



Original Article

Continuous extraction of prebiotics from jackfruit seeds

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Abstract

Prebiotics are functional foods with health-promoting properties that are currently used in many developed countries, such as the United States, Japan, and the EU. The synthesis method is still the main commercial production method. There are only a few direct extractions of natural oligosaccharides from plants in Thailand due to the lack of extraction devices. This research aims to design and construct the continuous extractor and study the optimum conditions of prebiotics extraction from jackfruit seed. Jackfruit seeds were extracted with 50% ethanol as a solvent. The response surface methodology was applied for experimental design to study the effects of temperatures (40-60°C), extraction times (15-45 min), and L/S ratios (6:1-10:1 v/w) in laboratory scale continuous extraction. The extraction efficiency was based on the extraction yield and the amount of non-reducing sugar, which is expected to be prebiotics. The optimum condition was the extraction time of 15 min at 60°C and L/S ratio 10:1 (v/w), which gave the maximum non-reducing sugar content of 491.70 mg/g extract from RSM modeling. This optimum condition was applied for pilot scale continuous extraction. The pilot scale continuous extraction unit composes of three 70-L stainless steel extraction tanks equipped with an indirect steam chest for process heating. The heating tank is an 88-L stainless steel vessel. Each extraction pot is connected to a solution pot. After extraction the solution was pumped to a large evaporation tank (60 L) and a small evaporation tank (7 L), respectively. With three-stage extraction the average extraction yield was 20.25% and the average non-reducing sugar content was 400 mg/g extract.

Keywords: continuous extraction, jackfruit seed, oligosaccharide, prebiotics, response surface methodology

1. Introduction

A prebiotics is defined as “nondigestible food ingredient(s) that beneficially affects host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” (Gibson and Roberfroid, 1995). The stimulated bacteria should be of a beneficial nature, namely bifidobacteria and lactobacilli (Gibson *et al.*, 1999). At least three criteria of prebiotics are required: (1) the substrate must not be hydrolyzed or absorbed in the stomach or small intestine, (2) it must be selective for beneficial commensally bacteria in the colon such as the bifidobacteria,

(3) fermentation of the substrate should induce beneficial luminal/systemic effects within the host (Manning and Gibson, 2004). Prebiotics are typically carbohydrates, such as oligosaccharides (Van Loo *et al.*, 1999).

Prebiotics are used in many developed countries, such as the U.S.A., Japan, and the EU. The market for prebiotics in food is growing rapidly. A 2007 report on the world prebiotics market states that there are over 400 prebiotics food products and more than 20 companies producing oligosaccharides and fibers used as prebiotics (<http://www.ubic-consulting.com/template/fs/The-World-Prebiotic-Ingredient-Market.pdf>). The European prebiotics market is currently worth €87 million, and will reach €179.7 million by 2010 (<http://www.frost.com>). Prebiotics can also be purchased in supplement form with some prebiotics commanding as much as €700 per kg of supplement capsules. The strict market for added prebiotics

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ingredients in functional foods are as defined, in the EU, U.S.A. and Asia, totals some 25,000 tones, which is forecast to rise in volume by more than 6% per year. However, the European market remains relatively small at €880m in terms of finished product sales values. Market potential though is considered high. (<http://www.ingredientsdirectory.com/reports/report2.pdf>)

Jackfruit (*Artocarpus heterophyllus, lamk*) is widely cultivated in Thailand. In 2003, Thailand has jackfruit produced 828,611 tons, with jackfruit seed as a by-product of about 120,000 tons (Department of Agricultural Extension, 2003). Jackfruit seed was selected as an agricultural material in this study.

