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Optimization of Enzymatic Production of Fructooligosaccharides from Longan Syrup

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Abstract: Fructooligosaccharides (FOS) are nutritive and low calorie sweeteners. They have been attracted and attributed to the expansion of healthy-sugar market due to prebiotic function. Longan is one of the traditional and economic fruits in the Northern of Thailand. In addition to its typical taste and floral flavor, it is a good source of sugars. In some years, over supply of longan is reported, consequently the extra produce was discarded. This research aimed to produce FOS from longan syrup and to optimize the yield of FOS using two enzymes of Pectinex Ultra SP-L and glucose oxidase from 60°Bx of the syrup. The sugar contents of the syrup consisted of sucrose (222.2±3.6 g L^{-1}), glucose (120.3±0.8 g L^{-1}) and fructose (104.7±1.7 g L^{-1}), respectively. Response surface methodology using central composite design was applied to optimize three parameters of FOS production, including pectinase (2.7-7.2 U g^{-1} sucrose), glucose oxidase (1022-4022 U g^{-1} sucrose) and reaction time (7-25 h). Results showed that Pectinex Ultra SP-L concentration had a significant effect ($p<0.05$) on nystose and 1-kestose contents. Optimal values of Pectinex Ultra SP-L, glucose oxidase and reaction time were 3.3 sucrose, 1022 U g^{-1} sucrose and 8 h 41 min, respectively which resulted in the highest amount of nystose (30.27 g L^{-1}) and 1-kestose (123.36 g L^{-1}).

Key words: Fructooligosaccharides, longan syrup, enzymatic production, pectinase, glucose oxidase

INTRODUCTION

Longan (*Dimocarpus longan* L.) is one of the traditional and economic fruits in the Northern part of Thailand, especially in Lamphun and Chiang Mai province. In 2010, Thailand exported longan and longan products about 298,600 Tons, the total value about 6,059.8 million baht (OAET, 2010). Longan is non climacteric fruit which is sweet, juicy and aromatic (Li *et al*., 2009). Generally, the total soluble solid and pH values of fresh longan are about 18.7°Bx and 6.8, respectively. The main sugar compositions of longan are glucose, fructose and sucrose (Yunchalad *et al*., 2008).

Nowadays, consumers pay more attention on functional food and weight control by limiting calories obtained from food in each meal (Yun, 1996). Fructooligosaccharides (FOS) are a nutritive and low calorie sweetener. They have been attracted and attributed to the expansion of healthy-sugar market due to prebiotic function (Jung *et al*., 1989). In the food industry, FOS are used as low-calorie food and nutraceutical ingredients (Vijn and Smeekens, 1999). Application of FOS and FOS-sucrose blends in Indian sweet (gulab jamun) reveals that both types of FOS do not affect color values and sensory scores of product (Renuka *et al*., 2010). Several beneficial aspects of FOS on human health include the increment of the number of bifidobacteria in the large intestine, reduction in total cholesterol and lipid in serum, relief of constipation and general improvement of human health such as immune system activation, resistance to some infections and synthesis of β-complex vitamins and calcium absorption (Hidaka *et al*., 1991; Tomomatsu, 1994). Furthermore, FOS could be used to treat breast cancer, diarrhea and constipation (Roberfroid *et al*., 1998).

FOS are generally found in several kinds of plants and vegetables such as banana, onion, asparagus roots, artichokes, shallot and wheat (Dohnalek *et al*., 1998; Flamm *et al*., 2001). The compositions of FOS consist of sucrose molecules to which 1, 2 or 3 additional fructose units are added by a β-(2-1)-glycosidic linkage to the fructose unit of sucrose, including 1-kestose (GF_2), nystose (GF_3) and 1^F-fructosylnystose (GF_4) (Jung *et al*., 1989). FOS can be produced from sucrose transformation by fructosyltransferase (FTase) or β-fructofuranosidase enzymes from bacterial and fungal sources (Franck, 2002;

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Ghazi *et al*., 2007; L'Hocine *et al*., 2000; Hidaka *et al*., 1988). FTase possess a higher transferring activity than β-fructofuranosidase (Koops and Jonker, 1994). The overall stoichiometry of FTase action on sucrose can be characterized by two parallel reaction paths (Jung *et al*., 1989). A set of disproportion reactions provides FOS and glucose from FTase activity whereas the enzyme hydrolytic activity results in the formation of glucose and fructose as by-products (Aboudzadeh *et al*., 2006). A typical composition of the reaction mixture on the mass basis is as follows: 65 FOS, 25% glucose, 5% fructose and 5% sucrose (Sangeetha *et al*., 2004).

