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Optimization of Rice (*Oryza Sativa*) Malting Process by Second-Order Experimental Design

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Abstract : The malting process of rice (OM4080 variety from Mekong Delta Rice Research Institute) was studied under pilot condition plan by means of the second-order experimental design. Processing parameters, such as the steeping time (0-60 hrs), steeping temperature (5-45^{0}C), germination time (0-8 days), germination temperature (5-45^{0}C) and gibberellin concentration (0-2 mg/kg) were investigated. As a result, all germination conditions, especially germination time, germination temperature, and gibberellin concentration had a significant effect on the malting loss, amylase activity and starch content. The protein content was not clearly affected by any conditions. The optimum conditions for malting process (with highest amylase activity) were as follows: 30 hrs of steeping time, 30-35^{0}C of steeping temperature, 5-5.5 days of germination time, 25^{0}C of germination temperature, and 1.5 mg/kg of giberrellin concentration.

Key Words : Rice, Malting, Steeping, Germination, Starch content, Amylase activity, The second-order experimental design.

1. Introduction

Various requirements must obviously be satisfied before germination can occur. In most cases, there must be sufficient oxygen to allow aerobic respiration, suitable temperature to permit various metabolic processes to continue at an adequate rate, and enough moisture for growth and development [4]. The quality of the germinated cereal flour is influenced by the nature of the raw material, steeping and germination conditions, chemicals, and plant hormones added during steeping and germination. The optimum quality of produced germinated cereal flour was difficult to produce because of the interrelated effects

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of different processing variables. The germination is a process used to make malt for the brewing industry, however germinated or malted flour can also be used in bakery products [5], nonalcoholic drinks [6] and weaning food formulation [5, 6]. The germinated flour from cereals has been reported to have better nutritional properties than those of flour from nongerminated cereals [4, 5, 6].

Supplementary foods made from germinated flours have low viscosity and high nutrient density and have properties acceptable to weaning infants in developing countries [10, 11, 12]. Cereals are an important source of dietary starch consumed in the world and constitute a major portion of human diet. Rice *(Oryza sativa)* is one of the most important domesticated cereals in Asia, where over 60% of the calories consumed by the population come from rice [7] and it is a major staple food crop of Vietnam.

2. Experimental

2.1. Materials

The Rice *(Oryza sativa,* OM4088) was supplied by Mekong Delta Rice Research Institute, Cantho Province, Vietnam. OM4088 was harvested in 2006 and was steeped for various times at various temperatures.

After the steeping process, the malt with about 40 wt% of moisture was wrapped on cheese cloth, kept in perforated plastic trays and germinated in the dark at 90±5% RH. The malt was then dried at 50^0C (moisture contents less than 10 wt%). The malting losses (%) were calculated on the base of the dried malt weight.

2.2. Chemical and enzyme assay

Determination of moisture and raw grain characteristics were as described by Nzelibe and Nwasike [1, 8]. Determination of protein was described by the Kjedahl method. Lipid content was determined by the Soxhlet method, and carbohydrate content was determined by acid hydrolysis method. Amylase activity was analyzed according to the Bernfeld method [1, 18]. The flour sample was extracted with acetate buffer (pH 4.3) for 1 h at ambient temperature (about 20^0C). Amylase activity was defined as the amount of maltose (mg) released by the action of malt enzyme extracted from 1 g malt flour in acetate buffer (pH=4.3) on soluble starch at 40^0C for 30 min.

The produced germinated rice flour was analyzed and the results were compared statistically with those of prediction by the mathematical model. Germination is a simple operation. However, the interactions between different variables have affect on the quality of the germinated cereal flour. The optimization of malting was studied by Box-Hunter using the second-order experimental design with 5 independent variables and 3 levels. The samples were analyzed for three times, the results were validated statistically by the mean of ± standard deviation, and the optimization was analyzed by the Matlab-2006 and the Excel-2007 software. Five independent variables were determined as steeping time(X_1), steeping temperature (X_2), germination time (X_3), germination temperature (X_4), and gibberllin content (X_5). Box-Hunter second-order experimental design included 32 experiments and six center points [14, 15, 16, 20, 21, 22].

The dependent variables were designed as malt loss (Y_1), starch content (%) (Y_2), protein content (%) (Y_3), and amylase activity (Y_4). The data were fitted to apply for the Taylor second-order approximating function:

$$Y = b_0 + \sum b_i X_i + \sum b_{ij} X_i X_j + \sum b_{ii} X_{ii}$$

Where, Y is the response function, b_0 the

center point of the system, b_i, b_j, b_{ij} represent the linear, quadratic and interactive effects, respectively. The linear, quadratic and interactive effects of the independent variables (steeping time, steeping temperature, germination time, germination temperature, gibberllin content) were designated as X_i, X_{ii} and X_iX_j, respectively.