Among the different existing techniques, single pot extraction in batch mode is the most widely practiced one in the herbal industry. The disadvantages of single pot extraction include high solvent consumption, long extraction time, and low extraction efficiency. Microwave or ultrasonic extraction provides better yield and faster speed but suffers from high-energy cost. Another attractive alternative is multi-stage countercurrent extraction, which combines circulatory dynamic extraction and continuous countercurrent extraction technologies (Shen, 2001). Studies on multi-stage countercurrent extraction have been reported as early as 1980 and since then the processing technique and equipment have been under continuous development (Shen and Dai, 1997). Wang *et al.* (2004) investigated many technologies for extraction of glycyrrhizic acid from licorice, including batch single pot extraction, batch double pot extraction, microwave-

assisted extraction, ultrasonic extraction, Soxhlet extraction, room temperature extraction, and multi-stage countercurrent extraction. It was found that the multi-stage countercurrent extraction process offers the highest glycyrrhizic acid extraction yield. The stage numbers of continuous extraction have a slight affect on extraction yield or extract concentration, as shown in the theoretical extraction yield reach of 93.3% and 98.4% with three-stage and five-stage, respectively (Wang *et al.*, 2004).

An extraction unit developed in Thailand is not available and imported units are extremely expensive. For example, the cost of Digmaz extractor RWBL model (10 L) of Olds College School in Alberta Canada, which comprises of a 10-L extraction tank, solvent tank, condenser, distillate vessel and EMSR measurement and control unit (Figure 1) is currently 7,500,000 baht. So, this research aims to develop a multi-stage countercurrent extraction process that is suitable for Thai agricultural products and can be applied to use in the industries. In addition, the extraction condition of prebiotics from jackfruit seeds was optimized using RSM technique.

2. Materials and Methods

2.1 Sample preparation

Prebiotics extraction from 32 Thai crops using 50% (v/v) ethanol as a solvent was pre-studied and the plants given high amount of indigestible polysaccharide were selected. Jackfruit seed gave high amount of indigestible

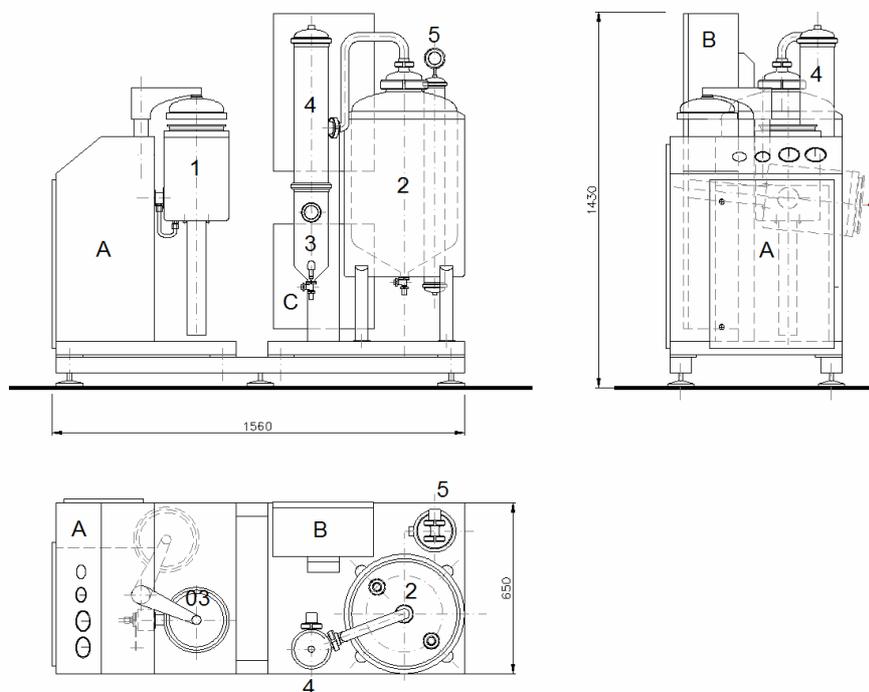


Figure 1. Schematic of the Digmaz-extractor RWBL model 10 L, A is Plant casing, B is Electrical-control cabinet, C is Vacuum pump, 1 is Extractor, 2 is Solvent vessel / Extract tank, 3 is Distillate vessel, 4 is Cooling unit, and 5 is Heat exchanger (Terry, 2006).

polysaccharide and moreover the extract could selectively stimulate the growth and/or activity of 3 kinds of probiotics, *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Bifidobacterium bifidum* (Paiboon, 2007).

