007).

Rangsriwong, P., Rangkadilok, N., Satayavivad, J., Goto, M., Shotipruk, A., Subcritical water extraction of polyphenolic compounds from *Terminalia chbula Retz.* Fruits, *Separation and Purification Technology,* 66, 51-56, (2009).

Renuka Devi, R., Arumughan, C., Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment, *Bioresource Technology,* 98, 3037-3043, (2007).

Salak Asghari, F., Yoshida, H., Acid-catalyzed production of 5-hydroxymethyl furfural from *D*-fructose in subcritical water, *Industrial & Engineering Chemistry Research,* 45, 2163-2173, (2006).

Salak Asghari, F., Yoshida, H., Kinetics of the decomposition of fructose catalyzed by hydrochloric acid in subcritical water: formation of 5-hydroxymethylfurfural, levulinic, and formic acids, *Industrial & Engineering Chemistry Research,* 46, 7703-7710, (2007).

Sasaki, M., Kabyemela, B., Malaluan, R., Hirose, S., Takeda, N., Adschiri, T., Arai, K., Cellulose hydrolysis in subcritical and supercritical water, *The Journal of Supercritical Fluids,* 13, 261-268, (1998).

Saunders, R. M., Rice bran: composition and potential food uses, *Food Reviews International,* 1, 465-495, (1985-86).

Shopova, N., Milkova, Tz., Thermal decomposition of α-tetralyl hydroperoxide in the presence of the phenylpropionic acids, *Thermochimica Acta,* 313, 165-174, (1998).

Taniguchi, H., Nomura, E., Tsuno, T., Minami, S., Method of manufacturing ferulic acid, *US Patent,* 5288902, (1994).

Tavakoli, O., Yoshida, H., Conversion of scallop viscera wastes to valuable compounds using

sub-critical water, *Green Chemistry,* 8, 100-106, (2006).

Tavakoli, O., Yoshida, H., Effective recovery of harmful metal ions from squid wastes using subcritical and supercritical water treatments, *Environmental Science & Technology,* 39, 2357-2363, (2005).

Velioglu, Y. S., Mazza, G., Gao, L., Oomah, B. D., Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products, *Journal of Agricultural and Food Chemistry,* 46, 4113-4117, (1998).

Villano, D., Fernandez-Pachon, M. S., Moya, M. L., Troncoso, A. M., Garcia-Parrilla, M. C., Radical scavenging ability of polyphenolic compounds towards DPPH free radical, *Talanta,* 71, 230-235, (2007).

Wang, L., Weller, C. L., Recent advances in extraction of nutraceuticals from plants, *Trends in Food Science and Technology,* 17, 300-312, (2006).

Wiboonsirikul, J., Hata, S., Tsuno, T., Kimura, Y., Adachi, S., Production of functional substances from black rice bran by its treatment in subcritical water, *LWT-Food Science and Technology,* 40, 1732-1740, (2007a).

Wiboonsirikul, J., Kimura, Y., Kadota, M., Morita, H., Tsuno, T., Adachi, S., Properties of extracts from defatted rice bran by its subcritical water treatment, *Journal of Agricultural and Food Chemistry,* 55, 8759-8765, (2007b).

Wiboonsirikul, J., Kimura, Y., Kanaya, Y., Tsuno, T., Adachi, S., Production and characterization of functional substances from a by-product of rice bran oil and protein production by a compressed hot water treatment, *Bioscience, Biotechnology, and Biochemistry,* 72, 384-392, (2008).

Yoshida, H., Terashima, M., Takahashi, Y., Production of organic acids and amino acids from fish meat by sub-critical water hydrolysis, *Biotechnology Progress,* 15, 1090-1094, (1999).

Zhao, Z., Moghadasian, M. H., Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: a review, *Food Chemistry,* 109, 691-702, (2008).

Zullaikah, S., Lai, C. C., Vali, S. R., Ju, Y. H., A two-step acid-catalyzed process for the production of biodiesel from rice bran oil, *Bioresource Technology,* 96, 1889-1896, (2005).

Chapter 5

Conclusions

This thesis has been devoted to study and develop an effective and environmentally friendly treatment technique to obtain valuable and edible substances from rice bran biomass. For this purpose, subcritical water which is a novel, clean and green medium for chemical processes was used. This study had three main objectives. The first objective was to develop the possibility of rice bran decomposition and hydrolysis, and consequently production of valuable substances such as organic acids, amino acids, and water-soluble sugars using subcritical water treatment. The next objective was the extraction of rice bran oil in subcritical water medium simultaneous with the inactivation of lipase enzyme of bran 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.

This thesis consists of five chapters. Chapters 2, 3, and 4 are the main components of this thesis. The major results and obtained findings of this investigation are summarized as follows:

In chapter 1, an introduction was given. Rice bran biomass and its composition, and the properties of water under and above its critical point were described. In addition, the related researches, and the outline of this thesis were presented.