Commercial enzyme preparation called Pectinex ultra SP-L derived from *Aspergillus aculeatus* contains several enzymes including pectinase, cellulase, β-galactosidase and FTase. This enzyme has been used for the production of FOS from sucrose due to a high level of FTase activity. The performance of this enzyme can be converted 450 g L^{-1} of sucrose 450 to 272 g L^{-1} of FOS which contained 224 g L^{-1} 1-kestose and 48 g L^{-1} nystose (Hang and Woodams, 1995; Del-Val and Otero, 2003; Hang and Woodams, 1996). However, glucose obtained from the reaction can be inhibited the FOS production. Efficiency of FOS production can be improved by simultaneous removal of glucose via an enzymatic reaction (Hang and Woodams, 1995; Del-Val and Otero, 2003).

Although, longan syrup is a good source of sugars, there is no information of the production of FOS from longan syrup. Therefore, this research aimed to produce FOS from longan syrup and to optimize the yield of FOS using two enzymes of Pectinex Ultra SP-L and glucose oxidase from 60°Bx of the longan syrup.

MATERIALS AND METHODS

Materials and reagents: Fresh longan fruits (cultivar Edor) were purchased from a local distributor in Chiang Mai which harvested in August 2010. The fruits with a diameter of 20-25 mm (AA size) and free from visual damages and diseases were used in the experiments. Pectinex Ultra SP-L (30 IU mL^{-1}) was purchased from Novo Nordisk Biochem North America Inc., USA. Glucose oxidase (16700 IU mL^{-1}) was kindly provided by Amano Enzyme Inc., Japan. Authentic pure standards (1-kestose, nystose, sucrose, glucose and fructose) were purchased from Sigma Chemical Co., USA. Other chemicals were of analytical grade purchased from commercial sources in Thailand.

Preparation of longan syrup: Fresh longan fruits were washed in tap water for two times and then peels and seeds were manually removed. Longan juice was extracted using a hydraulic press (Sakaya II, Sakaya Automate Co Ltd., Thailand). The obtained juice was boiled and then filtrated through four-layer cheesecloth (20 mesh). Longan juice was further vacuum evaporated in order to get a final solute concentration of 60°Bx.

Quality analyses of longan syrup: The quality of longan syrup was determined including Total Soluble Solids (TSS) was measured using a hand refractometer (Atago Co Ltd., Tokyo, Japan). Color (CIE L*, a* and b*) of longan syrup was measured using a Hunter LAB (Colorquest XE, Hunter Associates Laboratory, USA). Water activity was determined at 25°C using an AquaLab (model series 3, Decagon Device Inc., USA). A digital pH meter (F-22, HORIBA, Japan) was used for pH measuring. Sugar contents were analyzed using a high performance liquid chromatograph (HPLC Agilent series 1200, Waldbronn, Germany) coupled with a refractive index (RI) detector. All samples was diluted in HPLC water (RCI-Labscan, Thailand) and then filtered through a 0.45 μm membrane disc. Twenty μL of prepared sample were injected into a Rezex RSO-Oligosaccharides column (Ag^+ form, 200×10 mm, Phenomenex, Torrance, USA). The column and the RI detector temperatures were maintained at 40 and 32°C, respectively. HPLC water was used as a mobile phase at a flow rate of 0.25 mL min^{-1}. The interpretation and quantification were carried out by comparing with retention times and quantity of authentic sugar solutions. Standard sucrose, glucose, fructose, 1-kestose and nystose were prepared at the concentration 0, 0.5, 1, 2, 4, 6, 8 and 10 mg mL^{-1}.

