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Dextrans removal from sugarcane juice using dextranase from marine bacterium *Arthrobacter oxydans* KQ11

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RESEARCH ARTICLE

Abstract

Dextrans are microbially produced polysaccharides that detrimentally affect sugarcane juice processing. A decrease in sugar quality and yield can thus severely affect the sugarcane industry. Enzymatic hydrolysis is currently considered the most effective approach for removing dextrans from sugarcane juice. Dextranase produced from the marine microorganism *Arthrobacter oxydans* (KQ11) was exogenously added to sugarcane juice with an aim to remove dextrans. Following single-factor experiments, orthogonal experiments were performed, which determined the following optimal conditions for dextrans removal from sugarcane juice: pH 6.5, 50 °C, reaction for 20 min, and an enzyme concentration of 0.15 U/ml. These conditions resulted in 80.7% of dextran removal in sugarcane juice. Besides, the dextran removal was increased by 10% with power ultrasonication of 800 W for 15 min; 90% of dextran was hydrolysed, viscosity of sugarcane juice decreased by 20%. This study suggests the design of ultrasonic horns for dextrans removal in sugar mills.

Keywords: sugar industry, dextrans removal, enzymatic hydrolysis, ultrasound

1. Introduction

Dextrans are polysaccharides consisting of chains of α-1,6-linkedglucose units of varying lengths with multiple molecular weights ranging from 3,000 Da to 2,000,000 Da (Khalikova *et al.*, 2005). The larger dextrans (>60,000 Da) are excreted poorly from the kidney, so remain in the blood for as long as weeks until they are metabolised. Consequently, they have prolonged antithrombotic and colloidal effects. In this family dextran-40 (MW: 40,000 Da), has been the most popular member for anticoagulation therapy. Dextrans are also used in some eye drops as a lubricant, and in certain intravenous fluids to solubilise other factors, such as iron (in a solution known as Iron Dextran). Dextran has also been used as a plasma substitute (Bashari *et al.*, 2016), but extensive branchings in glucose units influence the viscosity and solubility of dextrans (Purushe *et al.*, 2012), which makes it a major problem in the sugar industry. A more than 14 h delay in the arrival of harvested sugarcane in sugar mills in warm and humid conditions favours the formation of dextrans (Imrie and Tilbury, 1972). Sugar losses in mills are mainly attributed to chemical inversion (13%), enzymatic effects (25%), and microbial inversions (62%) (Chen and Chou, 1993). Enzymatic effects are mainly due to resident microflora and strains that contaminate sugarcane juice (Robyt and Eklund, 1982). Dextrans are formed in sugarcane from sucrose by *Leuconostoc mesenteroides* and *Lactobacillus* species during post-harvest staling and juice extraction (Clarke *et al.*, 1980; Eggleston *et al.*, 2009). The presence of dextrans in juice increase the viscosity of the sugarcane juice, which in turn block filters and reduce the yield and quality of sugar (Fravet *et al.*, 2010). Furthermore, the pH of sugarcane juice decreases with the production of lactic and acetic acid from sucrose (Eggleston and Monge, 2005; Li *et al.*, 2017). The presence of dextrans increases the optical rotation of sugarcane juice, leading to errors in the analysis of sucrose content, which in turn interferes with filtration rates, settling and altering the shape of the sugar crystals, thereby causing economic losses for sugar mills (Fukumoto *et al.*, 1971). Physical methods, such as ultrafiltration, membrane dialysis, and reverse osmosis, have been proved to be efficient in dextrans removal, although they are relatively expensive (Jiménez, 2005). The only method of dextrans removal that is currently applicable to the sugar industry is enzymatic hydrolysis (Bhatia *et al.*, 2010; Xu *et al.*, 2004).

