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Optimization of Enzymatic Clarification of Sweet Potato Juice using Response Surface Methodology

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Abstract

A study on the optimization of enzymatic clarification of sweet potato juice (Ipomoea batatas) by alpha-amylase was performed using Response Surface Methodology (RSM). The experimental parameters studied focused on incubation temperature (35^{o}C – 45^{o}C), incubation time (31.6 minutes – 140.4 minutes) and the effects of different concentrations of the alpha-amylase enzymes (0.009% – 0.370%). The sweet potato juice was treated with industrial alpha-amylase enzyme according to the conditions set by MINITAB software. The clarity of the juices was measured using the optical density (OD) value obtained from UV-VIS spectrophotometer. From the RSM analysis, the feasible surface and contour plot are identified as that of the maximum value. From feasible target value, the processing conditions were found as; 39.991^{o}C incubation temperature, 0.195% enzyme concentration at 90 minutes of incubation time whereby the absorbance (Abs) of clarity of clarified sweet potato juice can be optimized by R^2 = 80.3%.

Keywords: Sweet potato juice; enzymatic clarification; Response Surface Methodology (RSM)

INTRODUCTION

Sweet potato (*Ipomoea batatas*) is a tuber type plant belonging to the Convolvulaceae family. *Ipomoea batatas* is considered a major crop plant amongst the approximately 50 genera and more than 1,000 species of Convolvulaceae (Nor Akma, 2011). Sweet potato is a very valuable crop, easy to grow and adaptable to various agro climates. Furthermore, it can produce high yield in short period. Presently, this crop is considered having low economic value, but significant social importance. The sweet potato is an important alternative source of carbohydrate, taking the fourth place after rice, corn, and cassava (Nani Zuraida, 2003).Globally, sweet potato plants grow easily all year round (Islam, 2006) and are planted primarily for their storage roots. Sweet potato is cheap yet provides a high nutrition diet with antioxidative traits (Hue et al., 2011).

Sweet potatoes are generally categorized into two types; “dry-fleshed” or “moist-fleshed”. These terms refer to the texture of the flesh when consumed. When exposed to heat, “moist-fleshed” types have the tendency to hydrolyze more starch to sugars and dextrin, thus becoming softer and sweeter than the “dry-fleshed” types (Lerner, 2001).Currently, there are two new varieties of sweet potatoes produced locally by Malaysia Agricultural and Development Institute (MARDI); VitAto and Gendut.

Sweet potatoes contain dietary fibre, minerals, vitamins, and antioxidants, such as phenolic acids, anthocyanins, tocopherol and β-carotene (Wireko-Manu et al., 2010; Suda et al., 2003; Woolfe, 1992). According to Hou et al., (2001), sweet potato is high in vitamin A and a good source of potassium and vitamin C, B6, riboflavin, copper, pantothetic acid and folic acid. Sweet potatoes contain many forms of bioactive compounds such as phenolics and carotenoids, which act as free radical scavengers (Teang pook et al., 2012). Sweet potato has been considered to have high antioxidants, which reduce premature aging and antimutagenicalkaloids along with other components which is essential to human health (Meira et al., 2012; Yoshimoto, 1999). According to Nor Akma, (2011) VitAto is high in β-carotene and is a suitable substitute for wheat flour in cake, bread making and juice production. The locally

produced VitAtovariet of *Ipomoea batatas* was used to make the clarified juice because of its high nutritional content and sweetness.

Sweet potato is a low cost carbohydrate source thatis usually labelled as rural food. In Malaysia, sweet potatoes are mainly consumed boiled or fried. The development of technology for sweet potato processing into a value added products would promote its production and consumption, and increase its economic value (Wireko-Manu et al., 2010). Coggins et al. (2003) reported that sweet potato juice may be consumed as a single, or combined with other juices to form a variety of juice blends. At present, there are many beverages made from fruits and vegetables on the market. McLellan (1990) stated that the demand for drinks and beverages are generally based on their nutritive values, flavors, aromas and colors. To penetrate the market, a novel sweet potato juice with high nutritional value urgently needs to be produced and this will certainly increase the sweet potato economic value (Wireko-Manu et al., 2010).

