



# Isolation and purification of D-mannose from palm kernel

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## ABSTRACT

An economically viable procedure for the isolation and purification of D-mannose from palm kernel was developed in this research. The palm kernel was catalytically hydrolyzed with sulfuric acid at 100 °C and then fermented by mannan-degrading enzymes. The solution after fermentation underwent filtration in a silica gel column, desalination by ion-exchange resin, and crystallization in ethanol to produce pure D-mannose in a total yield of 48.4% (based on the weight of the palm kernel). Different enzymes were investigated, and the results indicated that endo- $\beta$ -mannanase was the best enzyme to promote the hydrolysis of the oligosaccharides isolated from the palm kernel. The pure D-mannose sample was characterized by FTIR,  $^1\text{H}$  NMR, and  $^{13}\text{C}$  NMR spectra.

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## 1. Introduction

D-Mannose is a hexose that has wide application in the pharmaceutical industry, in the food and feedstuff industries, as well as in biological research. It is used as a feedstuff additive, which prevents infections caused by bacteria, especially by *Salmonella*.<sup>1</sup> Its inhibition of the inflammatory reaction in wound healing and a decrease in granulation tissue formation has also been reported.<sup>2</sup> In addition, mannan-binding lectins<sup>3</sup> and proteins have been proved to possess extraordinary biological activities as well.

There are two main processes to obtain D-mannose: (1) isomerization of other kinds of hexoses and (2) isolation from plants. Jun Hirose<sup>4</sup> reported the isomerization of D-fructose to D-mannose using immobilized *Agrobacterium radiobacter* as the catalyst at 60 °C and pH 7.5. Glucose can undergo the classic Lobry de Bruyn-Alberda van Ekenstein epimerization<sup>5</sup> to produce the corresponding mannose. Mannose can also be formed from D-glucose in the Bilik reaction with molybdate-supported catalysts on an ion-exchange resin in a yield of 28.4%.<sup>6</sup> Other similar reactions<sup>7</sup> have been reported as well. Compared to the isomerization of other hexoses, isolation of D-mannose from plant tissue could be more economical. It has been reported that D-mannose could be isolated from coffee grounds by acid hydrolysis in a yield of 48%.<sup>8</sup> Mannose-containing palm kernel meal, used as a feedstuff additive,<sup>1</sup> was produced by the enzyme-catalyzed hydrolysis of raw palm kernel meal or copra meal.

In this study, we present an economically viable method on the isolation and purification of D-mannose from palm kernel, in which

the raw material undergoes hydrolysis in acid, fermentation with enzymes, filtration on a silica gel column, desalination by an ion-exchange resin, and crystallization in organic solvent to produce pure D-mannose in a total yield of 48.4% (based on the weight of the palm kernel). Influential factors of each step were investigated, and the experimental parameters were optimized. The complete process is illustrated in Figure 1.

## 2. Materials and methods

### 2.1. Materials

Palm kernel of *Elaeis guineensis* was provided by Yunnan Hongyun Pharmaceutical Co., Ltd, PR China. All types of mannan-degrading enzymes were supplied by Shanghai Baofeng Biochemical Corporation, Ltd, PR China. D-Mannose used as a standard was purchased from Sinopharm Chemical Reagent Co., Ltd, PR China. Other chemical agents, such as sulfuric acid, ethanol, and silica gel, were purchased from Hangzhou Huadong Medicine Co., Ltd. Enzymes were food grade, and all chemicals were analytical grade. The ion-exchange resins were treated before use.

### 2.2. Characterizations

A digital polarimeter (Autopol V, Rudolph, USA) was used for the measurement of the optical rotation of D-mannose. To trace the reaction, TLC was carried out at room temperature with different solvent systems: system A: 8:2:2:1 EtOAc–EtOH–Py–H<sub>2</sub>O; system B: 2:1:0.7:0.3 BuOH–EtOAc–2-PrOH–H<sub>2</sub>O; system C: 6:3:1 EtOAc–2-PrOH–H<sub>2</sub>O. Concentration of the products was detected by HPLC (SPD-20A, Prominence, Japan) with a Phenomenex

