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Application of enzymes in feedstock preparation for the production of spray dried soursop (*Annona muricata* L.) powder

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Abstract

Soursop is a local fruit that is susceptible to spoilage. In this study, spray drying was used to preserve the fruit as a dry powder. Prior to drying, commercially available enzyme preparations, Pectinex® Ultra SP-L, Fungamyl® 800 L and Celluclast® 1.5 L (Novozymes, Denmark), were used in a pre-treatment step to macerate and liquefy soursop mesocarp. Results showed that there was a synergetic effect when the pectolytic enzyme was applied together with the cellulolytic enzyme where the liquefied puree was obtained within shorter incubation time and was smooth with low viscosity, making the puree suitable as feedstock for spray drying. RSM study on the effect of Celluclast® 1.5 L concentration (0 to 2 % v/w) at fixed Pectinex® Ultra SP-L concentration (1.5 % v/w), maltodextrin level (20 to 40 % w/w), and spray drying inlet temperature (130 to 160 °C) gave a polynomial model that was significantly ($p \leq 0.05$) fitted for process yield, moisture content, water activity, hygroscopicity, and stickiness. Multiple optimization of the process indicated Celluclast® 1.5 L concentration of 1.29 % (v/w) with maltodextrin of 37.42 % (w/w) that spray dried at inlet temperature of 156 °C was able to produce soursop powder that had optimal properties.

Keywords: soursop, enzyme, macerate, liquefy, Pectinex® Ultra SP-L, Celluclast® 1.5 L, spray drying, RSM

INTRODUCTION

Annona muricata L. is commonly known as soursop (English) or *durian belanda* (Malay) and originated from the Caribbeans (Nakasone and Paull, 1998; Alberto and Ganzel, 2004). In recent years, soursop has received considerable attention from the public because of the nutritional and health protective values of this fruit. Studies by Onyechi et al., (2012) found that soursop drink contains an appreciable amount of micronutrients. This suggests soursop can be processed and incorporated into human diet in order to improve nutritional status of consumer. Soursop fruit is harvested at the mature and firm stage before they begin to ripen fully (Janick and Paull, 2008). However, one of the apparent features of this fruit is soursop soften and bruises easily, and releases an unpleasant strong smell when ripe (Allen, 1967). It is associated with rapid respiration rate of soursop fruit. Worrell et al. (1994) reported that soursop fruit has a double sigmoidal growth pattern with a high respiration rate and this makes the fruit has a short shelf life.

Raw soursop fruit can be further processed into intermediate products such as puree by blending the fruit pulp with water (Umme et al., 1996 and Emmanuel et al., 2006). However, excessive energy is required to remove water for further processing such as drying. One way to overcome this problem is by incorporation of enzymatic maceration of fruit pulp which requires less water to produce puree. In food processing industry, enzymatic mash treatment is a well-known modern process to gain higher yield of

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extraction, increase press capacity, improve clarification and maceration. Pectinases, cellulases, and amylases are often used to catalyse the maceration reaction.

Spray drying is a commercial drying technique used to transform liquid raw material into solid product. The principle is generally involves pumping of food slurry into drying chamber by atomization and dispersed into fine droplet when contacted with current of heated air (Bhandari et al., 2008). Moisture in the droplet evaporated and the dried particle is separated from the drying medium in cyclone due to centrifugal force into collection vessel (Mujumbar, 2015). This operation is favourable because rapid drying process able to preserve the highest quality of raw material, hence it is suitable for heat sensitive material. Applications of spray drying operation are such as production of mango powder (Cano-Chauca et al., 2005), watermelon powder (Quek et al., 2007), acerola pomace extract powder (Moreira, 2009), and guava powder (Patil et al., 2014).

In this study, selected maceration enzymes were used to macerate and liquefy soursop pulp into puree which was then used as a base feed of spray drying process. The effect of independent variables (enzyme concentration, maltodextrin concentration, and inlet temperature) in the drying process on the physicochemical properties of soursop powder and optimization of the spray drying condition were investigated.

