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Short Communition

Effect of Spices and Herbs on the Growth of *Pichia burtonii* DA 69, and *Saccharomyces cerevisiae* Biot 88 Using the Application of Plackett-Burman Screening Design

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ABSTRACT

The aim of the present study was to screen the effect of thirteen spices and herbs on the growth of *Pichia burtonii* DA69, and *Saccharomyces cerevisiae* Biot 88. The two strains of yeast were isolated from a locally produced Chinese yeast cake, or *lookpang* in Phrae province. After applying the Plackett-Burman experimental design and linear regression model analysis, it was determined that only six spices and herbs showed a major affect on the growth of the strains. *Alpinia galangal* (Galangal), *Myristica fragrans* (mace), and *Elettaria cardamomum* (Cardamom) had a powerful inhibition of the growth of *Pichia burtonii* DA 69. For the strain Biot 88, *Elettaria cardamomum* (Cardamom), and *Zingiber offinale* (Ginger) inhibited the growth, whereas *Cinnamomum zeylanicum* (Cinnamon) and *Piper nigrum* (Pepper) supported the growth.

Keywords: plackett-burman experimental design, rice wine, chinese yeast cake, spices, herbs.

1. INTRODUCTION

Rice wine is one of the most popular alcoholic beverages in rural areas of Thailand. Small-scale production of rice wine can be found in almost every individual household, although recipes and processes for rice wine production vary between regions. In principle, the fermentation process consists of two steps: a saccharification of steamed rice starch by fungal enzymes, and a simultaneous alcoholic fermentation by yeasts derived from the traditional inoculum called *lookpang* in Thai or "Chinese yeast cake" in English. The ingredients of Chinese yeast cake are rice flour, cassava flour, and ground rice, or mixtures of the three. They are thoroughly mixed with spices and herbs which are believed to prevent the growth of undesirable microorganism but to have no effect on the growth of the inoculum [1,2]. However, the process of Chinese yeast cake production itself-especially the effect of spices and herbs on the growth of inoculum-has not been analyzed from the perspective of microbiological composition, due to the secrecy of the yeast cake ingredients in recipes passed from generation to generation. The aim of this study is to investigate

the effect of 13 kinds of spices and herbs traditionally used in *lookpang* on the growth of *Pichia burtonii* DA 69, and *Saccharomyces cerevisiae* Biot 88 using Plackett-Burman experimental design.

2. MATERIALS AND METHODS

2.1. Microorganisms and Cultivation

The microorganisms, *Pichia burtonii* DA 69, and *Saccharomyces cerevisiae* Biot 88 were isolated from local Chinese yeast cakes in the Phrae province, Thailand with a high level of the strains' capability in their production of ethanol at high levels, and their ability to impart attractive flavor and color to rice wine. Yeast inoculum was prepared by inoculating a loop of the yeasts in 10 ml of YM medium, and incubating the culture at 35 °C for 1 d. In order to test the spices as shown in Table 1, the starter was added into 90 ml of spice mixed YM medium, and then they were incubated at 35 °C and shaken at 250 rpm for 2 d.

2.2. Screening of Factors Affecting on Microbial Growth

Plackett–Burman factorial design was employed for screening types of spice for support growth of the microorganisms. Thirteen types of spices and herbs were evaluated the growth of the strains. Based on Plackett–Burman factorial design, each factor was examined at two levels: -1 for low level and +1 for high level [3,4]. Plackett–Burman experimental design is based on the first-order polynomial model:

$$Y = \beta_0 \Sigma\beta_i x_i \quad (1)$$

where Y is the response (growth of microorganisms), β_0 is the model intercept and β_i is the linear coefficient, and x_i is the level of the independent variable. The growth measurements were carried out in triplicate and the averages of the growth were shown as response Y (Table 1). High percentage of confidential values was considered to have a major effect on the growth. The direct count of *Pichia burtonii* DA 69, and *Saccharomyces cerevisiae* Biot 88 was performed using a haemacytometer for their growth measurement. The Plackett–Burman factorial design was statistically analyzed using Statistix *8*, Analytical Software, Tallahassee, FL, USA.