The jackfruit seeds used in the experiments are Tongprasert species, which were obtained from Tesco Lotus, Hat Yai. The jackfruit seeds were prepared by washing, drying, slicing, milling and sieving into a size of 2.00 mm. The prepared samples were frozen at -20°C and stored at this temperature.

2.2 Continuous extraction procedure

Extraction procedure in this work was modified from the continuous extraction process by Wang *et al.* (2004). The non-reducing sugar concentration gradients of the sample slurry and the extract in different extraction pots are illustrated in Figure 2. The sample slurry and the extract are represented by the symbols circle (○) and square (□), respectively, while the number of star symbols ‘*’ inside the circles or the squares represents the relative concentration of non-reducing sugar content in them. All the extracts transfer along the direction indicated by the arrows, while the sample slurry or extract discharges are expressed by (↓).

The continuous extraction process could be divided into two stages: the conditioning stage followed by the extraction stage. The conditioning stage consists of Pre-process 1 and Pre-process 2. In the sample conditioning stage, jackfruit seeds were mixed with the appropriate amount of 50% (v/v) ethanol in each extraction pot. The sample slurry inside the extractor was continuously extracted for a pre-determined time period. After the completion of the above conditioning operation, the extraction stage started according to the sequence of steps illustrated by the flow diagram in Figure 2. Processes 1–3 in the diagram illustrate the mass transfer of non-reducing sugar content between the solvent and the sample slurry in each step. Each step consists of four basic operations. For example, in process 1 the operations include (1) sample extraction for a pre-set time; (2) discharge of sample slurry from unit A and collection the extract from unit C; (3) transfer of solvents in the directions of A → C, B → A; (4) addition of jackfruit seed sample and fresh solvent to units A and B, respectively. Processes 1–3 illustrate the operational sequence of the first cycle of the process. This is followed by the second cycle in which the sequence of operations from processes 1 to 3 was repeated.

2.3 Analytical analysis

The yield of prebiotics was calculated as a percentage of the weight of extraction per weight of seeds (dry basis) with Extraction yield (%) = (extracted weight/dry raw material weight) × 100%.

The total sugar content was determined by the reaction of sugars with phenol in the presence of sulfuric acid using glucose as a standard (Dubois *et al.*, 1956). The reduc-

ing sugar content was determined by modified dinitrosalicylic acid method using glucose as a standard (Miller, 1959; Robertson *et al.*, 2001), and non-reducing sugar content was calculated as following

$$\text{Non-reducing sugar} = \text{Total sugar} - \text{Reducing sugar}.$$

2.4 Experimental design for continuous extraction at laboratory scale

Response surface methodology (RSM) was employed to optimize multiple variables to predict the best performance conditions with a minimum number of experiments (Lingyun

