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การประเมินแบบจำลองคุณภาพของการพาสเจอไรซ์น้ำก้านดอกเห็ดหลินจือ

Assessment of Quality Models of Lingzhi Stalk Juice Pasteurization

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ก้านดอกเห็ดหลินจือเป็นส่วนประกอบที่ถูกตัดและคัดทิ้งในกระบวนการแปรรูปอาหารและผลิตภัณฑ์ทางการเกษตร ดังนั้นเพื่อเพิ่มมูลค่าของก้านดอกเห็ดหลินจือ การแปรรูปผลิตภัณฑ์น้ำก้านดอกเห็ดหลินจือพาสเจอไรซ์จึงมีความน่าสนใจเพื่อที่จะใช้ประโยชน์จากวัตถุดิบเศษเหลือดังกล่าว อย่างไรก็ตามในระหว่างกระบวนการแปรรูปต้องมีการควบคุมคุณภาพของผลิตภัณฑ์ ดังนั้นจึงมีการประเมินเพื่อตรวจสอบความใช้ได้ของพารามิเตอร์ที่ควบคุมคุณภาพของผลิตภัณฑ์น้ำก้านดอกเห็ดหลินจือ ซึ่งในงานวิจัยนี้มีการใช้สมการที่สามารถสร้างจากแผนแบบแฟกทอเรียลเต็มรูป 3^2 จำนวน 2 ซ้ำการทดลอง เพื่อใช้ทำนายการเปลี่ยนแปลงค่าคุณภาพของผลิตภัณฑ์น้ำก้านดอกเห็ดหลินจือพาสเจอไรซ์ ภายใต้การกำหนดระดับของเวลา (15, 20, 25 นาที) และอุณหภูมิ (60, 65, 70 องศาเซลเซียส) ของกระบวนการพาสเจอไรซ์ จากผลการทดลองพบว่า ทุกสภาวะของการพาสเจอไรซ์สามารถควบคุมคุณภาพความปลอดภัยด้านจุลินทรีย์ให้สอดคล้องกับมาตรฐานของผลิตภัณฑ์น้ำผักและผลไม้ กรณีค่าความเป็นกรดและปริมาณของแข็งที่ละลายได้ไม่มีความแตกต่างทางสถิติในทุกสภาวะของการพาสเจอไรซ์ แต่ในทางตรงกันข้าม สภาวะการพาสเจอไรซ์มีผลกระทบต่อค่า pH ค่าสี และค่าความสามารถในการเป็นสารต้านอนุมูลอิสระของผลิตภัณฑ์น้ำก้านดอกเห็ดหลินจือ โดยสมการทำนายที่สร้างขึ้นจากพื้นผิวตอบสนองสามมิติมีค่าสัมประสิทธิ์การกำหนดสูง ซึ่งแสดงให้เห็นว่าพื้นผิวตอบสนองสามมิติสามารถใช้เป็นเครื่องมือต้นแบบในการทำนายค่าคุณภาพผลิตภัณฑ์น้ำก้านดอกเห็ดหลินจือพาสเจอไรซ์ได้

คำสำคัญ : พื้นผิวตอบสนองสามมิติ ; พารามิเตอร์ที่ใช้ในการควบคุมคุณภาพ ; น้ำก้านดอกเห็ดหลินจือพาสเจอไรซ์

Abstract

A part of Lingzhi stalk had been cut and rejected in food and agricultural production. To emphasize the value-added of this raw material, the pasteurized Lingzhi stalk juice was interested. However, the qualities of whose products should be controlled. Therefore, the validation of control measurement parameters for pasteurized Lingzhi stalk juices was assessed. The predictive equations could be formulated from 3^2 full factorial design with two replicates for predicting the alterations of various quality parameters after pasteurization. The independent factors of pasteurization, including times (15, 20, 25 min) and temperatures (60, 65, 70 ^{o}C), were then set. As results, the microbial quality revelated that all conditions of pasteurization could control the microbiological safety, which were relative to the standard of fruit and vegetable juice products. In case of acidity and total soluble solid characteristics, no significant difference was noted for all pasteurization treatments. In contrast, the conditions of pasteurization affected the value of pH, CIE color, and antioxidant capacity of pasteurized Lingzhi stalk juices. Among the mathematic equations, their predictive response surface models (RSMs) were considered based on high coefficients of determination, it indicated that those RSMs could be used as a prototype for predicting the quality of Lingzhi stalk juices under pasteurization.

