

Research Article

Evaluation of a Commercial β -Mannanase for Konjac Glucomannan Hydrolysis: Activity and Molecular Weight Analysis

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ABSTRACT

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β -Mannanase (endo-1,4- β -D-mannanase) is a glycoside hydrolase with potential for producing mannoooligosaccharides (MOS), which are recognized for their prebiotic properties. However, the applicability of commercial enzymes to specific substrates such as konjac glucomannan (KGM) remains insufficiently evaluated. This study investigated the hydrolytic activity and estimated the molecular weight of a commercial β -mannanase (Mannanase BGM "AMANO" 10) toward KGM. The enzyme exhibited measurable activity under the tested conditions, with a specific activity of approximately 100,574 U g⁻¹ protein and a corresponding reducing sugar yield. SDS-PAGE analysis indicated an apparent molecular weight in the range of 50–75 kDa. These findings provide preliminary insights into the performance of the commercial enzyme on KGM and establish baseline information for its potential application in MOS production.

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1. Introduction

β -Mannans are among the major hemicellulosic polysaccharides found in nature and exhibit considerable structural diversity, including linear mannan, glucomannan, galactomannan, and galactoglucomannan (Singh *et al.*, 2018b; Zhang *et al.*, 2021). Among these, konjac glucomannan (KGM) has attracted considerable interest as a promising substrate for the production of mannoooligosaccharides (MOS) because of its abundance, water solubility, and well-defined β -(1→4)-linked glucomannan backbone composed mainly of D-mannose and D-glucose residues (Singh *et al.*, 2018a; Sun *et al.*, 2023).

Controlled depolymerization of KGM can generate MOS, typically with a degree of polymerization (DP) of 2-6, which have received increasing attention due to their potential prebiotic functionality (Jana *et al.*, 2021; Singh *et al.*, 2019; Zhang *et al.*, 2019). Because MOS are selectively utilized by beneficial gut microorganisms and are associated with gut-health-related applications, the efficient conversion of KGM into structurally defined oligosaccharides is of growing interest in functional food research (Hughes, 2020; Liu *et al.*, 2022; Magengelele *et al.*, 2021).

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The enzymatic degradation of β -mannan-containing polysaccharides may involve several glycoside hydrolases, including endo- β -mannanase, β -mannosidase, and accessory enzymes acting on substituted side groups (Kaur and Dhawan, 2007; Malgas *et al.*, 2015), as classified in the CAZy database. Among these, β -mannanase (EC 3.2.1.78) is particularly important because it catalyzes the cleavage of internal β -(1 $\rightarrow$ 4)-mannosidic linkages within the mannan backbone, thereby initiating depolymerization and facilitating MOS production (Cai *et al.*, 2022; Kaur *et al.*, 2007; Malgas *et al.*, 2015). For this reason, β -mannanases have been widely studied for the conversion of mannan-rich substrates into functional oligosaccharides (Arunrattanamook *et al.*, 2020; Hu *et al.*, 2020; Magengelele *et al.*, 2021; Pomsang *et al.*, 2024). However, the efficiency of MOS generation is strongly influenced by substrate structure, enzyme source, and hydrolysis conditions (Hu *et al.*, 2020; Murillo-Franco *et al.*, 2025; Srivastava and Kapoor, 2017). Previous studies have primarily focused on β -mannanases derived from microbial sources or purified enzyme systems, whereas comparatively less attention has been given to the characterization of commercially formulated enzymes for KGM hydrolysis. Consequently, although enzymatic hydrolysis of mannan-containing substrates has been widely investigated, the substrate-specific performance of commercially available β -mannanase preparations toward KGM remains insufficiently characterized (Dawood and Ma, 2020; Magengelele *et al.*, 2021), with only limited information available for an enzyme such as Mannanase BGM “AMANO” 10 under defined experimental conditions. This study therefore provides baseline experimental data on the hydrolytic activity and molecular weight of a commercial β -mannanase toward KGM.