Optimization of enzymatic production of FOS: A central composite experimental design with five stars ($\alpha = 1.682$) and three replicates at the center point was employed. The purified Pectinex Ultra SP-L and glucose oxidase was used for batch production of FOS. The ranges of parameters were 2.7-7.2 U g^{-1} sucrose of Pectinex Ultra SP-L, 1022-4022 U g^{-1} sucrose glucose oxidase and 7-25 h time reaction being independent process variables. The resulting 17 experiments are shown in Table 1. For each reaction vessel, 40 μL 0.5 mol L^{-1} of sodium acetate buffer (pH 5.6) was added to 1 mL of longan syrup. The reaction temperature was controlled at 55°C. The enzyme was inactivated by heating 10 min in boiling water. Then, the sugars and their contents were measured using a HPLC . Response surface methodology was applied to optimize the experimental data using Design-Expert (version 6.0.2, Stat-Ease, Minneapolis, USA). A polynomial equation was fitted to the data to obtain a regression equation. The

Table 1: Central composite design matrix of FOS optimization

Sample	Pectinex ultra SP-L (U g^{-1} sucrose)	Glucose oxidase (U g^{-1} sucrose)	Reaction time (h)
1	2.70	1022	7.00
2	7.20	1022	7.00
3	2.70	4022	7.00
4	7.20	4022	7.00
5	2.70	1022	25.00
6	7.20	1022	25.00
7	2.70	4022	25.00
8	7.20	4022	25.00
9	1.17	2522	16.00
10	8.73	2522	16.00
11	4.95	0	16.00
12	4.95	5045	16.00
13	4.95	2522	0.86
14	4.95	2522	31.14
15	4.95	2522	16.00
16	4.95	2522	16.00
17	4.95	2522	16.00

statistical significance at 95% confidence in terms of regression equation was examined by analysis of variance (ANOVA). Response surface plots were generated with the same software. The average concentration of sugar mixture from a duplicate determination was used as a response.

RESULTS AND DISCUSSION

Physicochemical properties of longan syrup: Longan syrup obtained from evaporation was dark brown in color. Color L*, a* and b* were 21.48±0.69, 1.88±0.18 and 6.29±0.59, respectively. This is probably due to browning reaction from thermal decomposition of sugars. Caramelization develops the brown color which occurs in the absence of amines when sugars are heated. In the polymerization reaction of caramelization, sugars recombine to form large complex color structures (James and Roy, 1996). The syrup pH was 6.74±0.01 and water activity was 0.884±0.006. Sugar characterization demonstrated that the longan syrup composed of 222.2±3.6 g L^{-1} sucrose, 120.3±0.8 g L^{-1} glucose and 104.7±1.7 g L^{-1} fructose. The obtained longan syrup possesses the same range of maple syrup qualities which are pH and °Bx was 6.2-7.9 and 62.2-74.0, respectively (Stuckel and Low, 1996) and water activity was below 0.884, indicating that longan syrup could be safe for the bacterial spoilage (Fennema, 1996).

Enzymatic production of FOS: Three factors of Pectinex Ultra SP-L concentration (X_1), glucose oxidase concentration (X_2) and reaction time (X_3) were optimized to obtain the highest amount of FOS. In this experiment, there were two kinds of FOS, 1-kestose and nystose, produced by enzymatic reaction of longan syrup. The initial sugar concentrations in longan syrup were used for optimization of enzymatic production were 222.2±3.6 g L^{-1} sucrose, 120.3±0.8 g L^{-1} glucose and 104.7±1.7 g L^{-1} fructose. It was found that 1-kestose was in range 1-kestose 17.82-149.49 g L^{-1}, nystose 3.74-53.39 g L^{-1}, sucrose 7.46-160.54 g L^{-1}, glucose 143.52-240.19 g L^{-1} and fructose 66.73-112.98 g L^{-1}. The regression analysis revealed that the concentrations of Pectinex Ultra SP-L and glucose oxidase and reaction time affected sugar contents in longan syrup (Table 2).