3. RESULTS AND DISCUSSION

The Plackett-Burman screening design was used in this study to evaluate the main effects of thirteen independent variables. The design identified the preliminary effect on the microbial growth during the process of inoculum-making for rice wine production. As shown in Table 1 and equation 2, the response of the microbial growth was expressed as Y_1 for *Pichia burtonii* DA 69,

$$\begin{aligned} Y_1 = {} & -33.9X_1 - 79.8125X_2 - 40.7375X_3 - \\ & 17.4875X_4 - 54.2375X_5 + 40.25X_6 - \\ & 5.0125X_7 + 34.025X_8 + 30.4125X_9 - \\ & 37.7375X_{10} - 73.125X_{11} - 26.7375X_{12} - \\ & 3.575X_{13} + 245.875 \end{aligned} \quad (2)$$

To test the fit of the model equation**,** the nearer value of R^2 to 1 could be explained the better fit for variability of the experimental value to the predicted value from the equation. The coefficient R^2 for the equation 2 was 0.8937, interpreting 89.37 % of the variability in the response. The magnitude and direction of the factor coefficient in the equation explained the influence of the effect of the thirteen spices and herbs on growth of *Pichia burtonii* DA 69. The greater magnitude of the coefficient showed a high effect on the response with plus and minus symbols as defined positive and negative effects on the microbial growth. The variables with confidence level greater than 70 % were

Table 1. Plackett-Burman design showing thirteen variables and responses with the concentrations[a] of spices and herbs powders added into the medium during the process of *lookpang* making.

Run	*Allium sativum* (Garlic), X_1	*Alpinia galangal* (Galangal), X_2	*Cinnamomum zeylanicum* (Cinnamon), X_3	*Cymbopogon citratus* (Lemon glass), X_4	*Elettaria cardamomum* (Cardamom), X_5	*Eugenia aromatica* (Clove), X_6	*Glycyrrhiza glabra* (Licorice), X_7	*Piper chaba* (Long pepper), X_8	*Piper nigrum* (Pepper), X_9	*Plumbago indica* (Leadwort), X_{10}	*Myristica fragrans* (Mace), X_{11}	*Myristica fragrans* (Nutmeg), X_{12}	*Zingiber offinale* (Ginger), X_{13}	The number of viable cells of *Pichia burtonii* DA 69 (x 10^6/ml)	The number of viable cells of *Saccharomyces cerevisiae* Biot 88 (x 10^6/ml)
1	0.75	0.01	0.25	0.1	0.5	0.25	0.25	0.1	0.1	0.075	0.05	0.75	0.75	425.0	71.0
2	0.75	0.1	0.75	0.3	0.05	0.25	0.75	0.5	0.01	0.025	0.05	0.25	0.75	315.5	58.0
3	0.25	0.1	0.25	0.3	0.05	0.25	0.25	0.1	0.1	0.075	0.5	0.25	0.75	305.0	13.2
4	0.25	0.1	0.25	0.1	0.05	0.05	0.75	0.5	0.01	0.075	0.5	0.75	0.75	56.0	8.9
5	0.25	0.1	0.75	0.3	0.5	0.05	0.75	0.1	0.1	0.075	0.05	0.75	0.25	65.3	79.0
6	0.75	0.01	0.75	0.1	0.05	0.05	0.75	0.5	0.1	0.075	0.5	0.25	0.25	335.0	189.0
7	0.25	0.01	0.25	0.3	0.05	0.25	0.75	0.5	0.1	0.025	0.05	0.75	0.25	610.0	48.0
8	0.25	0.01	0.75	0.3	0.5	0.05	0.25	0.5	0.1	0.025	0.5	0.25	0.75	190.5	21.8
9	0.75	0.1	0.25	0.3	0.5	0.05	0.25	0.5	0.01	0.025	0.5	0.75	0.25	84.6	7.5
10	0.75	0.01	0.75	0.3	0.05	0.25	0.25	0.1	0.01	0.075	0.5	0.75	0.25	61.0	69.0
11	0.25	0.01	0.75	0.1	0.5	0.25	0.75	0.1	0.01	0.025	0.5	0.75	0.75	260.8	28.0
12	0.75	0.1	0.75	0.1	0.05	0.05	0.25	0.1	0.1	0.025	0.05	0.75	0.75	190.4	71.0
13	0.25	0.1	0.75	0.1	0.5	0.25	0.25	0.5	0.01	0.075	0.05	0.25	0.25	222.6	47.0
14	0.75	0.01	0.25	0.3	0.5	0.05	0.75	0.1	0.01	0.075	0.05	0.25	0.75	195.2	19.0
15	0.75	0.1	0.25	0.1	0.5	0.25	0.75	0.1	0.1	0.025	0.5	0.25	0.25	89.1	9.0
16	0.25	0.1	0.25	0.3	0.5	0.25	0.75	0.1	0.1	0.075	0.5	0.25	0.75	528.0	53.0