Keywords : response surface methodology ; quality control parameters ; Lingzhi stalk juice pasteurization

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Introduction

Lingzhi (*Ganoderma lucidum*) is a macrofungus widely used as the mushroom of durability in Asian countries for 2000 years. Currently, it has been used as folk medicine due to nutraceutical characteristics and pharmacological properties such as immune functions, hypocholesterolemic and coadjutant treatments in diseases, namely, hypertension, cancer, dizziness, insomnia, chronic hepatitis and anorexia, etc. (Taofiq *et al*., 2017; Wang *et al*., 2017; Lu *et al*., 2020). In addition, the *Ganoderma* species are popularly used in the formulation of functional foods because of whose antioxidant activity of phenolics (Saltarelli *et al*., 2009).

In food and agricultural industry, a part of Lingzhi stalk had been cut and rejected after production. Rashad *et al.* (2019) reported that *Ganoderma* mycelia on six agro-wastes namely, broad bean stalks, cotton stalk, maize straw, rice straw, sugarcane bagasse and wheat straw can be applied for value-added products. However, the qualities of whose products must be controlled. As mentioned before, pasteurized Lingzhi stalk product was certainly interested.

The implementation of response surface methodology (RSM) could be applied to predict the product quality during processing (Gasaluck & Mahidsanan, 2018; Lamo *et al*., 2019; Sawale *et al*., 2020; Sengupta & Bhowal, 2020). In term of quality assurance of product testing must be validated to achieve an appropriate condition based on a control measurement point. Moreover, no previous articles have presented the validation of parameters and rationally applied them for the predictive quality of pasteurized Lingzhi stalk juice product. The present study was to obtain the implantation of our control measurement parameters. The predictive RSM models of Lingzhi stalk juice would be formulated and validated after pasteurization.

Methods

Lingzhi stalk juice preparation and pasteurization treatments

Lingzhi was obtained from Suranakhon market in Nakhon Ratchasima province. A part of stalk was cut and cleaned. The cleaned Lingzhi stalk was dried at 50 ^{o}C for 24 h. Three grams of Lingzhi stalk powder were mixed with dried pandan leaves and lemon honey (120 mL) for decreasing undesirable odor to reach the final volume of 1000 mL. All treatments were carried out by pasteurization. The samples were then cooled to room temperature. Table 1 indicates the information of mathematical symbols, independent factors, and actual levels.

Table 1 The information of each independent factor and level used in the study

Independent factors (Unit)	Symbol of the variables	Levels of pasteurization temperature and time		
		-1	0	1
Temperature (°C)	X_1	60	65	70
Time (min)	X_2	15	20	25

Each quality parameter, temperatures and times were then modelled by RSM as written in Equation (1). It was described by Montgomery (1997).

$$y = beta_0 + beta_1 x_1 + beta_2 x_2 + beta_{11} x_1^2 + beta_{22} x_2^2 + beta_{12} x_1 x_2 + epsilon \ldots\ldots\ldots\ldots\ldots(1)$$

The value of Y is the model response (total soluble solid, pH, acidity, L*, a*, b*, Total phenols, and DPPH radical-scavenging activity), The values of X_1 and X_2 are independent variables. The $beta_{ij}$, $beta_{ii}$, $beta_i$ and $beta_0$ represent the interaction, the quadratic effect, the linear effects as well as the regression coefficients for the intercept, respectively.

