2014

ISSN : 0974 - 7435 Volume 10 Issue 20

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(20), 2014 [12102-12106]

Optimization of preparation parameter of yeast oligoglucosides by enzyme complex

Wang Qian*, Hu Ying, Dong Hai-Li
Dartment of Chemistry Engineering, Huainan Union University, Huainan, 232038
E-mail: anhuidonghaili@163.com

ABSTRACT

In order to develop a clean preparation method for yeast oligoglucosides, complex enzyme (β -glucanase and papain) was used to hydrolysis of oligoglucosides. Based on single factor experiments, L9(34) orthogonal test was used to optimum the preparation parameter. Enzyme dosage, enzyme temperature, time and pH were as factors and reducing sugar mass fraction was as index. The results shown that the optimization parameters were as follows: enzyme dosage 1.6%, enzyme temperature 55℃, time 30min and pH 5.5. The reducing sugar mass fraction reached at 92.1%. So complex enzyme method was a suitable method for preparation of oligoglucosides from yeast β -glucan.

KEYWORDS

β-glucan; Yeast; Oligoglucosides; Enzyme; Optimization.

INTRODUCTION

(1→3)-β-D-glucan, composed of glucose units is a homopolysaccharide contained in the cell walls of yeast[1-3]. It has been reported antitumor and antimicrobial, activities by enhancing the host immune function[4-6]. But (1→3)-β-D-glucan of yeast has lower water-solubility[7-8]. In oreder to increase its water solubility, degradation was a suitable method. Acid and alkaline hydrolyses, enzymatic digestion have been reported to be applied as methods for degradation of (1→3)-β-D-glucan[9-11]. Although these methods are effective in decreasing the molecular weight, they do exist some disadvantages. For acid or alkaline treatment, they may not only break the natural structure of β-glucan but also pollute the environment. For singal enzymatic digestion, the digestion ratio is lower and has longer time. Complex enzyme degradation has been reported using in polysaccharide degradation for it has higher digestion ratio and shorter time[12], but it hasn't been used in glucan degradation. In order to develop a clean and effective method, complex enzyme was used to preparation yeast oligoglucosides in this paper.

MATERIALS AND METHODS

Materials

(1→3)-β-D-glucan (yeast) was purchased from Zhengzhou Chaofan Co., Ltd (China). β- glucanase, cellulase, lysozyme, papain was purchased from Zhengzhou Jinpbang Co., Ltd (China). All reagent were analysis grade were purchased from Huainan Drugstore (China). T6 spectrophotometer was obtained from Beijing Pgeneral Co., Ltd. (China).

Methods

Signal test of hydrolysis of yeast glucan

(1→3)-β-D-glucan (yeast) was hydrolysed with β- glucanase, cellulase, lysozyme, papain and their combinations. Briefly, enzyme (β- glucanase, cellulase, lysozyme, papain) was added to the 20% glucan solution at the dosage of 0.5%, enzyme temperature 50℃, time 30min and pH 5.5. The degree of hydrolysis was determinated to ensure the constitute of complex enzyme. Then ensured complex enzyme was added to the 20% glucan solution at the dosage of 0.1-2.0%, enzyme temperature 40-65℃, time 10-50min and pH 4-8.

Orthogonal test

Based on the single factor experiment, select complex enzyme dosage, enzyme temperature, time and pH as factors, educing sugar mass fraction was as index, L9(34) orthogonal experimnt was used to optimum the hydrolysis parameter. The results were shown in Tab.1.

TABLE 1 : Factors and levels of orthogonal test

Level/Factor	(A)Enzyme dosage/%	(B) Temperature/℃	(C)Time/min	(D) pH
1	1.4	50	25	5
2	1.6	55	30	5.5
3	1.8	60	35	6

Determination of reducing sugar mass fraction

Reducing sugar mass fraction was determined by DNS method. Briefly, glucosum anhydricum 0.1000g was added to 100mL of distilled water to preparate the 1mg/mL solution. Then take 0, 0.1, 0.2,0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 mL solution to tube and add the distilled water to 1.0mL. Then add 2mL DNSsolution. The complex solution was boiled for 5min. After cooling, the complex was added distilled water to 10mL. The solution absorbance was determinated at 540nm. The standard curve was drawn.

Statistical analysis

All tests were conducted in triplicate and the data are presented as the means±SD. Statistical significance between the groups means were evaluated by ANOVA.

RESULT AND DISSCUSS

Effect of enzyme and their combinations on hydrolysis of yeast glucan

β- glucanase, cellulase, lysozyme, papain and their combinations was added to the 20% glucan solution at the dosage of 1.0%, enzyme temperature 50℃, time 30min and pH 5.5. Effect of enzyme and their combinations on reducing sugar mass fraction was as Figure 1.