[a]The concentrations of spices and herbs powders was prepared in the formula with % (w/v) was used for evaluation.

considered as a major influence on the growth of the strain.

Model coefficients estimated by the linear regression analysis for each variable were implied by the t-value and P-value. The higher value of t-value and the lower P-value indicated the high significance of the corresponding coefficients. From the P value, the first three spices and herbs that negatively influenced the growth of *Pichia burtonii* DA 69 were *Alpinia galangal* (Galangal), *Myristica fragrans* (mace), and *Elettaria cardamomum* (Cardamom) with 0.1671, 0.1905, and 0.285, respectively (Table 2).

$$Y_2 = 12.1625X_1-12.825X_2+20.825X_3-10.0875X_4-14.2375X_5-6.625X_6+5.3375X_7+6.875X_8+13.225X_9+12.4875X_{10}-6.225X_{11}-1.725X_{12}-13.1625X_{13}+49.525 \quad (3)$$

For the growth of *Saccharomyces cerevisiae* Biot 88 (Y_2), as shown in the equation 3 and Table 3, the top four strongest microbial effects were *Cinnamomum zeylanicum* (Cinnamon), *Elettaria cardamomum* (Cardamom), *Piper nigrum* (Pepper), and *Zingiber offinale* (Ginger) with P values of 0.156, 0.2675, 0.2931, and 0.2948,

Table 2. Statistical analysis of Plackett-Burman design showing coefficient values, t- and P- value for each variable and analysis of variance for fitted linear regression model for the growth of *Pichia burtonii* DA 69.

Spices	coefficient	t value	P value	Confidence level (%)
Intercept	245.875	6.56	0.0225	97.75
Allium sativum (Garlic)	-33.9	-0.9	0.4614	53.86
Alpinia galangal (Galangal)	-79.8125	-2.13	0.1671	83.29
Cinnamomum zeylanicum (Cinnamon)	-40.7375	-1.09	0.3909	60.91
Cymbopogon citrates (Lemon glass)	-17.4875	-0.47	0.6869	31.31
Elettaria cardamomum (Cardamom)	-54.2375	-1.45	0.285	71.5
Eugenia aromatica (Clove)	40.25	1.07	0.3955	60.45
Glycyrrhiza glabra (Licorice)	-5.0125	-0.13	0.9059	9.41
Piper chaba (Long pepper)	34.025	0.91	0.46	54
Piper nigrum (Pepper)	30.4125	0.81	0.5026	49.74
Plumbago indica (Leadwort)	-37.7375	-1.01	0.4202	57.98
Myristica fragrans (Mace)	-73.125	-1.95	0.1905	80.95
Myristica fragrans (Nutmeg)	-26.7375	-0.71	0.5498	45.02
Zingiber offinale(Ginger)	-3.575	-0.1	0.9327	6.73

Source	DF	SS	MS	F	P
Lack of fit Linear regression model	13	378,451	29,111.6	1.29	0.5183
Residual	2	45,006	22,503.1		
Total	15	423,457			

R^2 = 0.8937; Residual mean square (MSE) = 22,503.1; Adjust R^2 = 0.2029; SD = 150.010; DF = degree of freedom; SS = sum of square; MS = mean square.

Table 3. Statistical analysis of Plackett-Burman design showing coefficient values, t- and P- value for each variable and analysis of variance for fitted linear regression model for the growth of *Saccharomyces cerevisiae* Biot 88.

