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Process Development for Lipase Extraction and the Effect of Extracted Lipase on Triglyceride Base System

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ABSTRACT

The purpose of this research was to develope the process for prediction of rice bran lipase extraction condition and study the effect of the extracted lipase on triglyceride base system. The chemical composition of the Chainat 1 rice bran was examined. Esterification reaction was studied and the defatted rice bran lipase showed the highest specific activity of 0.4736 unit/mg protein. The optimum extraction condition was at 40°C reaction temperature and 1 h reaction time. The rice bran oil was used as the model for triglyceride base system. Neural network and fuzzy set approaches were used to predict the extraction temperatures from fatty acid, free fatty acid and specific enzyme activity values. The result showed the effectiveness of the method used with the error less than 4%. The properties of rice bran oil, which reacted with 20% defatted rice bran lipase at 40oC, were analyzed. The results showed the increases of free fatty acid and acid values, the decrease of reflective index, while the iodine and viscosity values were constant. The result of fatty acide profile showed the increase in gamma oryzanol 3.92%, decrease in linolenic and lignoceric fatty acids 87.18 and 25.86%, whereas increase in myristic fatty acid 10.34%.

Keywords: Rice bran, lipase, rice bran oil, neural network, fuzzy set

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INTRODUCTION

Rice bran is the by-product of rice mill process that contains regioselective lipase [6]. Rice bran can be used as the raw materials for rice bran oil extraction. Rice bran contains nutritional and antioxidant substances such as tocopherol, vitamin E and gamma oryzanol [5,10,13,15,17,22,24]. Rice bran is known as the source of lipase enzyme, which can be used as the catalyst in esterification reaction of fatty acid and glycerol. Lipase can change fatty acid composition and produce many important products such as mono-, di- and tri acylglycerols [18].These products can be applied in food and chemical industries [11,21]. One example of commercial lipase produced from hydrolysis and/or esterification reactions is diacylglycerol oil [7,12,23]. The utilization of lipases from defatted rice bran is an alternative way for adding the value of rice bran.

Biological processes are usually characterized by nonlinearities and uncertainties because of the complex physiochemical changes occurring in a process and the variability of raw materials. Most biological processes are multivariable systems, which mean that there are interactions between process inputs and outputs. In recent years, neural networks have received great attention because of their ability to represent nonlinear systems. A neural network can map a set of inputs to a set of outputs without prior knowledge of the underlying relationship. The mapping ability of neural networks is not limited by the level of complexity of the underlying relationship and does not require equality of the space dimensions. Fuzzy concept was applied for controlling product quality in food industry for more than 10 years. Kupongsak and Tan (2006) applied fuzzy set and neural network techniques in determining food process control set points. Therefore, the purpose of this study was to develop the process for predicting the extraction condition of rice bran lipase and to study the effect of the extracted rice bran on properties of triglyceride base system.

MATERILAS AND METHODS

Raw Materials

The Chai Nat 1 rice bran was ground and sieved through the 40 mesh sieve. The rice bran oil (King rice bran oil, Thai Edible oil, Co. Ltd., Thailand) was purchased from the local market (Top's supermarket, Bangkok, Thailand).

METHODOLOGY

Properties of Chai Nat 1 rice bran

The chemical composition of Chai Nat 1 rice bran such as moisture content [3], protein [2], fat [1], ash [2] and crude fiber [1] were determined.

Extraction of rice bran lipases

The rice bran was defatted in order to increase its stability at high temperature storage. Defatted rice bran was prepared by using the ratio of rice bran and hexane 1:3

(w/v).The reaction occurred at room temperature (30±2°C) for 1 h. The soluble protein was extracted with 0.05 M potassium phosphate buffer at pH 5.5 and 0.05 M calcium chloride. The reaction was further conducted at 4°C for 3 h using the ratio of rice bran and buffer 1:3 (w/v). Then, the soluble protein extracted from rice bran was determined by the method of Lowry *et al*., 1951.