Commercial enzyme preparations are of practical interest because they represent readily accessible biocatalysts for applied research and future process development (Arunrattanamook *et al.*, 2020; Murillo-Franco *et al.*, 2025; Zhou *et al.*, 2021). Therefore, evaluating their performance on specific substrates is important for reducing uncertainty in enzyme selection and for establishing baseline information for subsequent MOS production studies. In this context, Mannanase BGM “AMANO” 10 was selected as a commercially available β -mannanase for preliminary characterization against KGM. This study aimed to determine its hydrolytic activity using the DNS assay and to estimate its molecular weight by SDS-PAGE. The findings are expected to serve as a useful reference for the selection and utilization of commercial β -mannanases in future research on KGM hydrolysis and MOS production.

2. Materials and Methods

2.1 Enzyme activity assay

Enzymatic activity was determined using the 3,5-dinitrosalicylic acid (DNS) method by measuring the release of reducing sugars at 540 nm, as described by Miller (1959).

2.1.1 Substrate, chemicals, and enzyme

KGM was obtained from Thai Union (Thailand). Commercial β -mannanase (Mannanase BGM “AMANO” 10) was purchased from Amano Enzyme Inc (Japan). 3,5-dinitrosalicylic acid (DNS) and other analytical-grade chemicals were obtained from Sigma-Aldrich (USA).

2.1.2 Mannose standard curve

A standard curve was prepared using mannose solutions in the range of 0-2 mg mL⁻¹. Absorbance was measured at 540 nm using a microplate reader (BioTek Synergy HTX Multi-Mode Reader, BioTek Instruments, USA). All measurements were performed in triplicate and corrected using a reagent blank.

2.1.3 Substrate preparation

A 1% (w/v) KGM stock solution was prepared in 50 mM sodium acetate buffer (pH 5.0) and stirred until fully dissolved. For the enzyme assay, 100 μ L of the KGM solution was used as the substrate.

2.1.4 Enzyme preparation

The enzyme stock solution (2 mg mL⁻¹) was diluted with distilled water to obtain a range of working concentrations before the enzyme activity assay. Preliminary experiments were conducted to determine appropriate dilution levels for the DNS assay. Based on the observed absorbance values, enzyme dilutions in the range of 1000- to 6000-fold were selected to ensure that measurements fell within the linear range of the mannose standard curve. All selected dilution levels (1000-, 2000-, 3000-, 4000-, 5000-, and 6000-fold) were used as independent experimental conditions for activity measurements, as presented in Table 1. Heat-inactivated enzyme samples were prepared by boiling the enzyme solution for 10 min, followed by rapid cooling, and were used as negative controls.

2.1.5 Enzyme reaction

The reaction mixture consisted of 100 μ L enzyme solution and 100 μ L substrate solution. The mixtures were vortexed and incubated at 70°C for 30 min. All assays were performed in triplicate.

2.1.6 DNS assay

After incubation, 200 μ L DNS reagent was added, and the mixtures were boiled for 10 min. The reaction was stopped by cooling in an ice bath, then diluted with 2 mL of distilled water. Absorbance was measured at 540 nm using a microplate reader. (BioTek Synergy HTX Multi-Mode Reader, BioTek Instruments, USA). All measurements were performed in triplicate and corrected using a reagent blank.

2.1.7 Determination of enzyme activity

One unit (U) of β -mannanase activity was defined as the amount of enzyme required to release 1 μ mol of reducing sugar (mannose equivalent) per minute under the assay conditions. The total activity was calculated using Equation (1)

Total activity ($U\ mL^{-1}$)

$$= \frac{(OD\ sample - OD\ control) \times 1000 \times Dilution\ factor}{Slope \times MW \times Time \times Reaction\ volume} \quad (1)$$

OD sample = absorbance of the enzyme solution

OD control = absorbance of control

Dilution factor = dilution (times)

Slope = m value of the standard curve

Mw = molecular weight of mannose ($180.16\ g\ mol^{-1}$)

Time = reaction time (min)

Reaction volume = the total assay volume (mL)

2.1.8 Specific activity

Specific activity was calculated as the ratio of total activity to protein concentration using Equation (2). Protein concentration was determined by the Bradford method using bovine serum albumin (BSA) as a standard (Bradford, 1976).

