

## Combined Action of High Pressure, Temperature, pH and Time on Jack Bean $\alpha$ -Mannosidase Activity

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**Abstract:** The pressure, temperature, pH and treatment time are assumed to be key factors affecting the enzyme activity, and their combined influence on  $\alpha$ -mannosidase activity Jack bean was investigated by measuring  $\alpha$ -mannosidase activity under conditions of pressure at 0-500 MPa, temperature at 35-55°C, pH at 3.5-5.5 and treatment time at 24-168 h in this study. Taguchi orthogonal experiment was designed to optimize parameters. Results indicated that the pressure is most significant factor influencing the enzyme activity and progressive losses of activity of  $\alpha$ -mannosidase were observed with increasing hydrostatic pressures. Temperature and pH have also significant influences on enzyme activity. The optimal combinations of four parameters for Jack bean  $\alpha$ -mannosidase activity were: temperature at 50°C, pH at 5.0 and treatment time at 120 h in the absence of high pressure and temperature at 45°C, pH at 5.0 and treatment time at 48 h and hydrostatic pressure at 150 Mpa in the presence of high pressure, respectively.

**Keywords:** Activity,  $\alpha$ -mannosidase, high pressure, jack bean, parameter optimization

### INTRODUCTION

The manno-oligosaccharides have great potential as prophylactic agent, anti-adhesives and functional food ingredients, which stimulated increasing research interest (Côté, 2009; Maitin *et al.*, 2004; Maitin and Rastall, 2003; Suwasono and Rastall, 1998). However, the research and application of oligosaccharides was limited due to the limited quantity and their high cost. Therefore, it is important to develop efficacious and rather low cost methods for oligosaccharide synthesis to increase the repertoire of oligosaccharides available. The potentially promising alternative for oligosaccharide synthesis is to produce oligosaccharides by utilizing the enzymes that are involved in synthesis *in vivo*. Jack bean  $\alpha$ -mannosidase is capable of carrying out synthesis, hydrolysis and glycosyl transfer (Weber *et al.*, 2008). As a result, it appeared to be a potential candidate for use in oligosaccharide synthesis. Several oligosaccharide synthesized by  $\alpha$ -Mannosidase from jack bean have been reported (Atanasopoulos, 2004; Suwasono and Rastall, 1996; Rastall and Bucke, 1992). As the jack bean  $\alpha$ -mannosidase plays a key role in the enzymatic synthesis of oligosaccharides, it is, therefore, much interesting to know the effectors on its activity as well as the mutual interaction between effectors.

Enzyme activity, generally, is strongly dependent upon ambient factors. Li and Li (1968) reported that  $\alpha$ -mannosidase from jack bean has the optimum pH range at 4.0-5.0. Determinations on heat stability and pH

stability showed that the enzyme was quite stable at 70°C for 5 min and at pH 6.0-8.5 (Snaith and Levvy, 1968). Regarding pressure sensibility of enzymes, contradictory results have been found in the presence of temperature and treatment time (Zeuthen and Bogh-Sorensen, 2000; Zhou *et al.*, 2000; Weingand-Ziade *et al.*, 1999; Mozhaev *et al.*, 1994).

Although massive researches have been carried out to describe enzyme activity changes under hydrostatic pressure, combination effects of pressure, temperature and pH and treatment time on activity of Jack bean  $\alpha$ -mannosidase have not been reported so up to date. In this study, we examined the activities of Jack bean  $\alpha$ -mannosidase under various combined conditions of pressure, temperature, pH and treatment time by orthogonal experiment to obtain the optimal combination condition of parameters under which the activities of the Jack bean  $\alpha$ -mannosidase Reached the maximum.

### MATERIALS AND METHODS

$\alpha$ -mannosidase from Jack bean suspension in ammonium sulphate (M-7257, EC3.2.1.24), 4-nitrophenol  $\alpha$ -D-mannopyranoside (N2127) and 4-nitrophenol in free form (N7660) were provided by Sigma Chemical Company (Poole, Dorset, UK). Other chemicals such as sodium tetraborate, acetic acid and sodium acetate were of analytic grade and purchased from Sigma.