Enzymatic activity of extracted rice bran lipase

The effect of temperature on esterification reaction using rice bran lipase from the selected extraction method was studied. The reaction temperatures were varied at 30, 37, 40, 50 and 60°C with the 1 hr reaction time. Analysis of lipase activities (modified method of Chong *et al*., 2007) was performed. The reaction mixture consisted of oleic acid and glycerol (molar ratio of 2:1) in hexane. The defatted rice bran was added to the reaction mixture where the ratio of defatted rice bran and mixture was 1:2. The reaction was conducted at 50°C for 1 h and it was stopped by adding the mixture of acetone and ethanol at 1:1 ratio (v/v). Then the titration with 0.5 M NaOH was performed. Enzyme activity was calculated by using Equation 1 to 3 as the following

Esterified Free Fatty Acid; EFFA

$$\mathrm{EFFA\,(micromol)} = [\mathrm{NaOH\,control} - \mathrm{NaOH\,sample}] \times \mathrm{molarity\,NaOH} \times 100 \quad (1)$$

Lipase Enzyme Activity; LEA:

$$\mathrm{LEA}\left(\frac{\mathrm{U}}{\mathrm{g}}\right) = \mathrm{EFFA}\left(\frac{\mathrm{micromol}}{\mathrm{enzyme\,weight\,(g)}}\right) \times 60\,\mathrm{min} \quad (2)$$

Specific Enzyme Activity; SEA:

$$\mathrm{SEA}\left(\frac{\mathrm{U}}{\mathrm{g}}\right) = \frac{\mathrm{LEA}\left(\frac{\mathrm{U}}{\mathrm{g}}\right)}{\mathrm{Proteins\,in\,enzyme\,solution\,(mg)}} \quad (3)$$

Property of triglyceride base system

Rice bran oil was used as a triglyceride system model in this study. The physical and chemical properties of rice bran oil such as viscosity (FungilabAlpha series L, Spain), refractive index (Atago, NAR-1T model, Japan), free fatty acid value [4], acid value [4], iodine value [4], fatty acid profile (Gas Chromatography/Mass Spectrometry (GC-MS), Trace GC ultra, Italy/Polaris Q, USA), and gamma oryzanol (Reverse-Phase High Performance Liquid Chromatography, RP-HPLC) were determined.

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Develope the process for determining of lipase extration conditions by using neural network and fuzzy set approaches

In this study, fuzzy set technique was used to formulate the fuzzy characteristics of fatty acids of the rice bran oils, and neural network was employed to determine the extraction temperatures for given fatty acid, free fatty acid and specific enzyme activity values. The results of fatty acids from the esterification reactions of the rice bran oils with the extracted rice bran lipases at 30, 37, 40, 50 and 60 ^{o}C were formulated in the forms of fuzzy membership grades and fuzzy membership vectors (Equation 4). Then, the fuzzy membership matrix of multiple variables was formed (Equation 5).

$$\boldsymbol{\mu}(x) = [\mu_1(x) \quad \mu_2(x) \ldots \mu_n(x)]^{\mathrm{T}} \tag{4}$$

where fuzzy membership vector μ (x) represents the membership grades of *x* in all *n* fuzzy sets.

$$\boldsymbol{Y}= \begin{bmatrix} \mu_{1,1}(x) & \mu_{1,2}(x) & \mu_{1,3}(x) \ldots \mu_{1,q}(x) \\ \mu_{2,1}(x) & \mu_{2,2}(x) & \mu_{2,3}(x) \ldots \mu_{2,q}(x) \\ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \\ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \\ \mu_{p,1}(x) & \mu_{p,2}(x) & \mu_{p,3}(x) \ldots \mu_{p,q}(x) \end{bmatrix} \tag{5}$$

Each row in the ***Y*** matrix is the fuzzy membership vector. $\mu_{i,j}$ denotes the average membership grade in fuzzy set *j* of variable *i* . There are *p* variables and *q* fuzzy sets for each variable.

In order to predict the extraction temperatures (***Y***) from the results of fatty acid, free fatty acid and specific enzyme activity values (variables), a back-propagation algorithm neural network was trained in a Matlab environment (The Mathworks, Natick, MA). The gradient decent method was applied to minimize the error by adjusting the weight vector. Fuzzy membership matrix of fatty acids, free fatty acids and specific enzyme activities at 30, 37, 40, 50 and 60 ^{o}C were used as the inputs for the neural network whereas extraction temperatures were used as the outputs. A leave-one-out scheme was used to validate the performance of the trained neural network. The errors of the network prediction were then analyzed.