MATERIALS AND METHODS

Preparation of soursop puree

Soursop fruit (*Annona muricata* L.) were purchased from a farm located at Simpang Renggam, Johor, Malaysia. Fruit pulp was vacuum packed and stored at -20 °C ± 1 °C after the skin was removed. Before reaction with enzymes, the frozen pulp was thawed at room temperature. One hundred grams of pulp was placed in separate 250 ml beakers and added with different types of enzymes (Pectinex® Ultra SP-L® Ultra SP-L, Celluclast® 1.5 L and Fungamyl® 800 L) at different concentration (0 to 5% v/w). The pulp was then incubated at 50 °C and 100 rpm for 2 hours. Soursop puree that resulted was heated at 80 °C for 30 seconds to inactivate the enzymes and then stored at -20 °C ± 1 °C before analysis.

Spray drying of soursop powder

A mini spray dryer (Model B-290, Büchi, Switzerland) was used for the spray drying process. Based on Response Surface Methodology (RSM) experimental design, three independent variables namely concentration of Celluclast (X_1), concentration of maltodextrin (X_2), and inlet temperature (X_3) with five levels of each variable (Table 1) were used to study the effect on soursop powder. Different concentrations of Celluclast® 1.5 L (0 to 2 % v/w) was added to one hundred grams of soursop puree produced using 1.5 % v/w Pectinex® Ultra SP-L and incubated at 50 °C for 90 minutes to liquefy the puree. The liquefied puree was then added with different concentrations of maltodextrin (20 to 40 % w/w). Inlet temperatures ranged from 130 °C to 160 °C.

Table 1. Five levels of independent variable designed from central composite design (CCD).

Independent variable	Independent variable level				
	Axial (- α)	Low	Centre	High	Axial (+ α)
Concentration of Celluclast® 1.5 L (% v/w)	0	0.39	1.0	1.61	2.0
Maltodextrin level (% w/w)	20	23.88	30	36.12	40
Inlet temperature (°C)	130	136	145	154	160

Polynomial model was generated as Equation 1:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2$$

Where Y was response based on different dependent variables, β_0 was constant coefficient, β_1, β_2, and β_3 were linear coefficients, β_{12}, β_{13}, and β_{23} were interaction coefficients, β_{11}, β_{22}, and β_{33} were quadratic coefficients and X_1, X_2, and X_3 were independent variables.

Minitab version 16.0 was applied to create the experiment design matrix, analysed responses and optimized the level of each independent variables of the models. Multiple regressions were used to predict the linear, quadratic and interaction terms of independent variables in response surface models and generated final reduced models. Data collected was analysed and all variables were optimized using Minitab v16.0 statistical software (Minitab Pty Ltd, Sydney).

RESULTS AND DISCUSSION

Maceration and liquefaction of soursop

1. Overview.

Addition of an appropriate enzyme helps to hydrolyze the pectin matrix in the soursop pulp. Pectinex® Ultra SP-L (polygalacturonase) breaks the glysosidic bonds among the pectic substances that holding the plant cell (Aguilo-Aguayo et al., 2009). Once the cell wall matrix was disrupted, the plant tissues were softened. The cell content is then released due to the loss of attraction that holds cells together (Landbo and Meyer, 2001). Broek et al., (1997) who studied the effect of Pectinex® Ultra SP-L on potato tuber discovered that treatment with Pectinex® Ultra SP-L resulted in higher release of single viable cells and smaller clumps of cells. Besides, Landbo and Meyer (2001) proved that addition of Pectinex® Ultra SP-L able to enhance degradation of cell wall indicated by increasing amount of phenol compound and carbohydrate content.

2. Research data.

Control soursop pulp which was not treated with enzyme had no maceration effect because the soursop flesh was found retained with the pulp structure after incubation (Figure 1a). The same result was obtained when pulp was treated with Fungamyl® 800 L (Figure 1b). On the other hand, soursop pulp added with Pectinex® Ultra SP-L yielded macerated puree after incubation (Figure 1c). The soursop pulp was well hydrolyzed and the juice was released. For soursop added with Celluclast® 1.5 L, there was no maceration effect observed as the pulp remained intact. However, there was noticeably juice extracted out and made the soursop pulp slight watery (Figure 1d).

