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Study on Fructooligosaccharide (Fos) Production By Enzyme Pectinex Ultra Sp-l Immobilzed On Alginate

Binh T. Nguyen

Department of Biotechonology, VNUHCM- Bach Khoa University, Viet Nam

Abstract— *Fructooligosaccharide (FOS) is a new alternative sweetener with its characteristics such as low energy and safety for people with diabetes. Pectinex Ultra SP-L which has fructosyltransferase, catalyzes the reaction to produce short chain fructooligosaccharides. The research was conducted to enhance the high FOS content in process of FOS production by immobilized enzyme. Results achieved by Empirical planning Design Expert 7.0 - central composite method (CCD) identified at optimum conditions for process of immobilized enzyme with alginate 3.3 (%), ratio of enzyme: alginate was 0.79 (w/w), CaCl2 3.75 (%). Efficiency of loading protein reached 73.32 (%). Reaction conditions: 60 (oC), shaking velocity 90 (rpm), the initial sucrose concentrations of 50 (%), pH 5.75. When produced in 20 (h), the reaction obtained the highest level of FOS with column reaction system. The FOS for producted by immobilized enzyme achieved 47.87 (%), of which 1-kestose obtained 37.06 (%), the remaining was 10.81 (%) including nystose and fructofuranosylnystose. Efficiency of producing FOS by using immobilized enzyme compared to the free enzyme was high up to 89.49 (%).*

Keywords—Fructooligosaccharide, fructosyltransferase, Design Expert 7.0, nystose, fructofuranosylnystose.

I. INTRODUCTION

Functional sugars is one of functional food groups which focused on much in recent years and has the potential to become a new type of sweetener replaced for sucrose and traditional sugars. Among of them, Fructooligosaccharide (FOS) is low energy sweetener with prebiotic properties. FOS gradually displaces traditional sugars like normal sugar (fructose syrup). FOS is widely used in functional food, dairy, and confectionery manufacturing.

FOS offers health benefits to the consumers such as better digestion, lower cholesterol, phospholipids, triglycerides, anti-obesity, safe for people with diabetes, prevent cavities ... Especially, FOS also help increases absorption of Ca, Mg, Fe, ..., prevent anemia, iron deficiency, Mg2+ ion balance, Ca^{2+} in the body, protecting against osteoporosis [1].

Currently, in Vietnam obesity and diabetes are increasingly influential to life. Thus, studying of the alternative sweeteners which has less energy value and does not change the concentration of glucose in the blood to replace traditional sweeteners is an urgent necessity. Besides, the functional food industry is developing and enhancing high economic value, Vietnam has great potential for industrial production of FOS with sugarcane as source inputs for production.

From above reasons, study on immobilized enzyme for industrial production of FOS is essential subject today, bringing benefits to human health and economic resources.

II. MATERIALS AND METHODS

2.1. Materials

Albumin (Merck), Coomassie (Merck), sodium alginate (Kanto Chemical Co., INC, Japan) Bien Hoa Sugar sucrose concentrations > 99.8 (%)**.**

Pectinex Ultra SP - L liquid (Novoferm14) of Novozyme Nordisk Denmark.

2.2. Research Procedure

the introduction of the paper should explain the nature of the problem, previous work, purpose, and the contribution of the paper. The contents of each section may be provided to understand easily about the paper.

Method for determining protein content by Bradford method [2] Methods for determination of reducing sugars by DNS [2].

Determination of fructosyl transferase activity from immobilized and free Pectinex Ultra SP - L [3]

Fructosyl transferase activity is determined by reducing sugar by method of DNS in which amount of reduced sugar is evaluated.

Experiment planning: (Response Surface Methodology - RSM) [4].

Model Level 2- Central composite method (CCD): Option dial (Second - Oder Rotatable factorial Designs) [5]

Efficiency of immobilized enzyme (%) = (Total immobilized enzyme activity/ Total initial activity) x 100.

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Efficiency of loading protein (%) = (Total amount of loading protein/Total amount of initial protein) x 100.

III. RESULTS AND DISCUSSION

Fructosyl transferase activity of Pectinex Ultra SP -L is 352.03 (IU/ml), protein is 3027.88 (g/ml), the activity of free enzymes is 116.26 (UI/mg).

3.1. Optimization of enzyme immobilization process on alginate carrier

Table 1: Result of the conditions of optimization of enzyme immobilization process

Sample	Alginate (%)	Enzyme/Alginate	CaCl 2	Efficiency of immobilized enzyme (%)	Efficiency of loading protein
1	3,50	0,75	5,68	11,50	64,61
2	3,25	1,00	3,00	10,01	63,56
3	3,50	0,33	4,00	8,70	78,05
4	3,92	0,75	4,00	8,50	69,48
5	3,50	0,75	4,00	11,14	67,88
6	3,25	0,50	3,00	6,88	79,90
7	3,50	0,75	4,00	10,86	68,87
8	3,25	1,00	5,00	8,27	52,90
9	3,50	0,75	2,32	11,04	67,98
10	3,75	0,50	3,00	6,70	85,82
11	3,75	1,00	5,00	10,80	58,14
12	3,25	0,50	5,00	6,90	82,23
13	3,50	1,17	4,00	12,50	52,05
14	3,75	0,50	5,00	6,60	69,94
15	3,75	1,00	3,00	9,90	75,07
16	3,50	0,75	4,00	10,02	65,42
17	3,08	0,75	4,00	7,60	78,46
18	3,50	0,75	4,00	13,54	66,27
19	3,50	0,75	4,00	11,34	70,41
20	3,50	0,75	4,00	12,08	68,57