Microbial analysis

The microbiological parameters, such as total viable count, yeast and mold count, *Escherichia coli*, and *Staphylococcus aureus*, were monitored in Lingzhi stalk juice samples (FDA-BAM, 2001).

Measurement of total soluble solid, pH, acidity and color

Total soluble solid (°Brix) of Lingzhi stalk juice was observed by ATC hand refractometer.

A digital pH meter (Fisher scientific model AB15) was used to measure pH value of each Lingzhi stalk juice. Before analysis, the pH meter was calibrated by standard buffer solutions, such as pH 4 and pH 7.

Titratable acidity (% citric acid) of the Lingzhi stalk juice sample was measured (AOAC, 2000).

To measure the color, each sample was evaluated using a Chroma meter CR-410 (Konica minolta, Japan). The color parameters, L* (brightness), a* (redness / greenness), and b* (yellowness / blueness), were observed.

Measurement of total phenols

The amount of total phenols in each Lingzhi stalk juice sample was measured (Singleton *et al.*, 1999). The 1.0 mL aliquot of Folin-Ciocalteu (10-fold diluted solution) was mixed with sample (0.5 mL) and left for 6 min at room temperature. After inoculating 2.0 mL sodium carbonate (200 g/1000 mL), the mixture was incubated at room temperature for 60 min in a dark condition. The absorbance was then measured at 760 nm. Finally, the amount of total phenols in each sample was expressed by mg of gallic acid equivalents (GAE) per gram of sample.

Measurement of DPPH radical-scavenging activity (RSA)

RSA was analyzed by measuring the direct DPPH-scavenging activity in the samples (Modified from Krüzselyi *et al*., 2020). DPPH (0.25 mM) was prepared in ethanol, and 0.5 mL of this solution was incubated with 1 mL of various Lingzhi stalk juice samples. Sample blanks were prepared for each dilution (0.5 mL ethanol + 1 mL Lingzhi stalk juice) and a control tube of DPPH was used to determine the maximum absorbance (0.5 mL DPPH 0.25 mM + 1 mL ethanol). All mixtures were incubated at room temperature in the dark condition for 30 min. The absorbance of DPPH was measured at 517 nm. The percentage of RSA of each sample was calculated via the Equation (2):

$$\% \text{ RSA} = [(A_{\text{DPPH}} - A_{\text{sample}})/A_{\text{DPPH}}] \times 100 \ldots\ldots(2)$$

Statistical analysis and predictive model validations

A 3^2 full factorial design with two replicates was performed to predict the alterations in the various quality parameters of Lingzhi stalk juices. Analysis of variance (ANOVA) of each parameter was analyzed to estimate the significance ($P<0.05$) of the main effects, its interactions, regression coefficients, and coefficients of determination (R^2 values). Design-Expert® software (version 7.0) was performed for analyzing the three-dimensional plots of RSM. The data of normal probability plots of residuals were considered for all physico-chemical parameters of Lingzhi stalk juices.

Results

Microbiological qualities of each condition of Lingzhi stalk juices

The microbiological qualities of all Lingzhi stalk juices were considered after pasteurization. Total viable count, yeast and mold count, and *S. aureus* of all treatments were not detected in undiluted samples. In addition, the MPN index of *E. coli* were not found (<0.03 MPN/mL.) in all conditions.

The predictive models of total soluble solid, pH, acidity and color of Lingzhi stalk juices under each condition

Total soluble solid, acidity, pH and color are key factors that affect the physico-chemical quality of Lingzhi stalk juices. The linear model was significantly fitted to the total soluble solid (Fig. 1A), pH (Fig. 1B) and acidity (Fig. 1C) throughout pasteurization. The RSM models of pH can be expressed by Equation (3). In the parameters of colors, the quadratic models were fitted to L* (Fig. 1D) and b* (Fig. 1F) that were revealed by