Specific activity ($U\ mg^{-1}\ protein$)

$$= \frac{Total\ activity\ (U\ mL^{-1})}{Protein\ concentration\ (mg\ mL^{-1})} \quad (2)$$

2.1.9 Statistical analysis

All experiments were performed in triplicate. Results are expressed as mean $\pm$ standard deviation (SD). Data analysis was performed using Microsoft Excel (Microsoft Corp., USA).

2.2 Determination of mannanase molecular mass by SDS-PAGE

The molecular mass of the mannanase was determined by SDS-PAGE according to the method of Laemmli (1970).

2.2.1 Sample preparation

The enzyme sample was mixed with an equal volume of 2 $\times$ Laemmli sample buffer (0.125 M Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, 0.004% bromophenol blue, and 10% β -mercaptoethanol) at a ratio of 1:1 (v/v). The mixture was heated at 95°C for 10 min to denature the protein.

2.2.2 Gel electrophoresis

SDS-PAGE was performed using a vertical slab gel system with a 12% resolving gel. A volume of 25 μ L of the prepared sample and a molecular weight marker (10–250 kDa, Bio-Rad, USA) were loaded into the wells. Electrophoresis was carried out at a constant voltage of 100 V for approximately 2 h until the dye front reached the bottom of the gel.

2.2.3 Gel staining and destaining

After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 solution for 1h and subsequently destained using a solution of methanol: acetic acid: water (40:10:50, v/v/v) until clear protein bands were visible. The molecular mass of the enzyme was estimated by comparison with the protein marker. A protein molecular weight marker (10–250 kDa, Bio-Rad, USA) was used.

3. Results and Discussion

3.1. Enzyme assay of β -mannanase

A mannose calibration curve was constructed to quantify reducing sugars released during konjac glucomannan (KGM) hydrolysis using the DNS assay (Miller, 1959). The calibration curve showed a linear relationship between absorbance at 540 nm and mannose concentration over the tested range, with the regression equation $y = 0.3012x - 0.0103$ and an R^2 value of 0.9991 (Figure 1), indicating a high degree of linearity within the working range.

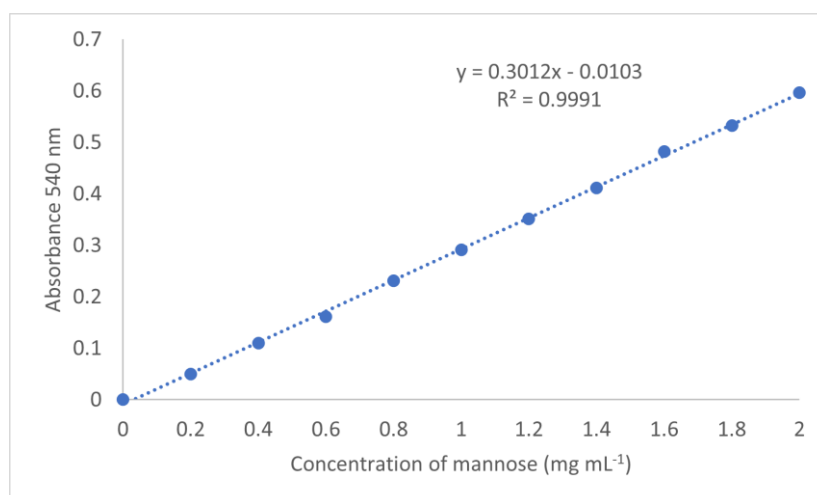


Figure 1 Linear calibration curve showing the relationship between absorbance at 540 nm and mannose concentration (0–2.0 $mg\ mL^{-1}$) obtained using the DNS method ($R^2 = 0.9991$).

Table 1 Absorbance values at 540 nm, reducing sugar concentration, total reducing sugar, reducing sugar yield, total activity, and specific activity of commercial β -mannanase determined by the DNS assay.