Absorbance is read on Ultrospec 1100 ProUV/visibles spectrophotometer provided by GE Healthcare. Food-Lab 900 High-Pressure Food Processor, supplied by Stansted Fluid Power, UK, was employed for pressurization of the enzyme. The system fluid contained 20% castor oil and ethanol. The systematic temperature is regulated by a cycle heater.

**Estimation of enzyme activity:** Dilutions of Jack bean  $\alpha$ -mannosidase were made in 0.1 M sodium acetate buffer with different pH. Then the enzymes were kept pressurized in pressure-resistant vials which were sealed in plastic bags for given intervals.  $\alpha$ -mannosidase activities were determined according to Li and Li (1970) and Li (1967) by using 0.9 mL of substrate solution (4-nitrophenol  $\alpha$ -D-mannopyranoside, 10 mM) incubated with 0.1 mL of the treated enzymes for suitable time periods at 30°C to determine the p-nitrophenol released by absorbance at 420 nm against the blank. The reaction was terminated by addition of 2 mL of 0.2 M of sodium tetraborate. The concentration of p-nitrophenol was calculated from prepared standard curve. One unit of activity of Jack bean  $\alpha$ -mannosidase was defined as 1  $\mu$ mole of p-nitrophenol liberated per mL per min at 30°C, U/mL.

**Experiment design:** The orthogonal experiment design is able to reduce experimental times and research cost by using orthogonal arrays. It is also a powerful tool to investigate the relative importance of various factors and identify the best levels for different factors (Taguchi, 1986). Therefore, the orthogonal experiment method was used to optimize these parameters responsible for the enzyme activity.

Four main parameters are reported to play dominant role on the enzyme activity, namely pressure, temperature, pH and treatment time. An orthogonal array  $L_{25}(5^4)$  was implemented and five levels were set for each of these factors. Table 1 lists the four factors and their levels as well.

## RESULTS AND DISCUSSION

The Jack bean  $\alpha$ -mannosidase activities of twenty five experiments are listed in Table 2.

For each factor,  $S_i$  refers to the sum of experiment results of various levels. The  $S_1$  represents the sum of values of all level 1,  $S_2$  for all level 2 and so on. For example, the sum of experiment results for factor pH at level 3 is:  $8.414 + 1.886 + 1.587 + 0.038 + 0.006 = 11.931$ . For each factor, the corresponding maximum value subtracting the minimum one among  $S_1$ - $S_5$  produced the Difference of Extreme Value (DEV) of each factor. The bigger the DEV is, the more significant the corresponding factor has effect on Jack

Table 1: Factors and levels of orthogonal experiment

Levels	Pressures (MPa)	Temperature (°C)	pH	Times (hours)
1	0	35	3.5	24
2	50	40	4.0	48
3	150	45	4.5	72
4	300	50	5.0	120
5	500	55	5.5	168

Table 2: Activity results of the orthogonal experiment and analyses of means

No.	Pressures (MPa)	Temperatures (°C)	pH	Times (hours)	Activities (U/mL)
1	1	1	1	1	0
2	1	2	2	2	9.9510
3	1	3	3	3	8.4140
4	1	4	4	4	29.519
5	1	5	5	5	2.2920
6	2	1	2	3	1.1570
7	2	2	3	4	1.8860
8	2	3	4	5	6.5130
9	2	4	5	1	9.9510
10	2	5	1	2	0
11	3	1	3	5	1.5870
12	3	2	4	1	2.0460
13	3	3	5	2	10.779
14	3	4	1	3	0
15	3	5	2	4	0
16	4	1	4	2	0.3290
17	4	2	5	3	3.9980
18	4	3	1	4	0
19	4	4	2	5	0
20	4	5	3	1	0.0383
21	5	1	5	4	0
22	5	2	1	5	0
23	5	3	2	1	0.0480
24	5	4	3	2	0.0060
25	5	5	4	3	0
$S_1$	50.177	3.0750	0	12.084	
$S_2$	19.508	16.345	11.157	21.067	
$S_3$	14.413	25.755	11.932	13.570	
$S_4$	4.3660	39.477	38.409	31.405	
$S_5$	0.0550	2.3300	27.021	15.610	
DEV	50.122	37.147	38.409	19.321	