1-kestose content: The concentration of Pectinex Ultra SP-L, glucose oxidase and reaction time had a significant effect ($p<0.05$) on 1-kestose content. Low level of concentration of Pectinex Ultra SP-L and glucose oxidase and short reaction time increased 1-kestose quantity (Fig. 1a). However, there were no interaction effects of the three interaction factors on 1-kestose content.

Nystose content: The concentration of Pectinex Ultra SP-L and reaction time had affected on nystose production ($p<0.05$). Longer reaction time and higher level of Pectinex Ultra SP-L concentration led to the higher amount of nystose (Fig. 1b). This was due to nystose is produced from 1-kestose provided sucrose and obtained fructosyl became nystose which long chain molecule. Therefore, there must use high level of Pectinex Ultra SP-L for converted 1-kestose to nystose (Jung *et al*., 1989; Hidaka *et al*., 1988).

Sucrose content: Sucrose was a substrate source for FOS production (Vijn and Smeekens, 1999). During synthesis, sucrose was converted to the mixtures of FOS (Tomomatsu, 1994). The regression coefficient in Table 2 demonstrated that higher level of Pectinex Ultra SP-L and greater reaction time significant decreased ($p<0.05$) sucrose content (Fig. 1c).

Glucose content: Glucose is by-product from FOS production and it can inhibit FTase (Vijn and Smeekens, 1999). From regression equation (Table 2), it was found that glucose oxidase and reaction time had different effects on glucose content. The utilization of high concentration of glucose oxidase resulted in decreasing of glucose content (Fig. 1d). Moreover, decreasing of glucose content was affected by short reaction time ($p<0.05$).

Fructose content: The high level of Pectinex Ultra SP-L concentration and long reaction time decreased fructose quantity (Fig. 1e) since fructosyl group was produced and connected to glucose to produce 1-kestose and nystose (Jung *et al*., 1989).