Enzyme dilution	Absorbance (540 nm)	Reducing sugar conc. (mg mL ⁻¹)	Total reducing sugar (mg)	Reducing sugar yield (μ mol)	Total activity (U)	Specific activity (U g ⁻¹ protein)
1000-fold	0.758 $\pm$ 0.007	2.552	0.510	1.418	0.0469	156,392
2000-fold	0.715 $\pm$ 0.015	2.409	0.482	1.338	0.0443	147,643
3000-fold	0.636 $\pm$ 0.023	2.147	0.429	1.193	0.0395	131,569
4000-fold	0.540 $\pm$ 0.017	1.828	0.366	1.016	0.0336	112,036
5000-fold	0.523 $\pm$ 0.024	1.769	0.354	0.983	0.0325	108,441
6000-fold	0.484 $\pm$ 0.011	1.641	0.328	0.912	0.0302	100,574

Note: One unit (U) of β -mannanase activity was defined as the amount of enzyme required to release 1 μ mol of reducing sugar (mannose equivalents) per minute under the assay conditions. The amount of protein used in each assay was 3.0×10^{-4} mg. Absorbance values are expressed as the mean $\pm$ standard deviation (SD) from triplicate measurements.

The hydrolysis of KGM by the commercial β -mannanase was evaluated under the tested conditions using different enzyme dilutions (1000–6000-fold), and the results are summarized in Table 1. The measured absorbance values (mean $\pm$ SD) were converted to mannose-equivalent reducing sugar concentrations using the calibration curve.

The release of reducing sugars increased with hydrolysis, observed at the 1000-fold dilution. Under these conditions, the highest absorbance (0.758 $\pm$ increasing enzyme concentration, with the highest 0.007), reducing sugar concentration (2.552 mg mL⁻¹), reducing sugar yield release (1.418 μ mol), total activity (0.0469 U), and specific activity (156.39 U mg⁻¹ protein) were obtained (Table 1). As the enzyme dilution increased from 1000- to 6000-fold, all measured parameters decreased accordingly, indicating a dilution-dependent reduction in hydrolytic activity.

For quantitative analysis, measurements within the linear range of the calibration curve were considered. The 6000-fold dilution (0.484 $\pm$ 0.011) was therefore selected as a representative condition, as it provided absorbance values within the central region of the calibration curve, thereby ensuring reliable quantification under the applied assay conditions. The relatively low standard deviations observed across replicates indicate good reproducibility of the assay.

The specific activity values obtained in this study were higher than the manufacturer's stated minimum activity. However, this comparison should be interpreted with caution, as total activity measurements are strongly influenced by assay conditions, including substrate type and detection method (Ghose, 1987; Zhu *et al.*, 2022). In the present study, KGM was used as the substrate, whereas commercial specifications are often based on galactomannan substrates. Structural differences between these polysaccharides may influence enzyme accessibility and hydrolysis behavior. KGM is a glucomannan composed of β -(1 $\rightarrow$ 4)-linked mannose and glucose units, whereas galactomannan contains a β -(1 $\rightarrow$ 4)-mannan backbone substituted with galactose side chains (BeMiller, 2019; Zhang *et*

al., 2021). Variations in side-chain substitution have been reported to affect enzyme–substrate interactions and catalytic performance (Chai *et al.*, 2020).

In addition, the DNS assay detects reducing ends generated during hydrolysis and is known to be sensitive to short-chain oligosaccharides (Miller, 1959; Zhu *et al.*, 2022). This differs from alternative methods such as the Somogyi–Nelson assay, which is based on copper ion reduction and may exhibit different sensitivities depending on sugar composition (Bailey *et al.*, 1992; Slavin, 2015; Song *et al.*, 2018). These methodological differences may contribute to variations in reported total activity values.

Furthermore, no information regarding enzyme purity was provided by the manufacturer, and purity was not independently verified in this study. Therefore, the reported specific activity values should be interpreted as apparent values rather than absolute indicators of enzyme purity. Protein concentration was determined using the Bradford assay, which may introduce variability depending on protein composition and the choice of standard protein.