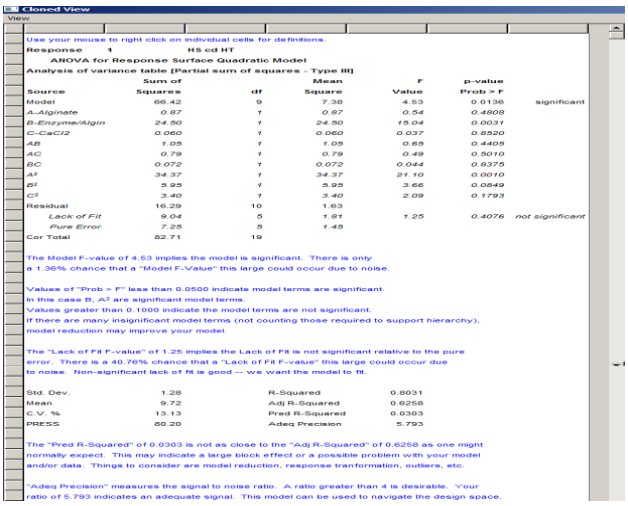

Fig. 1: Results of ANOVA analysis by Design-Expert 7.0

Results of ANOVA analysis, regression equation in the natural form:

Immobilized enzyme = -280.27307 + 164.59167 * Alginate + 2.00481 * (Enzyme / Alginate) - 0.30726 * 5.80000 * CaCl2 + (Alginate) * (Enzyme / Alginate) + 1.26000 * (Alginate) * (CaCl2) -0.38000 * (Enzyme / Alginate) * (CaCl2) - 24.71002 * (Alginate) 2-10.28504 * (Enzyme / Alginate) 2 -0.4854 * (CaCl2) 2

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According to ANOVA analysis, if the rate enzyme / alginate increase, the immobilized enzyme activity will increase. If concentrations of alginate increase, the immobilized enzyme activity will decrease. Besides, the concentration of CaCl2 did not affect considerably to activity

From the analysis of ANOVA and regression equation above, calculation program provides the optimal plans. We selected 5 plans from prediction equation to conduct empirical comparisons and choose the highest optimal plan.

Table 2: The optimal plan, prediction program and results of survey experiments

Alginate (%)	*Enzyme / Alginate*	*CaCl2(%)*	*Efficiency of immobilized enzyme was predicted by software*	*Efficiency of immobilized enzyme in experiment*	*Efficiency of protein experiment*
3,47	*0,73*	*4,69*	*11,09*	*9,97*	***74,78***
3,41	*0,94*	*3,11*	*11,63*	*9,89*	***59,83***
3,61	*0,89*	*3,46*	*11,84*	*9,68*	***80,28***
3,47	*0,81*	*3,18*	*11,55*	*10,79*	***76,33***
3,30	***0,79***	***3,75***	***10,53***	***11,02***	***73,32***

Our experimental examination shows the results which plan 5 is top with the highest efficiency of immobilized enzyme in practice.

3.2. FOS production

3.2.1. FOS production by using free enzyme

These parameters are the immobilization: 10 ml of sucrose 50 (% w/v) (pH = 5.75), 1 (ml) free enzyme, reaction temperature 60^0C**.**

According to the survey, the results showed the more reaction time prolonged, themore reducing sugar content increased, and reducing sugar content reached164.17 (mg / ml) in 12 (h) and then the amount of FOS began to stabilize. The time was shorter when compared with the results of Aziz Tanriseven, YakupAslan (2004) with a reaction time is 24 (h).

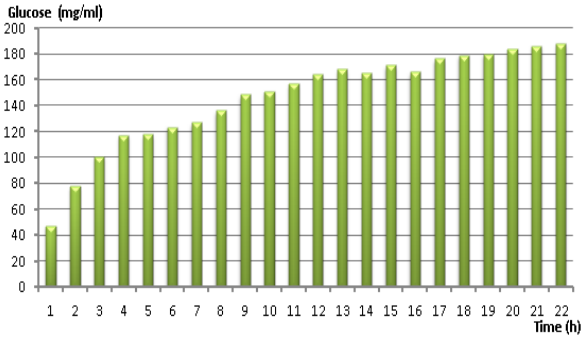

Fig. 1: The reducing sugar content generated by time when it was reacted with free enzyme

3.2.2. Compare the efficiency of the FOS production between shake flask reaction system and column reaction system

After the result of production process using free enzyme obtained, we proceed with the immobilized enzyme. FOS production process by immobilized enzyme applications in two systems: shake reaction system and column reaction system.