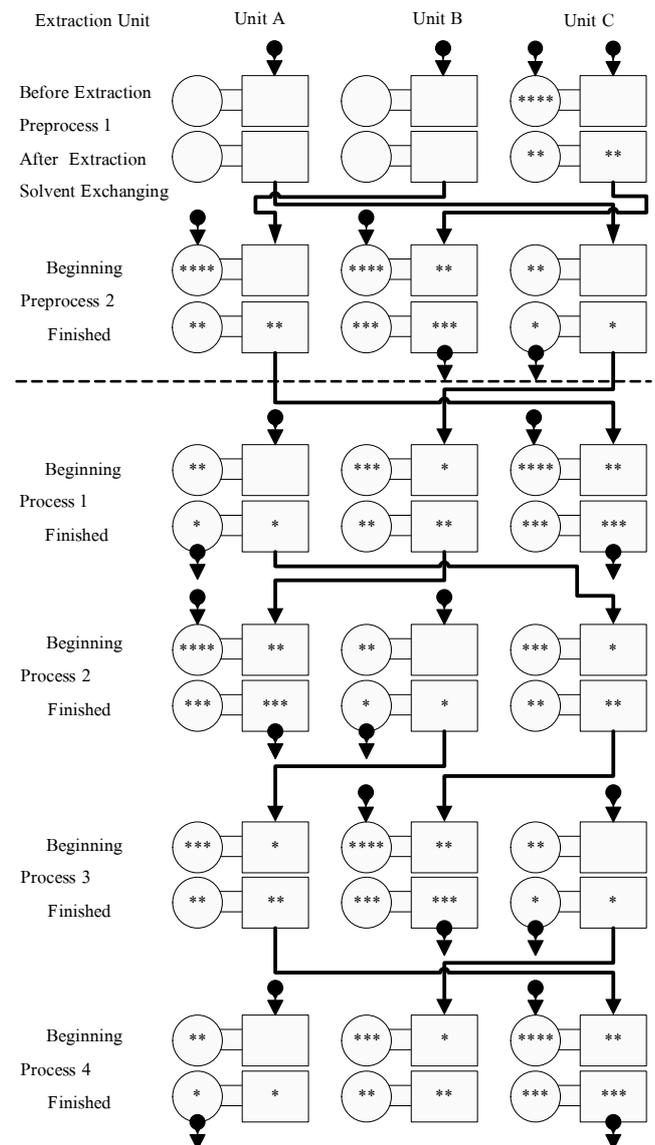


Figure 2. Flow diagram of the three-stage continuous extraction process, symbols circle (○) is raw material, symbols square (□) is extracted solvent, number of star symbols ‘*’ inside the circles or the squares is the relative concentration of non-reducing sugar content.

et al., 2006). The optimization process first entails identifying the most important factors in the extraction using the fractional factorial design, then focusing on the critical subset of factors. The steepest ascent design was used to determine the direction towards predicted higher responses (Lingyun *et al.*, 2006). This research investigates the effect of three independent variables (temperature, resident time, and liquid to solid ratio) using a Box-Behnken design (BBD) to optimize the critical factors and maximize the non-reducing sugar content.

The quadratic model for predicting the optimal point was expressed according to

$$Y = \sum A_0 + \sum_{i=1}^3 A_i X_i + \sum_{i=1}^3 A_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 A_{ij} X_i X_j \quad (\text{Eq. 1})$$

where Y is the response variable, $\sum A_0$ is the center point of the system, A_i , A_{ii} , A_{ij} are the regression coefficients of variables for linear, quadratic, and interaction terms, respectively, and X_i and X_j are independent variables ($i \neq j$).

Each experiment was performed in duplicate and the average of non-reducing sugar content was taken as the response, Y. The range of independent variables and their levels are presented in Table 1. The independent variables and their ranges were chosen based on preliminary experimental results. The variables were coded according to the following equation

$$X_i = \frac{x_i - x_0}{\Delta x_i}, \quad (\text{Eq. 2})$$

where X_i was a coded value of the variable; x_i was the actual value of variable; x_0 was the actual value of the x_i on the center point; and Δx_i was the step change value.

2.5 Continuous extraction of prebiotics from jackfruit seeds at laboratory scale

The laboratory scale continuous extraction system comprised of three 250-mL stainless-steel vessels. 20 g of prepared jackfruit seeds were well mixed with 50% (v/v) ethanol in each vessel by shaken at 200 rpm in oil bath. At the first stage the prepared jackfruit seeds were extracted with fresh solvent and this extracted solvent was used for the next stage. The extraction process was shown in Figure 2. After extraction, the solvent was evaporated by a rotary vacuum evaporator. The extract was analyzed for total sugars and reducing sugars. The RSM with Box-Behnken Design was used to decide the optimum condition.