Dextranase (EC3.2.1.11), an α-D-1,6 glucan-6-glucanohydrolase, hydrolyses the (1→6) α-D-glucosidic linkages presenting in dextran polysaccharides, forming oligosaccharides (Bhatia *et al.*, 2016; Gan *et al.*, 2014). Dextranase can hydrolyse dextrans to reduce the viscosity of sugarcane juice (Bashari *et al.*, 2013). Although some bacterial strains, fungi, and yeast produce dextranases (Galvez-Mariscal and Lopez-Munguia, 1991; Koenig and Day, 1989), only dextranases that are produced by *Chaetomium gracile* and *Chaetomium erraticum* fungi have been used in industrial applications (Zohra *et al.*, 2013). Properties of dextranase, such as its low activity and poor stability, high cost, and insufficient harvesting techniques, limit its application in the sugar industry (Hild *et al.*, 2007; Virgen-Ortíz *et al.*, 2015). *Arthrobacter oxydans* (KQ11) was found during screening from mud of marine by our laboratory. The molecular weight of the dextranase produced by *A. oxydans* is 66.2 KDa. The optimal reaction temperature of the dextranase is 50 °C, and above 60% of activity of enzyme is remained after storing for 1 h at 60 °C. There is an enzyme activity with the value of pH ranging from 4.0 to 8.0. The enzyme activity of the supernatant of fermentation culture medium can reach 28.1 U/ml (Wang *et al.*, 2014). The present study aimed to develop a method of removing dextrans in sugarcane juice using dextranase from *A. oxydans.*

2. Materials and methods

Chemicals

Dextran (MW: 20,000) was purchased from Guoyao Chemical Reagent Co., Ltd., Shanghai, China. Trichloroacetic acid, anhydrous ethanol, sodium hydroxide and hydrochloric acid were obtained from Nanjing Chemical Reagent Co., Ltd., Nanjing, China. Kieselguhr was from Guoyao Chemical Reagent Co., Ltd.

Sugarcane juice preparation

Sugarcane that was harvested and stacked for less than a week was milled, and the juice was centrifuged (24,000×*g*, 5 min). The supernatant was collected and used in the subsequent experiments. The viscosity of sugarcane juice was 1.0±0.05 mpa·s.

Dextranase preparation

The dextranase used in this study was produced by *A. oxydans* (KQ11), which was selected by screening from sea mud of Lianyungang, Jiangsu, China. Dextranase production was previously optimised at our laboratory. Dextranase was produced after 30 h of aerobic fermentations at 30 °C in a 1,000-l fermentation cylinder. The bacterial culture medium consisted of 10 g/l yeast extract powder, 7.5 g/l soymeal, 5 g/l cassava starch, 4 g/l wheat bran, 4 g/l dextran 20K, 4 g/l sodium chloride, and 0.4 g/l magnesium sulphate. The initial pH was 7.5 and not lower than 7.0 during the entire study. The culture medium was flocculated and centrifuged to remove the bacteria, and the enzyme activity of the crude dextranase was 28.1 U/ml. The supernatant was filtered and lyophilised for storage. Freeze-dried dextranase was resolved in deionised water and concentrated using a Labscale small tangential flow ultrafiltration system with a 10 K filter (Fisher Scientific, Hampton, NH, USA). Working dextranase activity was 9.2 U/ml. The optimal condition for dextranase activity was 50 °C and pH 7 (Wang *et al.*, 2014).

Analytical method

Dextranase assay

Dextranase activity was measured by 3,5-dinitrosalicylic acid (DNS) colorimetry (Miller method) using the increased ratio of the reducing sugar concentration in the reaction with DNS reagent. Approximately 10 μl of dextranase and 190 μl of 3% dextran 20,000 (50 mM sodium phosphate buffer, pH 7) were mixed and incubated at 50 °C for 15 min. Around 200 μl of DNS were added to the experimental and control groups to terminate the reaction, and 10 μl of dextranase were added to the control group. The reaction solution was boiled for 5 min, and 3 ml of deionised water were added. The absorbance was measured at a wavelength of 540 nm. The amount of enzyme required to release 1 μmol of isomaltose per min is an activity unit (U) (Wang *et al.*, 2014).