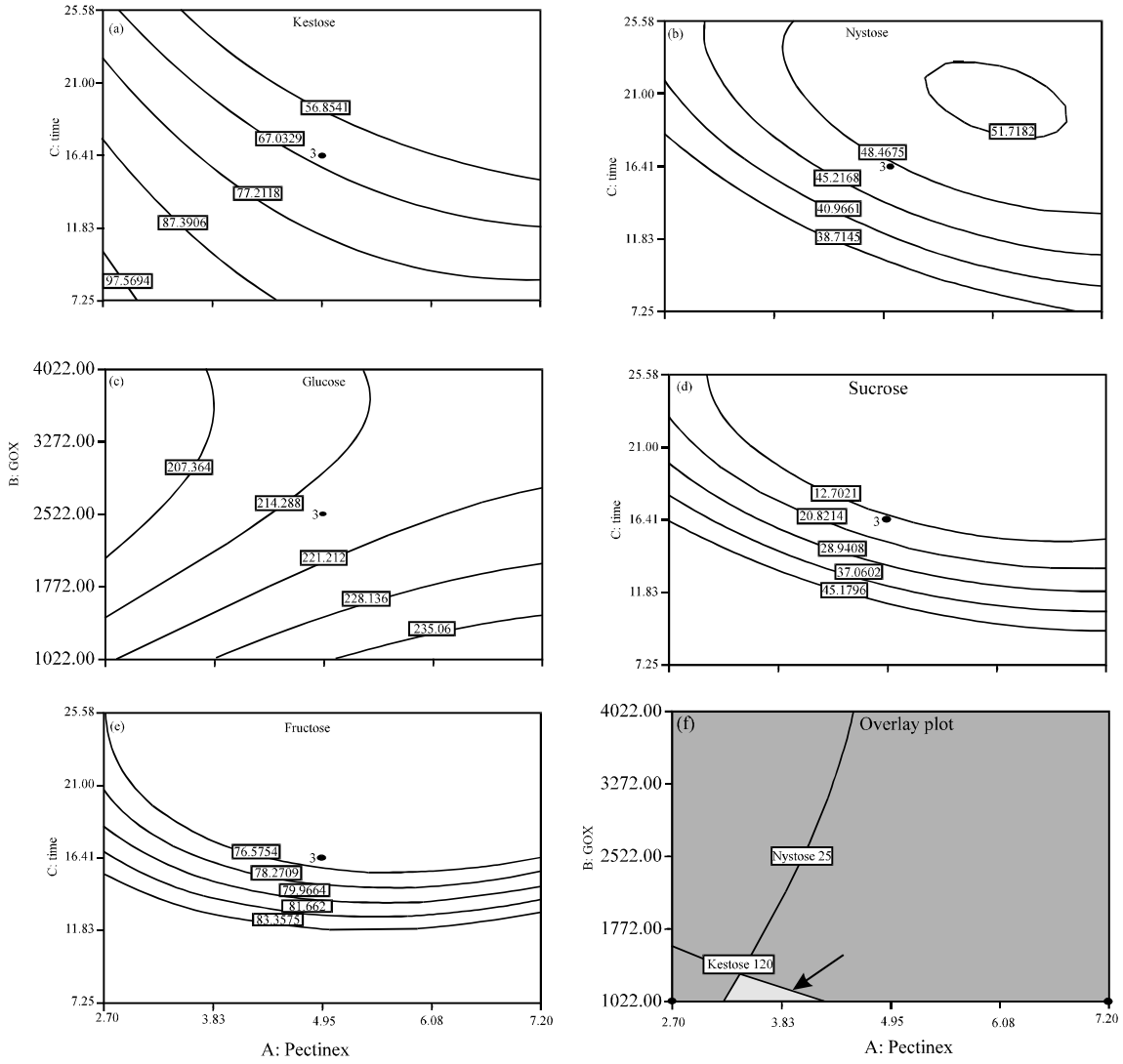

Fig. 1(a-f): Response contour plot showing the effect of concentration of Pectinex SP-L and time on (a) 1-kestose, (b) nystose, (c) sucrose, (d) glucose, (e) fructose contents and (f) optimal area of FOS production. Arrow indicates the feasible area

Table 2: Regression equation coefficients for 1-kestose (g L^{-1}), nystose (g L^{-1}), sucrose (g L^{-1}), glucose (g L^{-1}) and fructose (g L^{-1})

Variables	1-Kestose	Nystose	Sucrose	Glucose	Fructose
Constant	+211.81	-37.39	+259.08	+191.08	+137.19
X_1	-13.29*	+17.33*	-36.39*	+10.30	-6.25*
X_2	-0.04*	-8.57×10^{-3}	+0.02	-0.04*	-3.80×10^{-3}
X_3	-1.55*	+4.64*	-15.36*	+3.99*	-3.36*
X_1^2	+1.08	-0.95	+2.47*	-0.66	+0.75*
X_2^2	$+2.21\times10^{-6}$	$+7.67\times10^{-7}$	-3.57×10^{-7}	$+3.03\times10^{-6}$	$+5.32\times10^{-7}$
X_3^2	-0.05	-0.09*	+0.29*	-0.16*	+0.06*
$X_1 X_2$	$+5.85\times10^{-4}$	-1.52×10^{-4}	-1.79×10^{-3}	-3.59×10^{-4}	-1.57×10^{-4}
$X_1 X_3$	-0.45	-0.26	+0.51	+0.09	-0.12
$X_2 X_3$	$+1.04\times10^{-3}$	$+2.53\times10^{-4}$	-4.12×10^{-4}	$+9.63\times10^{-4}$	$+1.66\times10^{-4}$
p-value	0.0109	0.0076	0.0002	0.0371	0.0002
R^2	0.89	0.90	0.97	0.84	0.97

*Significant at 95% confidence interval, the R^2 value indicates the correlation coefficients. X_1 indicates Pectinex Ultra SP-L (U g^{-1} sucrose), X_2 indicates glucose oxidase (U g^{-1} sucrose) and X_3 indicates time (h)

Optimization: The aim of the production of FOS from longan syrup was the highest quantities of 1-kestose and nystose, the lowest concentration of Pectinex Ultra SP-L and glucose oxidase and the shortest reaction time. The equations from each parameter in Table 2 were used for optimization by superimpose technique. Optimization condition of FOS production by response surface area is shown in Fig. 1f. The optimal concentrations of Pectinex Ultra SP-L and glucose oxidase and reaction time were 3.3 U g^{-1} sucrose, 1022 U g^{-1} sucrose and 8 h 41 min, respectively. The prediction showed that 1-kestose, nystose, sucrose, glucose and fructose were 123.36, 30.27, 73.10, 216.69 and 92.43 g L^{-1}, respectively. These results are consistent with the previously