2.6 Continuous extraction at pilot scale

The schematic of a pilot scale continuous extraction instrument is illustrated in Figure 3. Photograph of the continuous extractor is represented in Figure 4 and photograph of the evaporator is illustrated in Figure 5. The instrument consists of three extraction units labeled as A–C. All units had the same configuration and dimensions, consisting of an extraction tank, a solution tank, and a pump. The extraction unit was run in a close loop configuration by letting the solvent from the solution tank flow down to the extraction tank, mixing with the plant material loaded in the sieved tank via nozzle. After extraction the solution was pumped to a large evaporation tank and a small evaporation tank, respectively. The extraction was carried out with a solvent to raw material ratio of 10:1 (v/w) at 60°C for 15 min.

Table 1. The Box-behnken experimental design and results of laboratory scale continuous extraction.

Run	Temperature (°C)	Resident time (min)	L/S ratio (v/w)	non-reducing sugar content (mg/g extract)
1	60	15	8	490.26
2	50	45	10	35.07
3	50	30	8	280.72
4	50	30	8	281.65
5	50	15	6	375.81
6	40	45	8	143.87
7	40	15	8	9.89
8	50	15	10	350.72
9	40	30	10	24.52
10	50	30	8	343.78
11	60	30	10	265.44
12	40	30	6	44.15
13	50	45	6	323.96
14	60	30	6	178.13
15	60	45	8	189.80

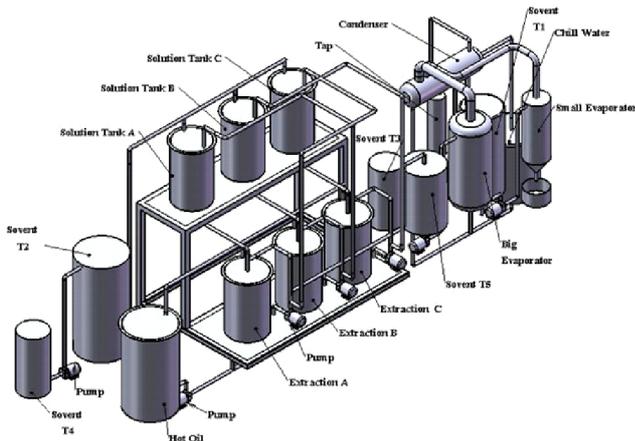


Figure 3. 3-D schematic of continuous extraction instrument.



Figure 4. Photograph of continuous extractor.



Figure 5. Photograph of evaporator.

3. Results and Discussion

3.1 Continuous extractions at laboratory scale

The experimental results of laboratory scale continuous extraction are in Table 1. From the experimental results the extraction with L/S ratio of 8 at 60°C for 15 min gave maximum amount of non-reducing sugar content. By applying multiple regression analysis on the experimental data, the response

variable and the test variable are related by the following second-order polynomial equation:

$$Y = -4138.1 + 149.26X_1 + 37.36X_2 - 1.163X_1^2 - 0.724X_1X_2 - 0.7X_2X_3 \quad (\text{Eq. 4})$$

Where the non-reducing sugar content (Y) can be expressed as a function of extraction temperature (X_1), resident time (X_2) and solvent to raw material ratio (X_3). This polynomial model equation was found to be adequate for prediction within the range of experimental variables as the determination coefficient, R^2 is 0.82. The closer the value of R^2 to the unity, the better the empirical model fits the actual data (Lee *et al.*, 2006). The P-value is used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. The smaller is the value of P, the more significant is the corresponding coefficient. It was found that the linear coefficients (X_1 , X_2), a quadratic term coefficient (X_1^2) and cross product coefficient (X_1X_2) were significant, with very small P values ($P < 0.05$). The other term coefficients were not significant ($P > 0.05$).