Effect of extracted lipase on property of triglyceride base system

The effect of lipase on properties of triglyceride base model was determined by application of 20% rice bran lipase in rice bran oil (weight of enzyme/weight of oil). The rice bran oil with the addition of 20% rice bran lipases were heated to 40 ± 2°C. Then, the physical and chemical properties of reacted rice bran oil (as described in property of triglyceride base system section) were investigated.

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RESULTS AND DISCUSSIONS

The results from chemical composition analysis of Chai Nat1 rice bran showed that the moisture content, carbohydrate, protein, fat, ash and crude fiber were 12.75% (wet basis), 51.86, 13.73, 12.15, 9.51 and 6.96% (dry weight), respectively. The defatted rice bran had 12.61% protein and 16.07 mg/mL soluble protein.

Lipases or triacylglycerol hydrolases (EC 3.1.1.3) are enzymes having an inherent capability to catalyze carboxy ester bonds cleavage and produce tri, di, monoacylglycerols and fatty acids [14]. The defatted rice bran contains many porous which can immobilize lipase in its molecule. These porous particles act as a matrix for immobilization of lipase molecules. The immobilized lipases in the defatted rice bran help to increase organic solvent and high temperature esterification stabilities [7].

The esterification activity of defatted rice bran increased as the temperature increased from 30 to 40°C and decreased with the elevation of temperature from 40 to 60°C. The result from Fig.1 demonstrated the activity of extracted lipase that showed the highest specific activity (0.4736 U/mg protein) for esterification reaction at 40°C.

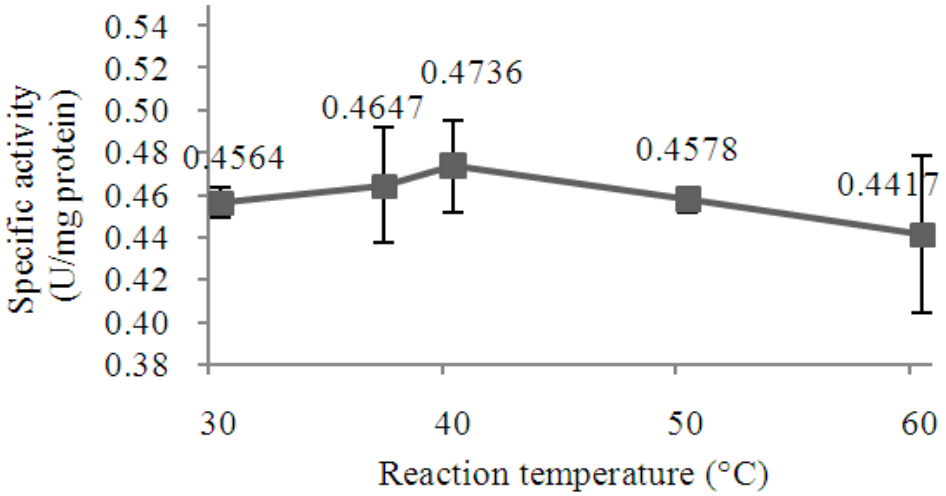

Figure 1. The specific activities of the extracted lipase enzyme at various esterication reaction temperatures (from 30 to 60°C)

The predicted extraction temperatures from the complex three-layer (15-24-1) neural network and fuzzy set approaches were 30.0, 36.0, 39.2, 51.4 and 62.3°C for 30.0, 37.0, 40.0, 50.0 and 60.0°C real extraction temperatures, respectively. The average prediction error was less than 4%. The result demonstrated the effectiveness of the method used for determining extraction conditions from given fatty acids, free fatty acid and specific enzyme activity values. Therefore, predicted extraction temperature can be used as the set point for the process control of lipase production based on desired variable responses (fatty acid, free fatty acid, and specific enzyme activity).

The physical and chemical properties of rice bran oil before and after esterification reactions were compared as shown in Table 1. The fatty acid profile of rice bran oils (before and after esterification reactions) was demonstrated in Table 2.

The results from Table 1 showed that the addition of lipase had no effect on oil viscosity. In this study, the lipase had the effect on the fatty acid composition of the triglyceride system . The addition of lipase enzyme affected the Refractive Index (RI) value (Table 1). The RI value of rice bran oil (after reaction) decreased due to the reduction of double bond fatty acid and the hydrolyzation of long chain to short chain fatty acids.

From Table 1, the addition of rice bran lipase in the rice bran oil increased free fatty acid and acid values. The defatted rice bran lipase hydrolyzed triglycerides in rice bran oil. The increase in free fatty acids (as the products from hydrolysis reaction) was obtained. The addition of lipase showed less effect on iodine value.