Equation (4) and (6), respectively. In contrast, a linear RSM model (Fig. 1E) was fitted to the parameter of a* and written in Equation (5). ANOVA were considered according to the consequences given in Table 2-5. It demonstrates that predictive models of L* and a* were found to be statistically significant (P<0.05), but the lack of fit was not (P>0.05). In term of the interactive effects, there was the significant role of time x temperature interaction in the level of L* (P = 0.0001), whereas the times significantly affected the level of pH (P = 0.0038) and a* (P = 0.0171). However, the b* was not affected by these two factors (P>0.05). Regarding lack of fit of pH and b* models, there were statistically significant (P<0.05) and might be improved for further study.

$$pH = 2.76250-(2.50*10^{-3}*X_1)+(4.67*10^{-3}*X_2)\text{.......}(3)$$

$$L^* = -267.81611+(10.26150*X_1)-(1.36542*X_2) +(0.025550*X_1X_2)-(0.085433*X_1^2)-(7.93333*10^{-3}* X_2^2)\text{.......}(4)$$

$$a^* = 5.02417-(0.028833*X_1)-(0.043000*X_2) \text{.......}(5)$$

$$b^* = -10.47611+(0.48333* X_1)-(0.40000*X_2)+(9.00*10^{-4}*X_1X_2)-(3.73333*10^{-3}*X_1^2)+(8.36667*10^{-3}*X_2^2)\text{........}(6)$$

The predictive models of total phenols and RSA of Lingzhi stalk juices under each condition

The phenolics and antioxidant activity had been emphasized in Lingzhi (Mishra *et al*., 2014). Total phenols and RSA of Lingzhi stalk juices were, therefore, monitored under each condition of pasteurization (Fig. 1G and 1H). The quadratic model was fitted to total phenols and expressed by Equation (7). On the other hand, a linear RSM model was applicable for RSA and presented in Equation (8). According to ANOVA results (Table 6-7), the tests of lack of fit were insignificant in total phenols and RSA (P > 0.05), which indicate that the models were adequately accurate to predict the level of these parameters. In term of the main effects, there was the significant role of temperature in the RSA reduction (P = 0.0060). In contrast to this, the total phenols were not affected by times and temperatures (P>0.05).

$$\text{Total phenols (mg GAE/g)} =-457.82667+(14.91117*X_1)-(2.07092*X_2)+(4.65*10^{-3}*X_1X_2)-(0.1165*X_1^2)+(0.0482*X_2^2)\text{......}(7)$$

$$\text{RSA (\%)} = 194.27306-(-2.55550*X_1)+(0.29333*X_2)\text{.......}(8)$$

Table 2 ANOVA of the modelled pH of Lingzhi stalk juices after pasteurization

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	8.408E-003	2	4.204E-003	7.51	0.0055
Temperature	1.875E-003	1	1.875E-003	3.35	0.0871
Time	6.533E-003	1	6.533E-003	11.68	0.0038
Residual	8.392E-003	15	5.594E-004		
Lack of fit	6.692E-003	6	1.115E-003	5.90	0.0094
Pure error	1.700E-003	9	1.889E-004		
Corrected total	0.017	17			

Table 3 ANOVA of the modelled L* of Lingzhi stalk juices after pasteurization

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	55.25	5	11.05	107.36	< 0.0001
Temperature	33.43	1	33.43	324.84	< 0.0001
Time	0.15	1	0.15	1.41	0.2579
Temperature x Time	3.26	1	3.26	31.71	0.0001
$Temperature^2$	18.25	1	18.25	177.29	< 0.0001
$Time^2$	0.16	1	0.16	1.53	0.2399
Residual	1.24	12	0.10		
Lack of fit	0.24	3	0.080	0.72	0.5646
Pure error	1.00	9	0.11		
Corrected total	56.48	17			

Table 4 ANOVA of the modelled a* of Lingzhi stalk juices after pasteurization

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	0.80	2	0.40	5.20	0.0192
Temperature	0.25	1	0.25	3.23	0.0925
Time	0.55	1	0.55	7.18	0.0171
Residual	1.16	15	0.077		
Lack of fit	0.78	6	0.13	3.11	0.0620
Pure error	0.38	9	0.042		
Corrected total	1.96	17			