3. Results and Discussion

3.1. The second-order experimental design

The effects of steeping time, steeping temperature, germination time and germination temperature on the malt loss, starch content, protein content and enzyme activity are shown in Table 2.

Table 1. Process variables and their levels in the five factors of the Box-Hunter second-order experimental design

Independent variables	Symbols		Levels		Independent variables	Symbols		Levels	
	coded	uncoded	coded	uncoded		coded	uncoded	coded	uncoded
Steeping time (h)	X_1	T (h)	-2	0	Germination temperature	X_4	t (^{0}C)	-2	5
			-1	15				-1	15
			0	30				0	25
			-1	45				1	35
			2	60				2	45
Steeping temperature (^{0}C)	X_2	t (^{0}C)	-2	5	Gibberellin content	X_5	Gib (mg/kg)	-2	0
			-1	15				-1	0.5
			0	25				0	1
			1	35				1	1.5
			2	45				2	2
Germination time (day)	X_3	T (day)	-2	0					
			-1	2					
			0	4					
			1	6					
			2	8					

Table 2. Result of experiments collected by malting process and the second-order experimental design

Expt run*	Variable levels					Responses			
	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_3	Y_4
1	15	15	2	15	1.5	2.65	58.7	7.85	4
2	45	15	2	15	0.5	3.45	59.6	7.65	8
3	15	35	2	15	0.5	2.15	58.4	7.84	12
4	45	35	2	15	1.5	4.65	57.9	7.72	42
5	15	15	6	15	0.5	4.22	59.7	7.55	58
6	45	15	6	15	1.5	6.75	66.7	7.65	79
7	15	35	6	15	1.5	5.83	61.1	7.76	95
8	45	35	6	15	0.5	6.53	60.3	7.42	102
9	15	15	2	35	0.5	2.25	64.8	7.86	15
10	45	15	2	35	1.5	3.78	62.1	7.54	10
11	15	35	2	35	1.5	2.83	65.2	7.86	25
12	45	35	2	35	0.5	4.35	61.4	7.97	36
13	15	15	6	35	1.5	7.45	59.8	7.85	90
14	45	15	6	35	0.5	8.37	60.1	7.84	95
15	15	35	6	35	0.5	8.16	57.1	7.55	115
16	45	35	6	35	1.5	12.17	54.2	7.98	130
17	0	25	4	25	1	3.24	67.4	7.37	100
18	60	25	4	25	1	9.15	57.2	7.51	140
19	30	5	4	25	1	6.45	60.5	7.65	120
20	30	45	4	25	1	7.65	59.9	7.51	135
21	30	25	0	25	1	2.06	63.4	7.65	12
22	30	25	8	25	1	15.93	49.6	7.75	155
23	30	25	4	5	1	2.47	61.8	7.55	21
24	30	25	4	45	1	7.96	56.7	7.66	9
25	30	25	4	25	0	6.48	58.4	7.44	120
26	30	25	4	25	2	8.23	66.8	7.55	165
27	30	25	4	25	1	5.52	61.2	7.15	145
28	30	25	4	25	1	6.53	58.5	7.43	132
29	30	25	4	25	1	6.85	61.8	7.45	136
30	30	25	4	25	1	6.75	57.5	7.64	141
31	30	25	4	25	1	6.85	61.1	7.53	130
32	30	25	4	25	1	5.48	58.2	7.22	148

* Experimental runs were performed in random order.

Table 3. Regression coefficients of the analyses of variance on the effect of germination conditions (X_k) as linear, quadratic and interaction (cross product) terms on the response variables (Y_k)

	Malt loss (%), Y_1		Starch content (%), Y_2		Protein content (%), Y_3		Amylase, Y_4	
	Coefficients	Significant level, p	Coefficients	Significant level, p	Coefficients	Significant level, p	Coefficients	Significant level, p
b_0	6.647	***	59.64	***	7.372	***	144	***
b_1	1.098	***	−0.955	*	−0.003		7.006	**
b_2	0.423	*	−0.713		0.001		9.508	**
b_3	2.548	***	−1.530	**	−0.020		37.447	***
b_4	1.005	***	−0.329		0.051		3.836	*
b_5	0.422	*	0.880	*	0.031		5.171	*
b_{12}	0.184	**	−0.844		0.032		2.375	
b_{13}	0.113		0.606		0.044		0.500	
b_{14}	0.091		−0.981		0.048		−2.250	
b_{15}	0.167		−0.331		−0.032		0.375	
b_{23}	0.253		−0.706		−0.042		2.625	
b_{24}	0.223		−0.119		0.014		−0.38	
b_{25}	0.122		−0.119		0.034		1.25	
b_{34}	0.782	**	−2.219	**	0.042		4.75	*
b_{35}	0.201		0.306		0.077		0.88	
b_{45}	−0.027		−0.531		−0.032		−2.875	
b_{11}	−0.393	*	0.288		−0.015		−10.620	***
b_{22}	−0.180		−0.236		0.020		−8.753	**
b_{33}	0.306	*	−1.160	*	0.050		−19.74	***
b_{44}	−0.638	**	−0.473		0.027		−36.83	***
b_{55}	−0.104		0.363		0.001		−5.009	*