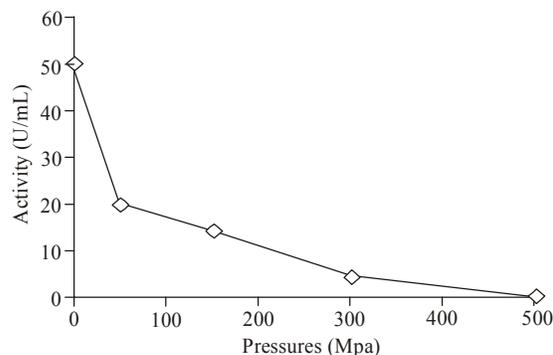


Fig. 1: The variation of Jack bean  $\alpha$ -mannosidase activity with pressure levels

bean  $\alpha$ -mannosidase activity. It was found that the DEV of pressure is highest among all of factors, indicating that the pressure was the most significant factor on the enzyme activity compared with the other three factors. According to the Table 2, the variation of Jack bean  $\alpha$ -mannosidase activity with five levels of pressure, temperature, pH and treatment time were plotted in Fig. 1, 2, 3 and 4, respectively. Figure 1 shows that the

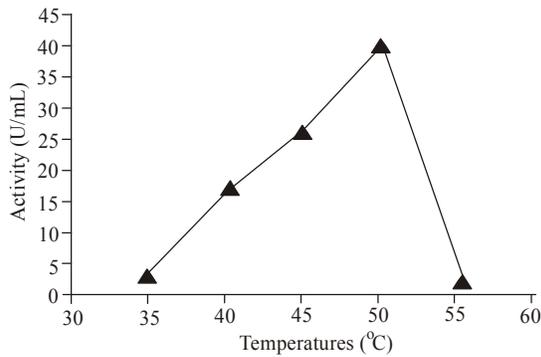


Fig. 2: The variation of Jack bean  $\alpha$ -mannosidase activity with temperature levels

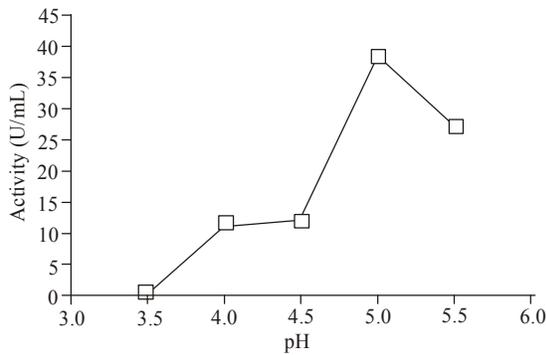


Fig. 3: The variation of Jack bean  $\alpha$ -mannosidase activity with pH levels

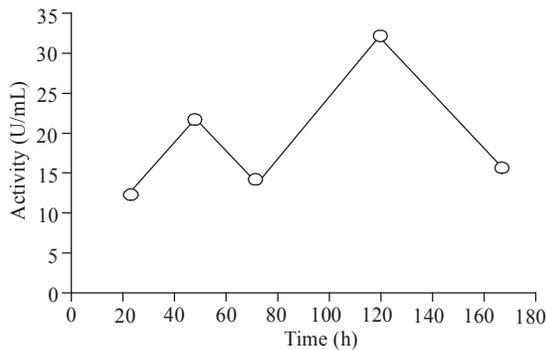


Fig. 4: The variation of Jack bean  $\alpha$ -mannosidase activity with time levels