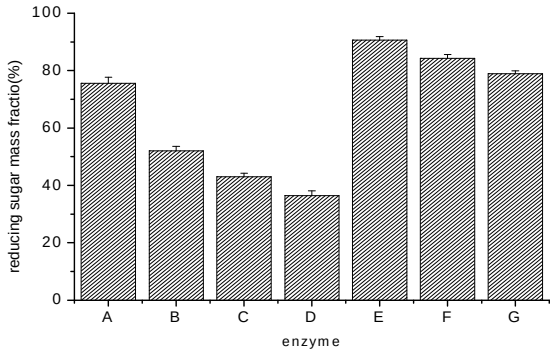

Figure 1 : Effect of enzyme and their combinations on hydrolysis of yeast glucan

A:β-glucanase,B:cellulase, C:lysozyme, D:papain, E:β-glucanase and cellulase, F:β-glucanase and lysozyme, G:β-glucanase and papain.

In Figure 1, In signal enzyme, β-glucanase has the higher reducing sugar mass fraction. It indicated β-glucanase has bettereffect on hydrolysis of yeast glucan. When β-glucanase was combinated to other enzyme, all the combinations were has higher reducing sugar mass fraction than signal enzyme. But β-glucanase and cellulase has significant differenc with others enzyme ($P<0.05$). So combinations of β-glucanase and cellulase was chosen as suitable hydrolysis enzyme.

Effect of enzyme dosage on hydrolysis of yeast glucan

Complex enzyme ($M_{\beta\text{-glucanase}}$:$M_{cellulase}$ =1:1), was added to the 20% glucan solution at the dosage of 0.4% to 2.0%, enzyme temperature 50℃, time 30min and pH 5.5. Effect of enzyme dosage on reducing sugar mass fraction was as Figure 2.

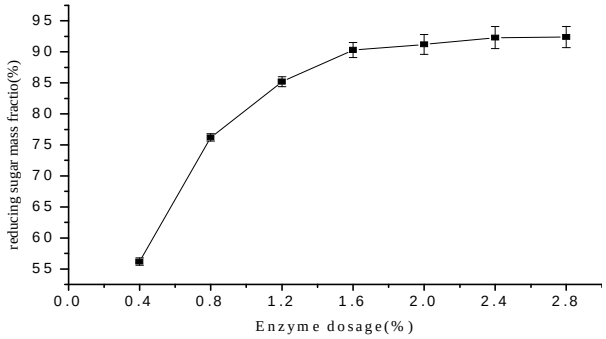

Figure 2 : Effect of enzyme dosage on hydrolysis of yeast glucan

In Figure 2, before enzyme dosage at 1.6%, reducing sugar mass fraction was increased with the increase of enzyme dosage ($P<0.05$). But when enzyme dosage to 1.6%, the reducing sugar mass fraction was was kept stable. Reducing sugar mass fraction of enzyme dosage 1.6%, 2.0%, 2.4% and 2.8% has no significant difference ($P>0.05$). So 1.6% was a suitable enzyme dosage for hydrolysis of yeast glucan.

Effect of enzyme temperature on hydrolysis of yeast glucan

Complex enzyme ($M_{\beta\text{-glucanase}}$:$M_{cellulase}$ =1:1) 1.6% was added to the 20% glucan solution at enzyme temperature 15℃ to 65℃, time 30min and pH 5.5. Effect of enzyme temperaturee on reducing sugar mass fraction was as Figure 3.

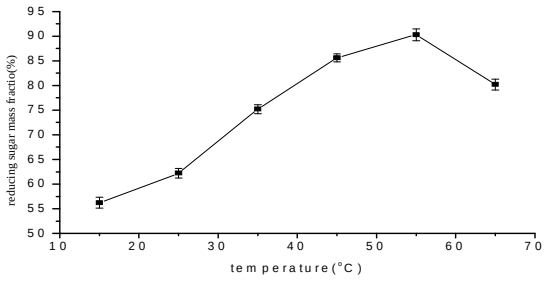

Figure 3 : Effect of temperature on hydrolysis of yeast glucan

In Figure 3, before 55℃, reducing sugar mass fraction was increased with the increase of temperature (P<0.05). But when enzyme temperature to 55℃, the reducing sugar mass fraction was decreased with temperature increase. This is enzyme inactivation at high temperature. So 55℃ was a suitable temperature for hydrolysis of yeast glucan.

Effect of pH on hydrolysis of yeast glucan

Complex enzyme (Mβ-glucanase:Mcellulase =1:1) 1.6% was added to the 20% glucan solution at enzyme temperature 50℃, time 30min and pH 4 to 8. Effect of pH on reducing sugar mass fraction was as Figure 4.

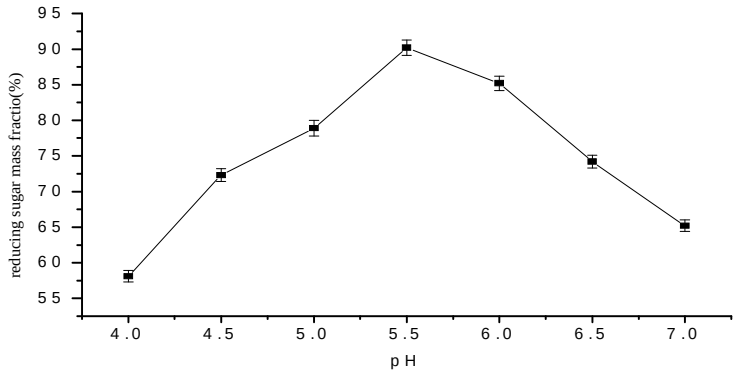

Figure 4 : Effect of pH on hydrolysis of yeast glucan

In Figure 4, before pH5.5, reducing sugar mass fraction was increased with the increase of pH (P<0.05). But when pH to 5.5, the reducing sugar mass fraction was decreased with pH increase. This is enzyme has "optimal temperature". So pH5.5 was a suitable pH for hydrolysis of yeast glucan.