The gamma oryzanol in the rice bran oil increased with the addition of extracted rice bran lipase. The increased gamma oryzanol may come from the rice bran. Since this is a mild condition, the gamma oryzanol was not destroyed.

The results from the physical and chemical properties of rice bran oil with defatted rice bran lipase at the reaction temperature 40°C showed the changes of fatty acid composition of the rice bran oil. Linolenic and lignoceric acids decreased 87.13 and 25.86%, respectively, while the myristic increased 10.34%. The SFA and MUFA increased 1.11 and 0.095% while PUFA decreased 3.32% (Table 2).

After the esterification reaction, the changes of fatty acid composition especially lignoceric (long chain fatty acid) and myristic acid (saturated fatty acid) may come from the lipase activity rather than the hydrolysis reaction. Since the hydrolysis reaction usually occurs in short chain and unsaturated fatty acids. The short chain fatty acids and unsaturated fatty acid more dissolve in water than the long chain and saturated fatty acids (Nawar, 1969).

Table 1. Comparison of physical and chemical properties of rice bran oil before and after esterification reactions (Addition of 20% defatted rice bran lipase)

Physical and chemical properties	Measured value (Before esterification)	Measured value (After esterification)
Viscosity (cP) 25°C	64.08±0.31	64.44±1.27
Refractive index (25°C)	1.4698±0.0002	1.4680±0.0000
Free fatty acid (%) as oleic acid	2.97±0.10	9.21±0.41
Acid value (mg NaOH/g oil)	5.91±0.20	18.32±0.81
Iodine value (g/100 g oil)	99.80±0.07	99.33±2.24
SFA: MUFA: PUFA (Saturated Fatty Acid, SFA Monounsaturated Fatty Acid, MUFA Polyunsaturated Fatty Acid)	19.00: 42.06: 38.96	19.21: 42.10: 37.7
Gamma oryzanol (mg/100 g oil)	174.67	181.52

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Table 2. Fatty acids of rice bran oils (before and after esterification reactions)

Fatty acid	Rice bran oil (Before esterification)	Rice bran oil (After esterification at 40°C)	Percentage of fatty acid change (%)
C12:0 Lauric	nd	nd	-
C14:0 Myristic	0.29	0.32	10.34
C16:0 Palmitic 15.1	15.1	15.47	2.45
C16:1 Palmitoleic	0.19	0.19	0
C18:0 Stearic	1.9	1.82	(-) 4.21
C18:1 n-9 cis Oleic	41.32	41.31	(-) 0.02
C18:2n-6 cisLinoleic	37.68	37.43	(-) 0.66
C18:3 n-6 GLA	0.11	0.12	9.09
C18:3n-3 Linolenic	1.17	0.15	(-)87.18
C20:0 Arachidic	0.87	0.89	2.3
C20:1n-9	0.55	0.6	9.09
C22:0 Behenic	0.26	0.28	7.69
C24:0 Lignoceric	0.58	0.43	(-)25.86
SFA	19	19.21	1.11
MUFA	42.06	42.1	0.095
PUFA	38.96	37.7	(-) 3.23
SFA : MUFA : PUFA	1:2.2:2	1:2.2:2	-

CONCLUSION

The optimum condition for rice bran lipase extraction was at 40°C for 1 h. Neural network and fuzzy set approaches were successfully developed to predict the extraction temperatures from the desired fatty acid, free fatty acid and specific enzyme activity data. The addition of defatted rice bran lipase in rice bran oil (triglyceride system) resulted in increasing of acid value, free fatty acid and decreasing in refractive index. There were no significant effects on iodine and viscosity values. The property of rice bran oil with the addition of 20% defatted rice bran lipase was changed. Long chain (linolenic and lignoceric) fatty acids were decreased while saturated (myristic) fatty acid was increased. Moreover, the gamma oryzanol in the rice bran oil was increased. There is potential use of defatted rice bran lipase from this reasearch. For example, the lipase extract can catalyze oil in hydrolysis reaction to obtain free fatty acids and glycerols. Glycerol products such as mo-, di-, and/or tri acylglycerol can be applied in food industry. In addition, the esterification by using extracted rice bran lipase can help to increase the nutritional values of the rice bran oil (or triglyceride base system).

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