Dextrans assay

The Sugar Processing Research Institute (SPRI) method (Chen *et al.*, 1993) was used to determine dextrans content. The principle of the SPRI method is that dextrans interacts with a hydrous ethanol at a 50% alcohol level. Dextrans do not dissolve in ethanol and thus induces turbidity. The absorbance value was determined spectrophotometrically. Dextrans content was determined using a standard curve. 1 g of kieselguhr and 3 ml of trichloroacetic acid were added to 10 ml of sugarcane juice, which was then thoroughly mixed and passed through a 0.45-mm filter membrane. The first 2 ml of the filtrate was then collected and then mixed with 2 ml of anhydrous ethanol. The absorbance of the mixture was determined at a wavelength of 720 nm to generate a standard curve, which was utilised in calculating dextrans content.

Viscosity of sugarcane juice assay

Sugarcane juice was added in the rotor (model 0) of rotary viscosimeter. The date was observed at 25 °C with a speed of 60 rpm. The viscosity was calculated using the equation:

$$\eta=\kappa\times\alpha \quad (1)$$

where η is absolute viscosity, κ is viscosity coefficient, α is the date observed.

Single-factor experiment

Dextrans content of 400 ml of sugarcane juice was determined using the SPRI method. Then it was dispensed into different conical bottles. For the pH experiments, the pH (4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) of sugarcane juice were adjusted by sodium hydroxide or hydrochloric acid. The catalysis reactions of dextranase were at 45 °C for 10 min. For the temperature experiments, different catalysis reaction temperatures (35, 40, 45, 50, 55, 60, 65 and 70 °C) were carried out at pH 6 for 10 min. Similarly, different reaction times (5, 10, 15, 20, 25, 30, 35 and 40 min) were employed. The catalysis temperature was 45 °C, and pH was 6.0. Approximately 0.1 U of dextranase was added to each millilitre of sugarcane juice in the above experiments. To optimise enzyme concentration, different concentrations of dextranase (0.01, 0.02, 0.05, 0.10, 0.15 and 0.20 U/ml) were added at pH 6 and allowed to react for 10 min at 45 °C. After the reactions, the sugarcane juice was boiled for 2 min to denature the enzyme. The mixture was then cooled to room temperature, and dextrans content was determined using the SPRI method. The hydrolysis rate of dextrans was calculated relative to the control. Three replicates were conducted in all the experiments.

Orthogonal experiments

Based on the results of single factor experiments, four factors and three levels of orthogonal experiments in Table 1 were conducted to optimise the effects of *A. oxydans* (KQ11) dextranase on dextrans removal in sugarcane juice.

Ultrasonication experiments

Sugarcane juice was adjusted to pH 6.5 using sodium hydroxide. Approximately 0.15 U of dextranase was added to each millilitre of sugarcane juice. The reaction occurred in an ultrasonic cell at 50 °C. Different reaction times (1, 5, 10, 15, 20 and 25 min) were used in the experiments with ultrasonic power of 300 W (ultrasonic treatment for 3 seconds and pause for 3 seconds during reaction time). The ultrasonic power (200, 400, 600, 800 and 1000 W. 20 kHz, wave amplitude was 52.1, 73.7, 90.3, 104.2 and 116.5 μm) for dextrans removal was also optimised. In addition, the ultrasonic temperature (35, 40, 45, 50, 55, 60, 65 and 70 °C) was conducted. After the reactions, the sugarcane juice was boiled for 2 min and then cooled to room temperature. The viscosity was determined by rotary viscometry at room temperature. Dextrans content was determined using the SPRI method, and the hydrolysis rate of dextrans was calculated relative to the control.

Table 1. $L_9(3^4)$ horizontal orthogonal experiment.

Levels	Factors			
	pH	Temperature (°C)	Time (min)	Enzyme dosage (U/ml)
1	6.0	40	10	0.05
2	6.5	45	15	0.10
3	7.0	50	20	0.15

3. Results

Changes of dextrans content in sugarcane juice

As shown in Figure 1, the content of dextrans in sugarcane juice showed an average daily increase of 8.67 mg/l (4.3%) at room temperature.