Effect of time on hydrolysis of yeast glucan

Complex enzyme (Mβ-glucanase:Mcellulase =1:1), was added to the 20% glucan solution at the dosage of 1.6%, enzyme temperature 50℃, time 10to 40min and pH 5.5. Effect of time on reducing sugar mass fraction was as Figure 5.

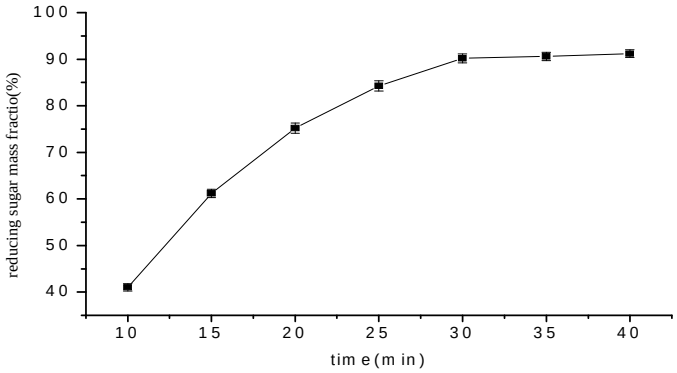

Figure 5 : Effect of time on hydrolysis of yeast glucan

In Figure 4, before 30min, reducing sugar mass fraction was increased with the increase of enzyme time (P<0.05). But when time to 30min, the reducing sugar mass fraction was was kept stable. Reducing sugar mass fraction of 30min, 35min and 40min has no significant difference (P>0.05). So 30min was a suitable time for hydrolysis of yeast glucan.

Optimization of hydrolysis of yeast glucan conditions

Orthogonal experiment to optimizate the hydrolysis of yeast glucan condition. The results were shown in Tab.2. According to the Tab. 2, the order of various factors influence the hydrolysis of yeast glucan were as follows: temperature> pH > enzyme dosage > time. Optimum conditions were A2B2C2D2, namely enzyme dosage1.6%, 55℃, 30min, pH5.5. Orthogonal experimental results were analyzed by ANOVA and F-test results are shown in Tab.3. Enzyme dosage, time and pH have no significant impact on the reducing sugar mass fraction. But temperature has significant impact on the reducing sugar mass fraction. Under optimized conditions, by the verification experiments, the reducing sugar mass fraction was 92.1%.

TABLE 2 : Result of orthogonal test

Order	(A)Enzyme dosage/%	(B) Temperature/℃	(C)Time/min	(D) pH	reducing sugar mass fraction(%)
1	1.4	50	25	5	72.3
2	1.4	55	30	5.5	88.6
3	1.4	60	35	6	78.1
4	1.6	50	30	6	80.1
5	1.6	55	35	5	88.2
6	1.6	60	25	5.5	84.6
7	1.8	50	35	5.5	81.6
8	1.8	55	25	6	90.2
9	1.8	60	30	5	79.2
k_1	79.667	78.000	82.367	79.900	
k_2	84.300	89.000	82.633	84.933	
k_3	83.667	80.633	82.633	82.800	
R	4.633	11.000	0.266	5.033	

TABLE 3 : Variance analysis

Factor	Sum of squared deviations	Degree of freedom	F/F0.05	Significance
A	37.869	2	0.552	no significant
B	197.936	2	2.887	significant
C	0.142	2	0.002	no significant
D	38.296	2	0.559	no significant
Deviation	274.24	8		

CONCLUSIONS

β-glucanase come from microorganisms and plants, is a hydrolytic enzyme whicn can decompose glucosidic bond of β-glucan. It can reduce the degree of polymerization of substrates, but don't change the natural structure of β-glucan. So it is a good enzyme for degradation of β-glucan. But it has selectivity and only decompose specially glucosidic bond. Cellulase is also a hydrolytic enzyme whicn can decompose glucosidic bond of polysaccharide. Combination of β-glucanase and cellulase can decompose glucosidic bond widely and increase the hydrolysis degree of yeast glucan. Optimum conditions for complex enzyme hydrolysis of yeast glucan were complex enzyme dosage ($M_{\beta\text{-glucanase}}$:$M_{cellulase}$ =1:1)1.6%, temperature 55℃, enzyme time 30min, hydrolysis pH5.5. The reducing sugar mass fraction was 92.1%. This work provide a guide for enhance the utility value forβ-glucan.

ACKNOWLEDGEMENT

This work was financial supported by Anhui University Natural Science Project (KJ2014A233).

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