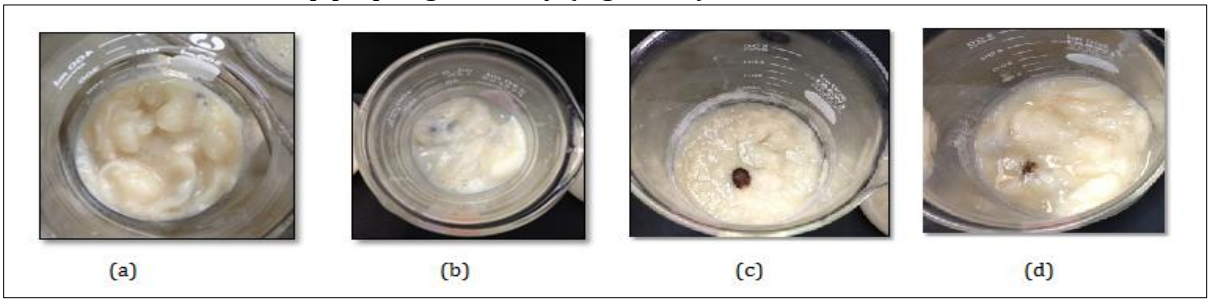

Figure 1. Effects of enzymes on raw soursop after incubation (a) Control (without enzyme) (b) Fungamyl® 800 L (2%) (c) Pectinex® Ultra SP-L (2%) (d) Celluclast® 1.5 L (2%)

Pectinex® Ultra SP-L was chosen as the best enzyme used to hydrolyze soursop fruit into macerated soursop puree. Puree produced when 1.5 % v/w pectinase was used was not significantly different from puree produced with pectinase 2.0 % v/w. Thus, the best treatment used to macerate puree to produce suitable spray drying base was Pectinex® Ultra SP-L at 1.5 % v/w.

Optimization of spray drying of soursop powder

1. Overview.

In present study, five important response functions (process yield, moisture content, water activity, hygroscopicity, and stickiness of powder) showed significant and fitted into the regression model. Optimization of multiple responses was carried out and the final conditions were considered optimum if the process yield was as high as possible, while for moisture content, water activity, hygroscopicity, and stickiness of powder were as low as possible. Generated results showed the soursop puree that was pre-treatment with cellulase concentration of 1.29 % (v/w) and added with maltodextrin 37.42 % (w/w) and subjected into spray drying inlet temperature of 156 °C was predicted to produce powder with overall optimum physical properties.

2. Research data.

A second order polynomial regression model was suitable to fit all response variables. All regression models showed statistically significant at 99 % confidence level ($p \leq 0.05$) [Table 3]. Besides, all five response variables had coefficient of determination (R^2) value more than 0.86 (Table 2). This suggested high proportion of the response can be explained and predicted from the final reduced model.

Table 2. Regression coefficients, coefficient of determination (R^2), F and p-value, and lack-of-fit test value for three independent variables (Minitab 16.1.0)

	Process Yield (%) Y_1	Moisture content (%) Y_2	Water activity Y_3	Hygroscopicity (g/100g) Y_4	Stickiness (g) Y_5
R^2	0.92	0.95	0.87	0.89	0.86
Regression	0.000	0.000	0.000	0.000	0.000

CONCLUSIONS

The following conclusions can be drawn from the study:

- Pectinase is able to hydrolyze soursop fruit to produce macerated soursop puree.
- Synergetic effect of Pectinex® Ultra SP-L with Celluclast® 1.5 L was favorable because the puree produced was smooth and liquefied within shorter incubation time.
- RSM results showed the polynomial model was significantly ($p \leq 0.05$) fitted response variables of process yield, moisture content, water activity, hygroscopicity, and stickiness.
- Multiple responses optimization result showed the soursop puree that pre-treatment with cellulase concentration of 1.29 % (v/w) and added with maltodextrin 37.42 % (w/w) subjected into spray drying inlet temperature of 156 ºC was predicted to produce high powder yield with low moisture content, water activity, hygroscopicity, and stickiness.

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