The fitted polynomial equation is expressed as surface and contour plots in order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions (Triveni *et al.*, 2001). In the plots two continuous variables were developed for the non-reducing sugar content, while another variable was held constant at its respective zero level (centre value of the testing ranges) (Wu *et al.*, 2007). Figure 6 and Figure 7 show the effect of resident time and extraction temperature on non-reducing sugar content. The non-reducing sugar content reduced with increasing of resident time, but increased with increasing of extraction temperature. The maximum non-reducing sugar content was reached at extraction temperature of 60°C and resident time of 15 min. The effect of extraction temperature and solvent to raw material ratio on non-reducing sugar content is presented in Figure 8 and Figure 9. Higher extraction temperature led to greater amount of non-reducing sugar content while the solvent to raw material ratio did not show significantly effect. The optimum extraction condition predicted by the polynomial equation is extraction with solvent to raw material ratio 10:1 (v/w) at 60°C for 15 min. Under this condition, the model gave predicted Y values (the non-reducing sugar content) of 491.70 mg/g extract. The work done by other researchers (Lee *et al.*, 1987; Nissreen and Mckenna, 1997) also reported that higher extraction temperature increased the effectiveness of oligo-saccharide extraction. However, extraction at too high temperature might cause denaturation of soluble proteins, which thereafter entrapped soluble sugars and impaired the extraction (Kim *et al.*, 2003).

3.2 Continuous extraction at pilot scale

During continuous extraction, the jackfruit seed can be viewed as a stationary phase that was extracted conti-

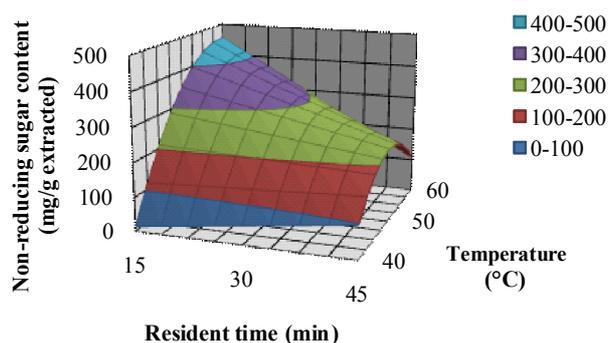


Figure 6. Response surface plots showing the effect of extraction temperature (X_1) and resident time (X_2) on non-reducing sugar content (Y) by extraction using solvent to raw material ratio 8:1 (v/w).

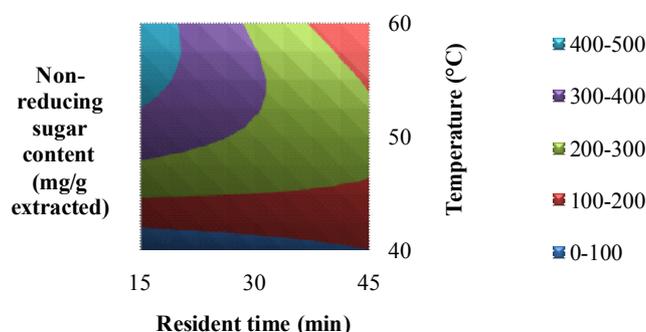


Figure 7. Response contour plots showing the effect of extraction temperature (X_1) and resident time (X_2) on non-reducing sugar content (Y) by extraction using solvent to raw material ratio 8:1 (v/w).

nuously by the solvent flow. The extract yields and non-reducing sugar contents obtained from pilot scale continuous extraction are shown in Table 2. At Preprocess 1 the extraction was only carried out in Unit C. All units (Unit A-C) were run since Preprocess 2. At Preprocess 2 the first extraction with fresh solvent was carried out in Unit A. In Unit B the fresh jackfruit seed sample was extracted with the extracted solvent from Unit C in the previous process. In Unit C the extracted jackfruit seed sample was extracted with fresh solvent. The three-stage extraction was firstly complete at Unit A in Process 2, which the extraction yield of 20.42% was obtained. After that the three-stage extraction was complete at Unit B in Process 3 and at Unit C in Process 4, respectively. It can be seen that the extraction yield increased with the number of stage. However, increasing number of stage also raises the capital and operation cost.