According to Keshani et al. (2010) demand for quality juice and juice type beverages have been increasing due to its appealing flavour and aroma, nevertheless, clarification process need to be done prior to commercialization because the turbidity and viscous texture of unclarified juice is very unappetizing. The turbidity and viscosity characteristics are mainly attributed by the starch and polysaccharides in the juice, thus the use of amylolytic and pectinolytic enzyme such as amylase and pectinase could effectively clarified the juice (Lee et al., 2006).

Response Surface Methodology (RSM) is important software in the design, development, and formulation of new products as well as in the improvement of present product designs (Diamante and Yamaguchi, 2012). RSM significantly reduces the number of experiments that need to be carried out to evaluate multiple parameters and their interactions, as a result, making the experiments convenient and time saving (Sevda et al., 2012; Surajbhan et al., 2012). The purpose of the present study are (1) to study the effect of enzyme concentration, incubation temperature and incubation time on the clarity of the sweet potato juice,(2) to optimize theenzymatic clarification of sweet potato juice using Response Surface Methadology (RSM).

MATERIALS AND METHODS

Material

Sweet potato (*Ipomoea batatas*) was obtained from Batang Berjuntai, Selangor, Malaysia. Commercial enzyme, alpha-amylase (BAN® 480L) which was obtained from Science Technics Sdn. Bhd, is a brown in color liquid endo-enzyme. This enzyme function tohydrolyzes (1, 4)-alpha-D-glucosidic linkages in starch polysaccharides. It has been used in a variety of industrial processes within food manufacturing.

Juice Processing

Juice extraction process was done according to method adapted from Wireko-Manu et al. (2010). The tuber of sweet potato were sorted out, weighed and washed, knife peeled and cut into smaller pieces and steamed for 20 minutes or until they were soft. Excess water was discarded. The steamed sweet potatoes were homogenized using a food processor with distilled water at a ratio of 1:1 (w/w). Addition of water with the steamed sweet potatoes in 1:1 ratio gives more than 100% of juice recovery and concurrently diluted the juice (Panda, 2010). Next, the mixture was treated with Alpha-amylase enzyme.

Enzymatic treatment

Alpha-amylase enzyme was added according to the experimental conditions and the incubated at the temperature and time period as the condition set by the software MINITAB based on the range of chosen variables (Table 1). After incubation, the mixture was put into a water bath at 90^{o}C for 10 minutes to deactivate the enzyme before being strained with cheesecloth.

Determination of the Clarity of Sweet Potato Juice

The strained juice was let to cool at room temperature before centrifuged for 10 minutes and the supernatant was collected and put into another centrifuge tube. The supernatant was then centrifuged again at 1400 rpm for 10 minutes (Liew Abdullah et al*.,* 2007; Zainal, 2001). Lastly, the supernatant was collected and the absorbance was read at wavelength of 660 nm using UV-VIS spectrophotometer (Hitachi) (Lee et al*.,* 2006). Control, in this study is the absorbance of the tested sample which when thru all the processing steps(X_1= 45°C; X_2 = 120 min) except for enzymatic treatment.

Experimental Design

Response Surface Methodology (RSM) (MINITAB Software Version 14) was used to generate the experimental designs, statistical analysis and regression model. Central composite design with quadratic model was employed. Each independent variable has 5 levels and only -1 and +1 level was chosen (Table 1). The range values of the three independent variables were determined by preliminary study. A total of 20 different combinations of treatment were chosen randomly order according to Central Composite Design (CCD) configuration of three factors. The expression of design is coded (x), actual is (X) levels of variables and (Y) for the response (absorbance). These three test variables were used to optimize the condition for clarified juice treatment by determining the value of absorbance as a response. Experiments were carried out in replicate and the average values were used as the response, Y.

The coding variables that were used in optimization experiments by MINITAB software to determine the experimental design is shown in Table 2. This experimental design is the relevant combination of all test variables which were predicted by MINITAB software in order to obtain the optimum condition for the absorbance of the clarify sweet potato juice.