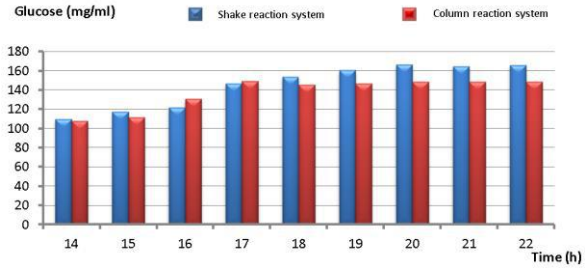

Fig. 2: Shows the reducing sugar content generated by time in shake reaction and column reaction system

Results of FOS production in shake reaction system reached equilibrium at 17 (h), whereas in column reaction system, the amount of reducing sugars column after 17 (h) remains elevated for up to 20 (h) to reach equilibrium (Figure 2).

Compared to the free enzyme, capability of producing FOS through reducing sugar in both systems are quite high, namely: in shake reaction system at 17 (h) it was up to 84.66 (%), and in column reaction system at 20 (h) it reached 90.72 (%).

The ability of immobilized enzyme activity in column reaction system was higher than shake reaction system.

3.2.3 Analytical results of FOS production by high pressure liquid chromatography (HPLC)

Product samples of FOS produced by using free enzyme and immobilized enzyme (reaction in the column reaction system) were analyzed in HPLC and Quality Management Division Nanogen Biopharmaceutical Company Ltd.

Parameters HPLC: C18 column size: 4.6 (mm) x 250 (mm), Probe RI, Inject 100 (l), 25 (°C), mp (mobile phase): ACN: H_2O, pump speed: 0.7 (ml / min)

The analytical results show that the total concentration of FOS in product sample using enzyme free 53.49 (%),

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including 1-kestose content is 192.621 (mg / ml) accounted for 40.13 (%), while to 13.36 (%) is nystose and fructofuranosylnystose (because of standard solution was not known, the amount of content could accurately not accurately calculate). For the FOS sample by using immobilized enzyme, the total amount of FOS reaches 47.87 (%), including 1-kestose content is 196.04 (mg / ml) accounted for 37.06 (%), the remaining 10, 81 (%) is nystose and fructofuranosylnystose. Capability of immobilized enzyme to produce FOS when compared to the free enzyme is quite high, up to 89.49 (%).

3.3. Study the reuse ability of immobilized enzyme

According to survey results of reuse ability of immobilized enzyme, using immobilized enzyme in column reaction system showed that the immobilized enzyme was reused multiple times higher than in the shake reaction system. For the shake reaction system, at the 4th reuse of it, the activity remained only 48.77 (%), as compared to the column reaction system, at the 11th reuse, its activity just reduced to 49.93 (%). The result is equivalent to the results of Zs. C sanadi, Cs. Sisak (2006) in which immobilized enzyme was reused 12 times (reusable condition: temperature 55°C, pH 5.6, the reaction time of 120 minutes).

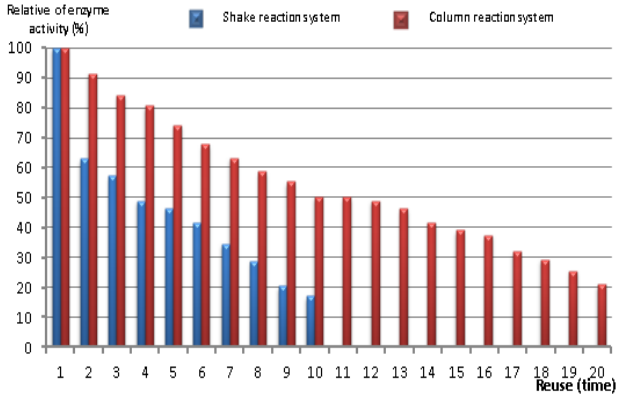

Fig. 3: The reuse ability of immobilized enzyme

IV. CONCLUSION

Immobilized enzyme is more heat-resistant than free enzyme, using immobilized enzyme easily applied in modern reactive systems with its automatic ability and controlling the process. The ability of immobilized enzyme activity in the column reaction system is stable and more effective than in the system reactions shake.

REFERENCES

[1] Iraj ghazi, LucíaFernández-arrojo, Aranzazu Gomez de Segura, Miguel Alcalde, Francisco j. Plou, Antonio Ballesteros. Beet sugar syrup and molasses as low-cost feedstock for the enzymatic production of fructo-oligosaccharides. J. *Agric. Food chem* 54, 2964-2968 (2006)

[2] Nguyen Duc Luong, Cao Cuong, Biochemistry Experiment, HCMC National University Publishing House (2003)

[3] Zs. Csanádi, Cs. Sisak. Immobilization of Pectinex Ultra Sp-L pectinase and its application to production of fructooligosaccharides. *Actaalimentaria* 35(2), 205–212 (2006)

[4] Raymond H.Myers, A.Khuri and Walter H.Carter. Response Surface Methodology. *Technometrics* 31(2), 1966- 1988 (1989).

[5] Nguyen Canh, Empirical planning, HCMC National University Publishing House (2004)