4. Conclusion

The optimum condition of continuous extraction of prebiotics from jackfruit seeds at laboratory scale was in-

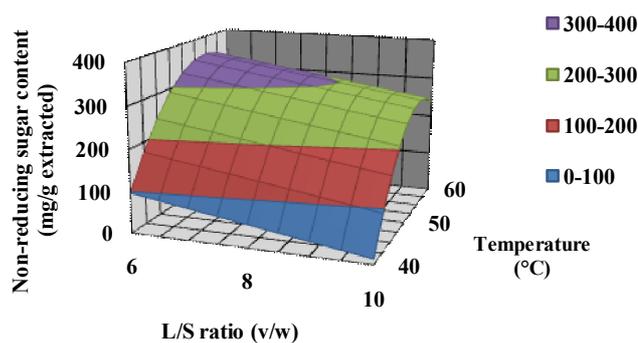


Figure 8. Response surface plots showing the effect of extraction temperature (X_1) and solvent to raw material ratio (X_3) on non-reducing sugar content (Y) by extraction with resident time 30 min.

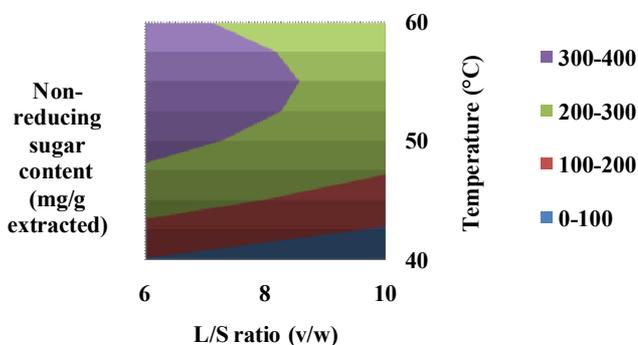


Figure 9. Response contour plots showing the effect of extraction temperature (X_1) and solvent to raw material ratio (X_3) on non-reducing sugar content (Y) by extraction with resident time 30 min.

vestigated by RSM to obtain the desired levels of non-reducing sugar content. The optimum condition was extraction temperature 60°C, extraction time 15 min, and L/S ratio at 10:1 (v/w) using 50% ethanol as a solvent. This condition was applied for pilot scale continuous extraction. The extract stage number of 3 gave the average extraction yield of 20.25% and the average non-reducing sugar of 400 mg/g extract. The pilot scale continuous extraction unit and the extraction procedure developed in this research can reduce the cost, which is a strong bearing in the manufacturing of herbal extraction in Thailand.

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Table 2. Results of pilot scale continuous extraction.

Process	Results of continuous extraction		
	Unit A	Unit B	Unit C
Preprocess 1	Unit A	Unit B	Unit C
Extraction yield (%)	-	-	11.06
Non-reducing sugar (mg/g extract)	-	-	394.28
Preprocess 2	Unit A	Unit B	Unit C
Extraction yield (%)	14.39	20.24	2.14
Non-reducing sugar (mg/g extract)	413.21	391.72	412.64
Process 1	Unit A	Unit B	Unit C
Extraction yield (%)	2.35	8.15	20.08
Non-reducing sugar (mg/g extract)	413.82	401.33	421.13
Process 2	Unit A	Unit B	Unit C
Extraction yield (%)	20.42	1.88	6.39
Non-reducing sugar (mg/g extract)	414.24	460.39	437.71
Process 3	Unit A	Unit B	Unit C
Extraction yield (%)	5.79	20.24	1.36
Non-reducing sugar (mg/g extract)	424.89	361.00	435.07
Process 4	Unit A	Unit B	Unit C
Extraction yield (%)	1.26	6.05	20.08
Non-reducing sugar (mg/g extract)	473.37	417.58	424.75

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