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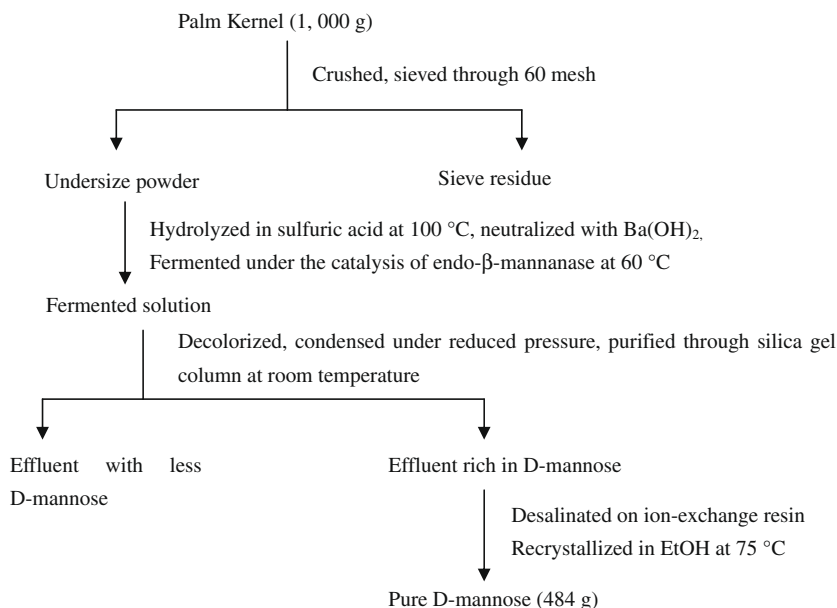


Figure 1. The isolation process.

NH<sub>2</sub>-column with 7:3 CH<sub>3</sub>CN–H<sub>2</sub>O as the eluent at 60 °C at a flow rate of 0.8 mL/min. Detection was accomplished by UV absorbance at 190 nm. The structure of D-mannose was confirmed by <sup>1</sup>H NMR (Bruker AC-400, 400 MHz, DMSO), <sup>13</sup>C NMR (Bruker AC-400, 101 MHz, DMSO), and IR (Nicolet NEXUS-670 FTIR).

### 2.3. Acid and enzymatic hydrolysis

Palm kernel was dried at 50 °C and then crushed to powder. A fraction (100 g) with a particle size smaller than 60 mesh was dispersed in 1000 mL of water, and the system was heated to reflux for 3 h, after which time concd sulfuric acid (5 mL) was added dropwise at 100 °C. After 30 min of reaction, the system was cooled to room temperature and then neutralized with Ba(OH)<sub>2</sub>. The insoluble solid was filtered out and washed twice with 100 mL of deionized water. The washings were combined with the previous filtrate, and the mixture was then evaporated to 100 mL. The concentration of D-mannose in the solution was determined by HPLC.

The hydrolysate was then added with 1 g of mannan-degrading enzymes (0.01 g/mL) and fermented at 60 °C and pH 7 for 60 min. The concentration of D-mannose in the new solution was detected under the same conditions and compared with the previous results.

### 2.4. Isolation

The solution obtained in the previous step was first decolorized with charcoal at 50 °C for 30 min and then filtered to obtain a clear solution that was then evaporated under reduced pressure. The solution was initially monitored by TLC and the results were compared with the peak of standard mannose to select a suitable eluent. Then the concentrate was mixed with the same weight of silica gel, and the mixture was stirred and heated to remove the water. The dry powder thus obtained was then purified through a silica gel column (24 × 500 mm) using solvent system B (2:1:0.7:0.3 BuOH–EtOAc–2-PrOH–H<sub>2</sub>O) at a flow rate of 5 mL/min. As the product was colorless, the effluent was collected in a series of small bottles (50 mL each time), and each batch was analyzed by HPLC to identify the concentration of D-mannose. The solution in the first four bottles (200 mL) was processed in the following step.

### 2.5. Desalination

Two experiments were carried out in this step with two columns (24 × 800 mm, labeled as A and B) filled with different ion-exchange resins connected in series. In the first experiment, a cation-exchange resin (#001 × 4) was filled in column A, and an anion-exchange resin (#201 × 7) in column B. The effluent obtained in step 2.4 (100 mL), which contained D-mannose, was purified, in turn, through columns A and B. The conductivity of the effluent was then detected. The order of the two columns was reversed in the second experiment with other operations remaining the same as the first one.