It is important to note that total activity in this study was evaluated under a single reaction condition (pH 5.0 and 70°C) using only KGM as the substrate. Therefore, no conclusions can be drawn regarding catalytic efficiency, substrate specificity, optimum conditions, thermostability, or pH stability. Furthermore, hydrolysis products were not characterized; thus, the potential for MOS production is discussed only as a possible application rather than a confirmed outcome.

Further studies should include kinetic analysis (K_m and V_{max}), substrate comparison, stability profiling, and product characterization to provide a more comprehensive understanding of the catalytic properties and application potential of the commercial β -mannanase.

3.2 Molecular mass of mannanase

SDS-PAGE was performed under reducing conditions to estimate the molecular mass of the commercial β -mannanase (Figure 2)

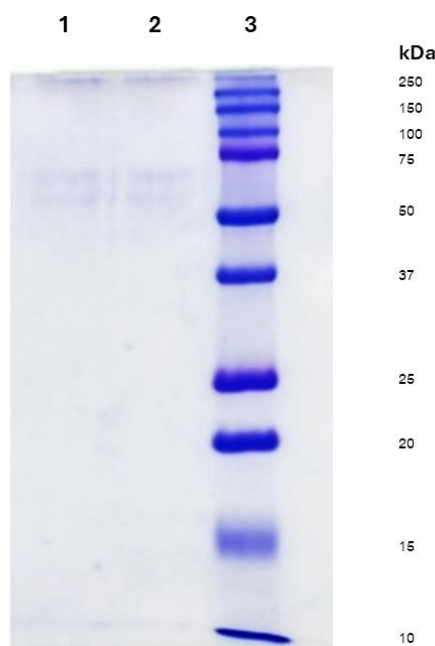


Figure 2 SDS-PAGE analysis of commercial β -mannanase (BGM “AMANO” 10) under reducing conditions. Lane 1–2: β -mannanase samples (1 mg mL^{-1}). Lane 3: molecular weight marker (10–250 kDa, Bio-Rad, USA). Faint protein bands were observed in the approximate molecular weight range of 50–75 kDa, consistent with the reported size range of β -mannanases. The low band intensity may be attributed to limited protein loading or staining sensitivity.

The electrophoretic profile revealed two distinct protein bands within the molecular weight range of 50–75 kDa. Based on comparison with the molecular weight marker, the bands were estimated to have molecular masses of approximately 55 kDa and 70 kDa. The presence of two protein bands suggests that the commercial enzyme preparation is not a single purified enzyme but rather a mixture of protein components, which may represent isoenzymes or co-purified proteins. Similar observations have been reported for β -mannanases derived from fungal sources, where multiple isoforms and post-translational modifications, such as glycosylation, can influence electrophoretic mobility (Chai *et al.*, 2020; Yang *et al.*, 2015).

The banding pattern was consistent across replicate lanes, indicating reproducibility of the electrophoretic analysis. However, SDS-PAGE under reducing conditions provides information only on the apparent molecular mass of denatured proteins and does not allow conclusions to be drawn regarding native structure, enzyme purity, or subunit composition.

Therefore, although the observed molecular weight range is consistent with previously reported β -mannanases, further analyses such as non-reducing SDS-PAGE, zymography, or LC-MS would be required to confirm protein identity and to better characterize the enzyme composition.

4. Conclusion

This study evaluated the total activity and molecular mass of a commercial β -mannanase using KGM as a substrate under defined assay conditions. The enzyme exhibited measurable

activity, with activity values dependent on enzyme concentration. SDS-PAGE analysis revealed two protein bands with approximate molecular masses in the range of 50 and 75 kDa. As the enzyme activity was assessed under a single reaction condition and no product characterization was performed, the results should be interpreted as preliminary. Therefore, no conclusions can be drawn regarding catalytic efficiency, substrate specificity, or stability. These findings provide initial evidence supporting the potential use of the commercial β -mannanase for KGM hydrolysis. Further studies are required to evaluate its catalytic properties, substrate interactions, and product profile for potential applications.

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