Jack bean  $\alpha$ -mannosidase activity decreases with the elevating pressure. Jack bean  $\alpha$ -mannosidase activity has a sharp drop when the pressure increases from atmosphere to 50 MPa. Above 50 MPa, the Jack bean  $\alpha$ -mannosidase activity decreases slowly. Slight enzyme activity was found even at 500 MPa. Figure 2 reveals that, below 50°C, the enzyme activity rise with the increasing temperature. The activity of Jack bean  $\alpha$ -mannosidase reached the maximum at 50°C. However, the enzyme might be in quasi-complete inactivation at 55°C. Figure 3 suggests that Jack bean  $\alpha$ -mannosidase is unstable at a high acid case and could be completely

Table 3: Analysis of variance showing significance of independent variables on enzyme activity

Source	df	S.S.	M.S.	F	Sig.
Pressure	4	311.60	77.90	4.97	**
Temperature	4	187.34	46.83	2.99	*
pH	4	181.02	45.26	2.89	*
Time	4	87.680	21.92	1.40	
Error	16	250.69	15.66		

\*: Significant at 10% level; \*\*: Significant at 1% level

deactivated in pH 3.5 while pH 5.0 is optimal. This result was consistent with previous study (Snaith and Levvy, 1968). In Fig. 4, we found two peak values at 120 h and at 48 h. That the possible reason for this could be that the effect of treatment time on enzyme activity is not important in comparison with the other factors. From Table 2, we can conclude that the optimum conditions for Jack bean  $\alpha$ -mannosidase activity are: pressure at 1 atmosphere, temperature at 50°C, pH at 5.0 and treatment time at 120 h.

Noticeably, although a sharp ascendance in the enzyme activity occurred as the pressure rose from the atmosphere to 50 MPa, we can see from Table 2 that Jack bean  $\alpha$ -mannosidase activity under the conditions: 150 MPa, 45°C, 5.0 and 48 h and 100 MPa, 50°C, 5.5 and 24 h, is 10.779 and 9.951 U/mL, respectively, despite less than that under the optimum condition which is significant higher than those under the other conditions. This indicates that Jack bean  $\alpha$ -mannosidase will keep comparatively high activity at moderate pressure coupled with appropriate temperature, pH and treatment time. Thus, the 150 MPa, 45°C, 5.0 and 48 h can be considered as the optimal combination for Jack bean  $\alpha$ -mannosidase activity in the presence of hydrostatic pressure. Especially, our resultant data are helpful in explaining that the hydrostatic pressure is favorable for the enzymatic synthesis of mannooligosaccharides.

To further investigate which factor significantly affects the Jack bean  $\alpha$ -mannosidase activity, ANOVA (Zhao, 2006) was carried out and the results were showed in Table 3. Statistical analyses indicated that pressure, temperature and pH are principal factors due to significant effects on the enzyme activity these results are good agreements with the previous analysis of means.

Finally, in order to validate the optimum conditions suggested by the matrix experiments, another two experiments were performed under the two proposed optimum combined conditions: one in the absence of high pressure and the other in the presence of high pressure. The similar results from duplication showed that the optimal parameters for Jack bean  $\alpha$ -mannosidase activity were pressure at 1 atmosphere, temperature at 50°C, pH at 5.0 and treatment time at 120 h in the absence of high pressure and 150 MPa, 45°C, 5.0 and 48 h is the optimum combined condition in the presence of hydrostatic pressure.

## CONCLUSION

The enzyme activity of Jack bean  $\alpha$ -mannosidase was significantly affected by hydrostatic pressures, temperature and pH. The treatment time did have less effect on  $\alpha$ -mannosidase activity and the highest activity was obtained with temperature at 50°C, pH at 5.0 and treatment time at 120 h in the absence of high pressure. In the case of presence of high pressure, the combination: temperature 45°C, pH 5.0, treatment time 48 h and hydrostatic pressure 150 Mpa is the optimum condition for Jack bean  $\alpha$ -mannosidase activity.

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