### 2.6. Crystallization

The desalinated solution was then condensed to a concentration of 75% (w/w) under reduced pressure. The concentrate was filtered to separate the impurities. The filtrate (100 mL) was transferred to a sealed container, and 500 mL of 95% (w/w) of EtOH was added. The mixture was then shaken for 3 h in a shaking table in a water bath at 75 °C. After that, the solution was allowed to stand for 24 h to yield the pure D-mannose, 48.4 g; mp 122–126 °C, lit.<sup>9</sup> 129–131 °C;

$[\alpha]_D^{20} +13.9$  (c = 10 g/100 mL, water), lit.<sup>9</sup> +14.1 (c = 10 g/100 mL, water), IR (film);  $\sigma_{\max}$  3464.6, 3375.8 and 3302.2 (–OH), 2902.8 (–CH<sub>2</sub>–), 1087.7 and 1072.5 (–O–C); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz):  $\delta$  6.14–6.16 (d, 1H), 4.67–4.68 (d, 1H), 4.52–4.54 (t, 2H), 4.41–4.45 (q, 2H), 3.64–3.68 (m, 1H), 3.54 (d, 1H), 3.37–3.43 (m, 1H), 3.25–3.26 (d, 2H), 2.98–3.01 (t, 1H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 101 MHz):  $\delta$  (61.86, C-6), (67.43, C-4), (72.00, C-2), (74.19, C-3), (77.60, C-5), (94.55, C-1).

## 3. Results and discussion

### 3.1. Effect of different enzymes

It has been reported that more than 81% of palm kernel carbohydrate is in the form of non-starch polysaccharides,<sup>10</sup> and HPLC results indicated that the palm kernel was hydrolyzed in the sulfuric acid to yield large amount of low-molecular-weight oligosac-

**Table 1**

Three types of polysaccharide-degrading enzymes, weight ratio of enzymes used to the palm kernel, reaction times, and concentration of D-mannose after fermentation at 60 °C and pH 7

Types of enzymes	Weight ratio to the palm kernel (%)	Reaction time (min)	Concentration of D-mannose (g/mL)
—	0	50	0.256
<i>endo</i> -β-Mannanase	1.0	50	0.639
α-Mannosidase	1.0	50	0.582
Xylanase	1.0	50	0.285

charides, including mannan, galactomannan, glucomannan, hemicelluloses, among other components. Different enzymes (listed in Table 1) were employed to decompose these oligosaccharides to D-mannose, and the results are listed in Table 1.

The results are that *endo*-β-mannanase and α-mannosidase showed much better performance in the process, and D-mannose was readily produced under the two catalysts at the same temperature for the same reaction time. We could obtain only a little of the target product without using an effective enzyme. When xylanase was employed, the concentration of glucose was shown to be higher than that of D-mannose. It successfully removed the glucose and galactan side-chains but left the main chain of hemicellulose connected with 1,4-mannoside bonds.

The *endo*-β-mannanase<sup>11–15</sup> has long been demonstrated to be effective in the hydrolysis of the cross-link network of hemicellulose. In our experiments, compared with the other two catalysts, it showed a significant effect in dissociating the 1,4-mannoside bonds, and a remarkable amount of D-mannose was released.

### 3.2. Operational conditions for hydrolysis

Key parameters such as temperature, reaction times, and concentration of catalyst in both acid and enzymatic hydrolysis were selected in a series of parallel experiments. The results are briefly described below.

The acid hydrolysis was tried at 40, 60, 80, 100, and 120 °C. A temperature of 100 °C was shown to be best with the highest concentration of D-mannose at 0.256 g/mL. With respect to reaction time, the concentration of D-mannose resulted in a drastic increase in the first 50 min to 0.256 g/mL, but remained the same value for the next 20 min. Therefore, 50 min of hydrolysis was appropriate.