Table 5 ANOVA of the modelled b* of Lingzhi stalk juices after pasteurization

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	0.30	5	0.061	1.34	0.3142
Temperature	0.077	1	0.077	1.68	0.2189
Time	0.014	1	0.014	0.31	0.5897
Temperature x Time	4.050E-003	1	4.050E-003	0.089	0.7709
Temperature^2	0.035	1	0.035	0.76	0.3994
Time^2	0.18	1	0.18	3.83	0.0739
Residual	0.55	12	0.046		
Lack of fit	0.48	3	0.16	22.39	0.0002
Pure error	0.065	9	7.189E-003		
Corrected total	0.85	17			

Table 6 ANOVA of the modelled total phenols of Lingzhi stalk juices after pasteurization

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	53.41	5	10.68	1.52	0.2552
Temperature	5.95	1	5.95	0.85	0.3755
Time	7.62	1	7.62	1.08	0.3183
Temperature x Time	0.11	1	0.11	0.015	0.9033
$Temperature^2$	33.93	1	33.93	4.83	0.0483
$Time^2$	5.81	1	5.81	0.83	0.3811
Residual	84.30	12	7.03		
Lack of fit	23.38	3	7.79	1.15	0.3803
Pure error	60.92	9	6.77		
Corrected total	137.72	17			

Table 7 ANOVA of the modelled RSA of Lingzhi stalk juices after pasteurization

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	1984.99	2	992.49	5.19	0.0194
Temperature	1959.17	1	1959.17	10.25	0.0060
Time	25.81	1	25.81	0.13	0.7184
Residual	2868.43	15	191.23		
Lack of fit	357.13	6	59.52	0.21	0.9632
Pure error	2511.30	9	279.03		
Corrected total	4853.42	17			