RESULTS AND DISCUSSION

Statistical Analysis

(a) Optimization of treatment conditions for sweet potato juice:
The clarity was determined under four different combinations of incubation temperature (X_1), incubation time (X_2), and enzyme concentration (X_3)

(b) Response surface regression for the absorbance of clarified sweet potato juice:
At $p < 0.05$, the test variables (X_1, X_1, X_1, X_3, X_3) are considered to give the significant effect on the response of the absorbance of clarified juice. Thus significant regression equation at 5% level for the absorbance (Abs) value in clarified banana juice that can be accepted and expressed as follows:

$$Y = 0.09809X1 – 0.00121\ X_1X_1 – 3.38880\ X_3X_3$$

(c) ANOVA for the overall of absorbance of the clarity of sweet potato juice:
It was found that the only linear and squares test variables that gave significant ($p < 0.05$) effects in absorbance of clarity in sweet potato juice were X_1, X_1X_1 and $X_3\ X_3$. The experimental data were very close to the predicted responses because the correlation value was R^2 = 80.3% as calculated by the MINITAB Software. The model shows that variation of clarity banana juice content was 80.3% and that only 19.7% of variation was due to the other factor that not included in the model.

(d) Response optimizer and overlaid contour plots of the percentage on absorbance (a) of clarity of clarified sweet potato juice:
The overlaid contour plot indicated feasible and possible to carry out and the value was 40^{o}C for the incubation temperature, a period of 90 minutes for the incubation time and 0.195% of enzyme concentration.

(e) Surface and contour plots for optimizer condition for absorbance (a) of clarity on the hold values of clarified sweet potato juice:
The three dimensional surface plots and two dimensional contour plot show the effect of feasible optimum treatment conditions on the absorbance (Abs) of clarity of clarified sweet potato juice. The hold value fixed by MINITAB software is decided on incubation time, which is 90 minutes. From the contour plots (goal: maximum), the colour of the contour gets darker when the value response absorbance (Abs) become increased. The increase of the temperature with the increase of enzyme concentration make the value response absorbance (Abs) become less or lower. The shape of surface plots and the shape of contour were based on regression model and were very close to the data obtained since R^2 = 80.3%.

CONCLUSIONS

The result indicates that sweet potato juice which was treated with alpha-amylase at concentrations 0.195% with 39.991^{o}C incubation temperature for 90 minutes were recommended as the optimum treatment conditions by which the absorbance (Abs) reading for clarity of clarified sweet potato juice can optimized by 80.3%.

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Appendices

Table 1: Coded and uncoded variables used in optimization experiment

Coded	-2.00 (-α)	-1	0	+1	2.00 (α)
Incubation Temperature (^{o}C)	30	35	40	45	50
Incubation Time (min)	30	60	90	120	150
Enzyme Concentration (%)	0.06	0.09	0.1	0.3	0.6

Table 2: Test independent variables and dependent variable (response) of banana juice

Test Variables			Response (Y)
X_1	X_2	X_3	Absorbance (Abs)
35.00	60.00	0.30	0.076
45.00	60.00	0.09	0.107
40.00	90.00	0.02	0.123
45.00	120.00	0.30	0.115
35.00	60.00	0.09	0.080
35.00	120.00	0.30	0.085
40.00	90.00	0.20	0.194
45.00	120.00	0.09	0.118
40.00	90.00	0.20	0.193
45.00	60.00	0.30	0.104
48.41	90.00	0.20	0.124
40.00	90.00	0.20	0.195
40.00	90.00	0.37	0.088
40.00	90.00	0.20	0.195
35. 00	120.00	0.09	0.086
40.00	39.55	0.20	0.213
31.59	90.00	0.20	0.127
40.00	90.00	0.20	0.194
40.00	90.00	0.20	0.194
40.00	140.45	0.20	0.098

Note: X_1: Incubation temperature (^{o}C), X_2: Incubation time (minute), X_3: Enzyme concentration (%).