In chapter 2, the decomposition and hydrolysis of rice bran over the whole temperature range of subcritical water was investigated. Subcritical water could effectively hydrolyze rice bran in a short reaction time without using any organic solvent, acid, base, and/or enzyme. Four phases were isolated after reaction: hexane-soluble, acetone-soluble, water-soluble, and remained solid. Since rice bran contains bio-macro polymers like polysaccharides and proteins, decomposition of rice bran under subcritical water conditions caused significant increase in total organic carbon (TOC) and total nitrogen (TN) in the obtained water-soluble phase after subcritical water reaction. Various kinds of valuable water-soluble compounds were identified after subcritical water treatment of rice bran. Decomposition of protein part of this biomass 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 aqueous phase. Among the identified amino acids, lysine gave the highest yields. Subcritical water could also produce

five water-soluble organic acids (acetic, citric, formic, glycolic, and levulinic acids) from decomposition of carbohydrates and amino acids at temperatures higher than 463 K. Production of acidic compounds such as amino acids and organic acids in subcritical water medium may autocatalyze the hydrolysis reaction and consequently increase the liquefaction of rice bran and production of the valuable compounds.

It was realized that subcritical water not only could convert protein part of rice bran into amino acids but also could effectively hydrolyze its cellulosic part into water-soluble sugars; fructose, glucose, glyceraldehyde, and sucrose were identified in the aqueous phase. Total soluble sugars gave the highest yield (nearly 20% of initial dry matter) at the optimum temperature of 463 K.

In chapter 3, the possibility of the edible rice bran oil production in subcritical water medium was investigated. In the first part of this chapter, the effect of the activity of lipase enzyme of rice bran on the quality of its oil was studied, and several enzyme inactivation methods were investigated to stabilize oil. In order to evaluate the efficiency of the treatment methods, their total free fatty acids concentration as a criterion of oil quality was evaluated over the storage period of samples. In fact, it was found that total free fatty acids concentration in rice bran oil increased drastically soon after milling process; total free fatty acids concentration in untreated sample increased from initial value of 5.6% to 36.0% within 12 weeks storage. Results showed that total free fatty acids concentration in untreated sample reached above 10.0% in less than one week from rice milling date which made oil unfit for human consumptions. Among the evaluated methods, subcritical water could effectively and irreversibly inactivate lipase enzyme in a very short reaction time, and the level of total free fatty acids in the treated sample remained constant over the storage period. In contrast, conventional solid-solvent extraction methods could not completely inactivate enzyme to produce the stabilized oil, and they had damping effect on the activity of enzyme. Meanwhile, 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 bran prior to its deterioration.

In the second part of this chapter, extraction of rice bran oil was carried out using

subcritical water. Rice bran oil could be efficiently extracted simultaneous with enzyme inactivation. It was found that subcritical water temperature and reaction time affected the extraction yield. Approximately 94% of total oil of rice bran was successfully extracted by subcritical water.

In chapter 4, subcritical water conditions were tuned in order to produce phenolic compounds as well as other valuable substances from rice bran. Subcritical water could effectively hydrolyze and decompose the lignin/phenolics-carbohydrate complexes of rice bran and produce various phenolic compounds. Up to 92% of rice bran could be converted to water-soluble compounds with application of subcritical water and without utilization of any catalyst, enzyme, acid, base, and/or organic solvent. Several phenolic compounds could be identified; caffeic, ferulic, gallic, gentisic, p-coumaric, p-hydroxybenzoic, protocatechuic, sinapic, syringic, vanillic acids and vanillin. These valuable compounds have antioxidative properties, and they have various applications in pharmaceutical industries.

Based on the experimental data obtained from the decomposition of rice bran and defatted rice bran, and also production of phenolic compounds, it was concluded that phenolic compounds were mainly produced from decomposition of lignin/phenolics-carbohydrate network and not from rice bran oil.

Compare to the conventional treatment methods which are time consuming, subcritical water could quickly hydrolyze rice bran in a very short reaction time. Results indicated that phenolic compounds production was a function of subcritical water temperature and reaction time; therefore, it was realized that the production of these useful compounds can be selectively and optimally performed with temperature and time changes.

Furthermore, the released carbohydrates from lignin/phenolics-carbohydrate complexes in subcritical water medium could be effectively converted into water-soluble sugars which may be a good feed stock for bioethanol production industries.

In chapter 5, general conclusions of the present work were given.

Based on the results obtained in this thesis, it is believed that subcritical water produces cheap, safe, clean, and environmentally friendly techniques which decompose and hydrolyze rice bran to valuable substances, and extract edible oil which is not denatured after treatment. In addition, 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.