Spices	coefficient	t value	P value	Confidence level (%)
Intercept	49.525	5.29	0.0339	96.61
Allium sativum (Garlic)	12.1625	1.3	0.3233	67.67
Alpinia galangal (Galangal)	-12.825	-1.37	0.3041	69.59
Cinnamomum zeylanicum (Cinnamon)	20.825	2.23	0.156	84.4
Cymbopogon citrates (Lemon glass)	-10.0875	-1.08	0.3938	60.62
Elettaria cardamomum (Cardamom)	-14.2375	-1.52	0.2675	73.25
Eugenia aromatica (Clove)	-6.625	-0.71	0.5523	44.77
Glycyrrhiza glabra (Licorice)	5.3375	0.57	0.626	37.4
Piper chaba (Long pepper)	6.875	0.73	0.539	46.1
Piper nigrum (Pepper)	13.225	1.41	0.2931	70.69
Plumbago indica (Leadwort)	12.4875	1.33	0.3137	68.63
Myristica fragrans (Mace)	-6.225	-0.67	0.5744	42.56
Myristica fragrans (Nutmeg)	-1.725	-0.18	0.8707	12.93
Zingiber offinale(Ginger)	-13.1625	-1.41	0.2948	70.52

Source	DF	SS	MS	F	P
Lack of fit Linear regression model	13	27,456.2	2,112.02	1.51	0.4683
Residual	2	2,802.1	1,401.06		
Total	15	30,258.3			

R^2 = 0.9074; Residual mean square (MSE) = 1,401.06; Adjust R^2 = 0.3054; SD = 37.4308; DF = degree of freedom; SS = sum of square; MS = mean square.

respectively. The coefficient values obtained from the equation could explain that *Elettaria cardamomum* (Cardamom), and *Zingiber offinale* (Ginger) inhibited the growth of yeast with the values of -14.2375 and -13.1625, respectively.

Using this experimental design, it was revealed that four spices and herbs (*Alpinia galangal* (Galangal), *Elettaria cardamomum* (Cardamom), *Myristica fragrans* (mace), and *Zingiber offinale* (Ginger)) showed high inhibitory effects on the growth of the two microorganisms, and they might decrease the presence of undesirable microorganisms during the production of inoculum production. There was a report in using several herbs, up to 10, by Dung [1], they studied the effect of oriental herbs on Vietnamese rice wine production. Four herbs (*Atratylodes macrocephala* (Bai Zhu), *Mentha arvensis* (mint), *Curcuma longa* (turmeric), and *Foeniculum vulgare* (fennel)) appeared in the Vietnamese recipes but do not appear in Thai recipes. The researchers used herb extracts with the concentration of 0.1%, but the influence of herb combinations was not investigated. By the experiment with one factor at a time, they revealed that fennel (*Foeniculum vulgare*) had the strongest stimulatory effect on the growth of *Amylomyces rouxii* and *Saccharomyces cerevisae* because of its mineral contents. In the traditional process of rice winemaking, fennel and clove are applied to enhance fragrant flavor and prevent fermentation failure, guarding against microbial spoilage due to their anti-microbial properties. Nevertheless, the rest of herbs (mace, cinnamon, cardamom, licorice, ginger, and clove) also gave an inhibitory effect on the growth of microorganisms. The researchers' findings agreed with the results of this study of Thai rice wine inoculum production: in this study, it was found that adding spices and herbs to *lookpang* inhibited microbial growth, particularly microbial spoilage, and decreased the two strains of starters.

4. CONCLUSIONS

Using of experimental design, the models were proposed and illustrated the quantitative effect of spices and herbs on the growth of *Pichia burtonii* DA 69, and *Saccharomyces cerevisiae* Biot 88. The three spices and herbs which inhibited the growth of *Pichia burtonii* sp. DA 69 were *Alpinia galangal* (Galangal), *Myristica fragrans* (mace), and *Elettaria cardamomum* (Cardamom). For the strain Biot 88, *Elettaria cardamomum* (Cardamom), and *Zingiber offinale* (Ginger) inhibited the growth. For further study, the contamination of rice wine by undesirable microorganisms should be studied during the inoculum production, and the effects of these types of spices and herbs on the production of Chinese yeast cake will be further investigated.

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