In the step of enzymatic hydrolysis, 0.1 g, 0.5 g, 1 g, and 2 g of *endo*-β-mannanase were added to four identical parts of acid hydrolysate (100 mL), respectively, to identify the appropriate concentration. The results showed that a lesser amount (0.1 g, 0.5 g) of enzyme led to a much longer reaction time (3 h) to obtain the same yield, while a larger amount (2 g) led evidently to no better performance (0.635 g/mL of D-mannose) than 1 g of *endo*-β-mannanase (0.639 g/mL of D-mannose) within a reaction time of 60 min. Therefore, 0.01 g/mL of enzyme was selected. Similarly, the temperature and pH of the enzymatic reaction were optimized to gain a higher yield of D-mannose. A temperature of 60 °C at pH 7 was shown to be the best combination with 0.639 g/mL of D-mannose obtained in 1 h.

### 3.3. Order of desalination

The effluent was desalinated in two columns in which both anion- and cation-exchange resin were used (as described in Section 2.6). The effect of both arrangements is listed in Table 2.

**Table 2**

Effects of the two desalination columns arranged in different order

	Conductivity (us/cm)	Salinity
<i>Cation-exchange resin before anion-exchange resin (process 1)</i>		
Before desalination	1950	3.2
After desalination	125.3	0.2
<i>Anion-exchange resin before cation-exchange resin (process 2)</i>		
Before desalination	1950	3.2
After desalination	1890	3.1

From Table 2 we could see a drastic decrease in the conductivity after process 1 and the uselessness of process 2. The isolated effluent first went through the cation-exchange resin to yield an acidic solution with most cations eliminated. The acidic solution was then neutralized by anion-exchange resin. The ion concentration was very low in the solution after the treatment. If reversed, precipitates formed from the cations, and protons released by the resin hindered the ion-exchange process.

## 4. Conclusions

According to the above results, we have developed an economically viable procedure in which pure D-mannose can be readily isolated from palm kernel, the fruit of a kind of plant widely distributed in south China. The key step in the procedure is the consecutive acid and enzymatic hydrolysis of the raw material. The purity of D-mannose was verified by the melting point and specific rotation data. The structure of the target molecule was confirmed by IR, <sup>1</sup>H NMR, and <sup>13</sup>C NMR spectroscopy. The total yield of D-mannose was 48.4% based on the weight of palm kernel used. The yield is much higher than the normal process (20–30%) and the two-step hydrolysis does not add much additional effort or cost to the operation. Thus, the economic viability is guaranteed.

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## References

- Yokomizo, Futoshi. W.O. Patent 2,002,081,724, 2002.
- Kössi, J.; Peltonen, J.; Ekfors, T.; Niinikoski, J.; Laato, M. *Eur. Surg. Res.* **1999**, *31*, 74–82.
- Rest, R. F.; Farrell, C. F.; Naidu, F. L. *J. Leukocyte Biol.* **1988**, *43*, 158–164.
- Hirose, J.; Kinoshita, Y.; Fukuyama, S.; Hayashi, S.; Yokoi, H.; Taksaki, Y. *Biotechnol. Lett.* **2003**, *25*, 349–352.
- Angyal, S. J. *Top. Curr. Chem.* **2001**, *215*, 1–14.
- Köchriz, A.; Kant, M.; Walter, M.; Martin, A. *Appl. Catal., A: Gen.* **2008**, *334*, 112–118.
- Watanabe, Y.; Miyawaki, Y.; Adachi, S.; Nakanishi, K.; Matsuno, R. *J. Mol. Catal. B: Enzym.* **2000**, *25*, 241–247.
- Charles, V. F.; Howard, D. S.; Evan, J. T.; Renee, B. U.S. Patent 4,508,745, 1985.
- Sallam, M. A. E. *Carbohydr. Res.* **1977**, *53*, 134–135.
- Sekoni, A. A.; Omenge, J. J.; Bawa, G. S.; Esuga, P. M. *Pakistan J. Nutr.* **2008**, *7*, 614–619.
- Warren, R. A. *J. Annu. Rev. Microbiol.* **1996**, *50*, 183–212.
- Richard, B.; Bewley, J. D. *Plant Physiol.* **2002**, *130*, 1254–1262.
- Christian, H.; Daniel, B.; Hidde, L. P. *EMBO J.* **2003**, *22*, 1035–1046.
- Kensch, O. *Spec. Chem.* **2008**, *28*, 18–19.
- Dhawan, S.; Samriti, K. *Rev. Biotechnol.* **2007**, *27*, 197–216.