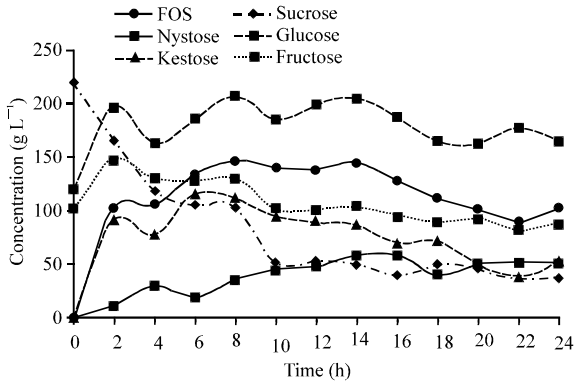

Fig. 2: Production of FOS under optimal concentrations of Pectinex Ultra SP-L and glucose oxidase

Table 3: Comparison of experimental and predicted values of FOS production

Sugars (g L^{-1})	Exp. value	Pred. value	Diff (%)
1-kestose	119.88±2.93	123.36	-2.8
Nystose	28.22±2.66	30.27	-6.8
Sucrose	75.37±8.66	77.46	-2.7
Glucose	198.71±17.0	216.31	-8.1
Fructose	107.11±4.93	94.33	13.5

Exp indicates experimental value, Pred: Predicted value, Diff: Calculated from (Exp-Pred/Pred)×100

published research. Hang and Woodams (1995) found that the optimized conditions for production of FOS from sucrose could be used 0.5 mL Pectinex Ultra SP-L (3.4 U mL^{-1}) and 1.5 mL of 45% sucrose in 0.05 mol L^{-1} sodium acetate buffer (pH 5.6). After 14 h of reaction 65°C, Pectinex Ultra SP-L converted 450 g L^{-1} sucrose to 224 g L^{-1} 1-kestose and 48 g L^{-1} nystose. Csanadi and Sisak (2008) also found that Pectinex Ultra SP-L mixed with glucose oxidase from saccharose could increase the performance of FOS production from 60 to 74%.

Model validation: The optimal process was selected and run to valid the model (Table 3). Figure 2 demonstrates the changing of sugar content during the enzymatic reaction. Firstly, sucrose rapidly decreased in 4 h and remained until constant 10 h of reaction time. 1-kestose was rapidly produced from sucrose in the early state of sucrose consumption and its quantity was highest at 6-8 h of reaction time and then decreased in 10 h of reaction time. Nystose increased due to 1-kestose received fructosyl group and converted which increase continuously and the highest in 8-14 h of reaction time and decrease. Glucose was rapidly increased in 2 h and fluctuated throughout the reaction. There was 104.7 g L^{-1} of fructose in the starting longan syrup then rapidly increased due to enzymatic hydrolysis of sucrose in first 2 h. Hu (1999) stated that predicted equation model should have error percentage of predicted value and experimental value less than 10%. The error percentages of sucrose, 1-kestose, nystose, glucose and fructose were 2.7, 2.8, 6.8, 8.1 and 13.5, respectively (Table 3). Confidentiality of predicted model of four sugars were acceptable except fructose because fructose was a by-product from reaction of FOS production.

CONCLUSIONS

Sixty degree brix of longan syrup can use as a substrate for the production of FOS with the mixtures of enzymes. The optimal values for FOS production were Pectinex Ultra SP-L 3.3 U g^{-1} sucrose, glucose oxidase 1022 U g^{-1} sucrose and reaction time 8 h 41 min. As a result, the amount of nystose and 1-kestose were ranged from 28-30 g L^{-1} and 119-123 g L^{-1}, respectively. It is suggested that FOS from fruit syrup can be produced and used as healthy ingredients. Future work is required to investigate the flavor of this FOS and apply FOS from longan syrup in the food products.

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