* Significant at 5% level
** Significant at 1% level
*** Significant at 0.1% level.

The data were fitted with the second-order approximating functions:

$$Y_1 = 6.647 + 1.098 * X_1 + 0.423 * X_2 + 2.548 * X_3 + 1.005 * X_4 + 0.422 * X_5 + 0.782 * X_3 * X_4 - 0.393 * X_1^2 - 0.338 * X_3^2 - 0.606 * X_4^2$$

$$Y_2 = 59.64 - 0.955 * X_1 - 1.530 * X_3 + 0.880 * X_5 - 2.219 * X_3 * X_4 - 1.16 * X_3^2$$

$$Y_3 = 7.372$$

$$Y_4 = 144 + 7.006 * X_1 + 9.508 * X_2 + 37.613 * X_3 + 4.003 * X_4 + 5.171 * X_5 + 4.75 * X_3 * X_4 - 10.62 * X_1^2 - 8.753 * X_2^2 - 19.74 * X_3^2 - 36.83 * X_4^2 - 5.009 * X_5^2$$

Table 4. Variance analysis of the fitting model ($F_{0.95}(20,5)=4.5$ and $F_i=s_{tt}^2/s_{th}^2$)

	Y_1	Y_2	Y_3	Y_4
s_{tt}^2	1.981	12.906	0.144	175.590
s_{th}^2	0.427	3.430	0.035	51.867
$F_i=s_{tt}^2/s_{th}^2$	4.48	3.76	4.18	3.39

$F_i=F_{0.95}(20,5)$ indicated that the data were fit and were statistical representing the response surface.

3.2 Malting loss

The viability of germination can be realized with high yield and low malting loss. Malting loss includes losses due to leaching of solids during steeping and losses due to increased metabolic growth during germination. Increase in malting loss may subsequently decrease the level of water soluble nutrients in the germinated flour. However, in order to germinate the seed, adequate moisture must be attained to hasten metabolic development of the roots and shoots. The seed should attain by suitable moisture content with steeping for the optimum time and temperature. The effects of steeping and germination conditions on malting loss (Y_1) are shown in Table 2. The mathematical model clearly suggests that any increase in germination time and temperature will significantly increase malting loss (Table 3). There was a significant interaction between the independent variables ($b_{34}=0.782$, $p=0.01$). The increase in steeping time was independent of the effect of steeping temperature on malting loss, however a significant quadratic effect was noted for germination time and temperature. The increase or decrease in malting loss is mainly due to the linear and quadratic terms of the independent variables (Fig. 1). The effects of steeping temperature and gibberllin on malting loss influenced were showed lower than that of other parameters (Table 3).

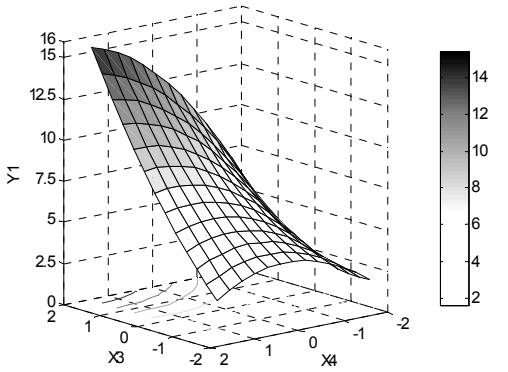

Fig. 1. Response surface plots of effect of malting conditions (X_3 and X_4) on malting loss (%).

3.3. Starch content (%)

Germination is one of the methods that can be used to reduce the viscosity of the grain extracts [2, 3, 13, 23]. During germination, the starch is degraded by the action of enzymes present in the seed. Amylases break down the amylose and amylopectin components of the starch producing smaller dextrins, maltose and glucose [2, 26, 28], thus to reduce the viscosity. A good correlation between viscosity or falling number and amylase activities in cereals has been reported [23]. The factor affecting the starch of rice during germination were investigated and are presented in the model equation (Y2) in Table 2. The germination times significantly reduced the starch content ($b_3=-1.5330$, $b_{33}=-1.160$, $p=0.01$). The

gibberllin content and germination time also influenced to decrease the starch content. Analysis of variance are shown in Table 3, moreover, this result was clearly showed in Fig. 2.