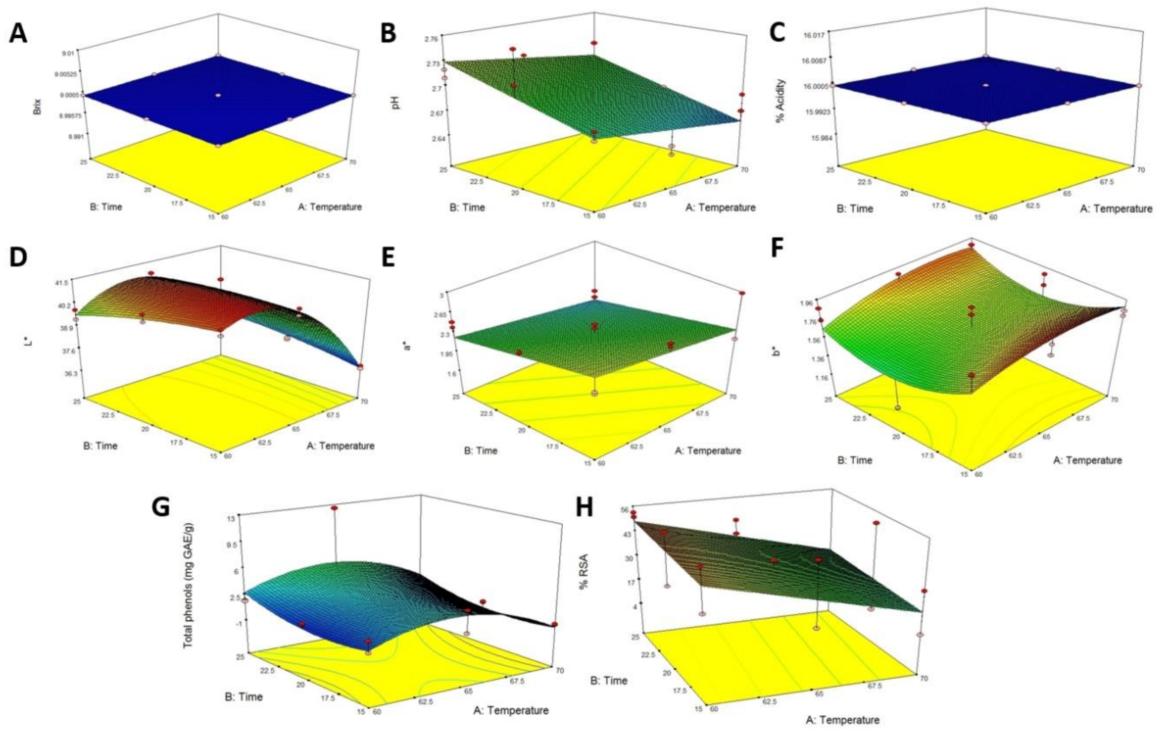

Figure 1 Response surface plots presenting the effects of the investigated times and temperatures on total soluble solid (A), pH (B), acidity (C), L* (D), a* (E), b* (F), Total phenols (G), and RSA (H) of pasteurized Lingzhi stalk juices.

Validation method of control measurement parameter prediction for pasteurized Lingzhi stalk juices

Table 8 reveals Y-intercepts, slope coefficients, and coefficients of determination (R^2) of normal probability plots of residuals for quality parameters in pasteurized Lingzhi stalk juices. It explains that those assumptions of mathematic model errors were proved via normally distributed random from each predictive model (Figure not shown). The coefficients of determination (R^2) of any control measurement parameter prediction accorded to the reliability of predictive response. Those values nearly equal to 1 represent the measured and predicted values.

Table 8 Y-intercepts, slope coefficients, and coefficients of determination (R^2) of normal probability plots of residuals for quality parameters in pasteurized Lingzhi stalk juices

Parameters	Values		
	Y-intercepts	Slope coefficients	R^2
pH	50.116	27.648	0.9285
L*	49.810	28.327	0.9519
a*	49.889	26.939	0.8976
b*	49.721	28.557	0.9659
Total phenols	56.725	47.119	0.9369
RSA	50.097	28.151	0.9467

Discussion

The aim of pasteurization is to destroy microorganisms, to improve safety of food products. In this study, all conditions of pasteurization could control the microbiological safety, that meet the standard criteria of fruit and vegetable juice products (Berthold-Pluta *et al*., 2017). In term of acidity and total soluble solid aspects, no significant difference was revealed for all treatments of Lingzhi stalk juice. The results are in agreement with the research of Aguilar-Rosas *et al.* (2007), they also presented no significant alterations in the level acidity of pasteurized apple juice. In contrast, the pH value was affected by times. The results obtained from CIELAB parameters of pasteurized Lingzhi stalk juices, the conditions of pasteurization could affect the product colors. This might be interpreted as the consequence of thermal processing on the pigment degradation in juices (Choi & Nielsen, 2005). According to antioxidant capacity of Lingzhi stalk juices, it was decreased due to the increase in the temperatures. Most of the previous study in the literature on the antioxidants of fruits and vegetables have revealed that processing operations could decrease the level of antioxidants of foods (Nayak *et al*., 2015; Benattouche *et al*., 2020).

In case of predictive assessments, many researchers confirmed that the R^2 of each RSM predictive model under normal probability plots has appropriate approach, since acceptable levels should be close to 1 (Diniz *et al*., 2014; de Oliveira *et al*., 2016; Gasaluck & Mahidsanan, 2018). Therefore, this consequence can be implied as an approach to verify the RSM equation models and their validation for quality parameters of pasteurized Lingzhi stalk juices.

Conclusion

All pasteurization treatments could maintain the microbiological safety of Lingzhi stalk juices, which were relevant to microbial standard of fruit and vegetable product. With the implementation of RSM models, those validated mathematic equations would be advantageous for further juice pasteurization to control the product quality assurance in the fruit and vegetable production. However, further studies are required to evaluate the sensory characteristics of Lingzhi stalk juice products after pasteurization, while these thermal treatments did not affect the several sensory attributes.

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