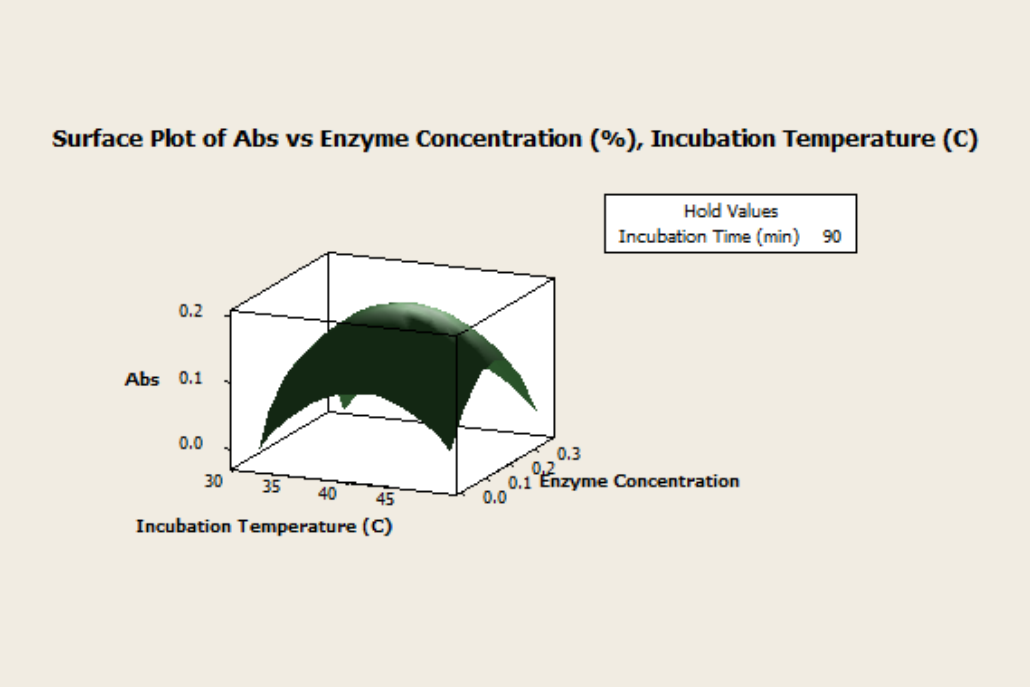

Figure 1 (a)

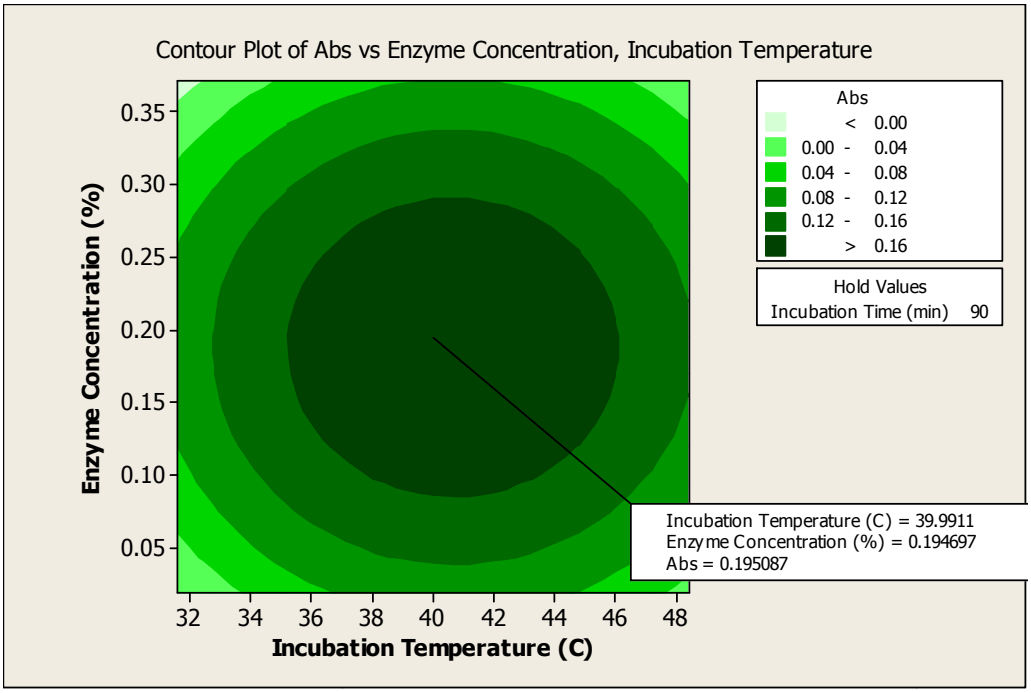

Figure 1 (b)

Figure 1: Contour plot (a) and Surface plot (b) for the Absorbance (Abs) of clarity of clarified banana juice for the feasible optimum condition; hold values: time: 30 minutes incubation time, centrifugation at 2000 g.