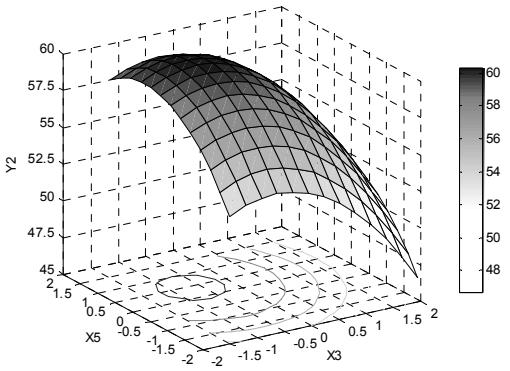

Fig. 2. Response surface plots of effect of malting conditions (X_3 and X_5) on starch content, % (Y_2).

3.4. Amylase activity

The relationship between amylase activity and the factors that contribute to its change during germination are presented in Table 3. The result of the model equation (Y_4) revealed that, not only germination time (bk_3=37.447), steeping time (b_1=7.006), steeping temperature (b_2=9.508), germination temperature (b_4=3.836) and gibberllin content (b_5=5.171) were contributed significantly to the increase in enzyme activity, but also the quadratic terms of steeping time (bk_{11}=–10.620), steeping temperature (bk_{22}=–8.753), germination time (bk_{33}=–19.74) germination temperature (bk_{44}=–36.83) and the gibberllin content (bk_{55}=–5.009). The enzyme activity increased dramatically as the other parameters were increased. α- and β-amylase were sufficient by required in the moisture to hydrolyze starch in the rice grain. The range of steeping and germination temperatures to which the rice seeds have been subjected will give rise to increase their enzymatic activity at different rates. Similar observations were reported in germinated millets, kaffircorn, barley and wheat [16, 17, 27, 28, 29, 30]. The relationship of the independent variables to amylase activity are shown in Fig. 3.

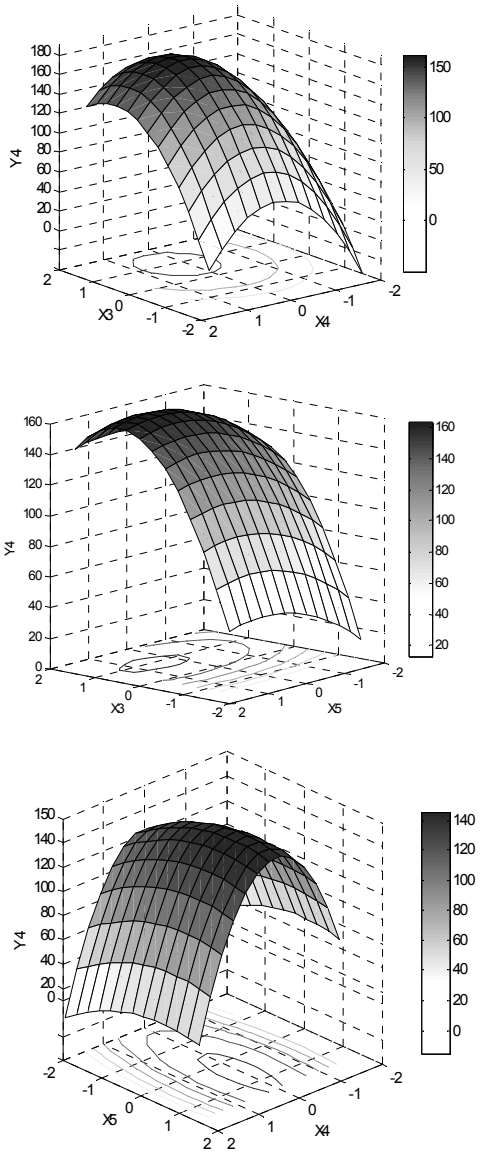

Fig. 3. Response surface plots of effect of malting conditions (X_3, X_4 and X_5) on amylase activity, % (Y_4).

4. Conclusions

Germination conditions were significantly affected by the malting loss, starch content, protein content, and amylase activity. However, the protein content was not significantly affected by any conditions investigated. The model equation developed can be used for predicting the quality of malting. As a result, all germination conditions, especially germination time, germination temperature, and gibberellin concentration have significantly affected on the malting loss, amylase activity, and starch content. The optimum conditions for malting (with highest amylase activity) are; 30 hrs of steeping time, 30-35^{0}C of steeping temperature, 5–5.5 days of germination time, 25^{0}C of germination temperature, and 1.5 mg/kg of giberrellin concentration .

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