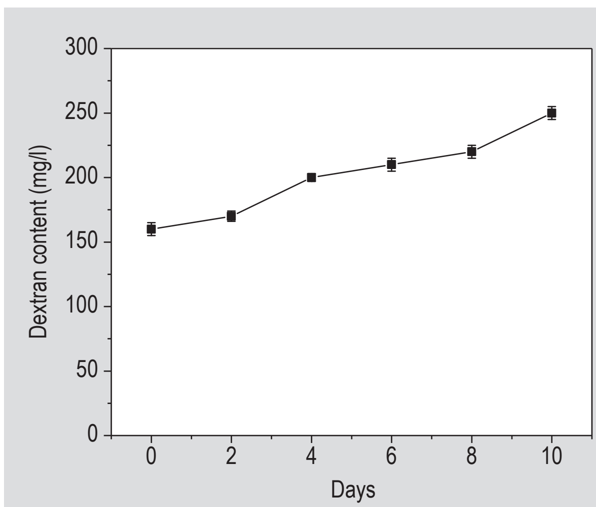

Figure 1. Changes of dextran content in sugarcane juice at room temperature.

Hydrolysis of dextran in sugarcane juice

Influence of pH value

Figure 2 shows that the hydrolysis rate of dextrans enhanced slowly with increased in pH value, reaching the maximum value at pH 6.0, followed by a dramatical decrease after pH 6.5.

Influence of temperature

Figure 3 shows that the hydrolysis rate of dextrans in sugarcane juice remained relatively stable when the reaction temperature was between 35 and 60 °C, and reached a peak at 81.4% using 45 °C. When the temperature was above 60 °C, the hydrolysis rate of dextrans rapidly decreased.

Influence of reaction time

Figure 4 shows that the hydrolysis rates of dextrans in sugarcane juice were almost at the same levels in reaction times ranging from 5 to 40 min. 70% dextran could be removed in five min, and the rate of dextran removal did not change by the time lasting.

Influence of enzyme dosage

Figure 5 shows the effects of dextranase at various concentrations within the range of 0.01-0.20 U/ml. The rate of hydrolysis dextran peaked at 80.15% at an enzyme concentration of 0.10 U/ml.

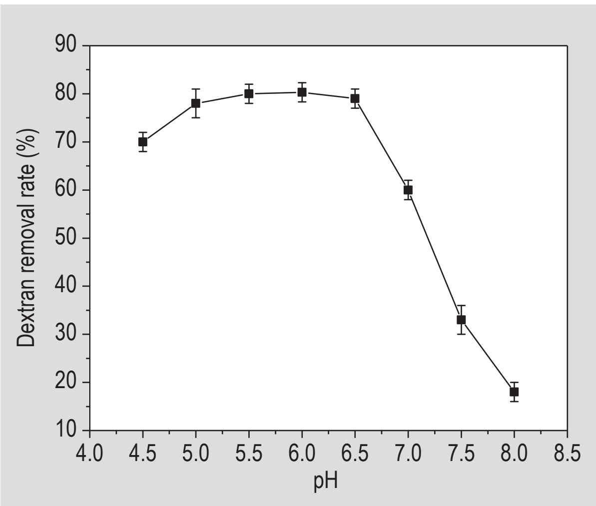

Figure 2. Influence of pH value on the rate of hydrolysis of dextran.

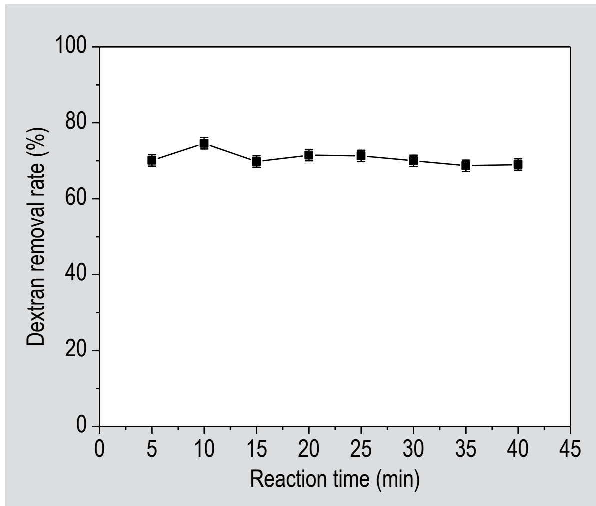

Figure 4. Influence of reaction time on the rate of hydrolysis of dextran.

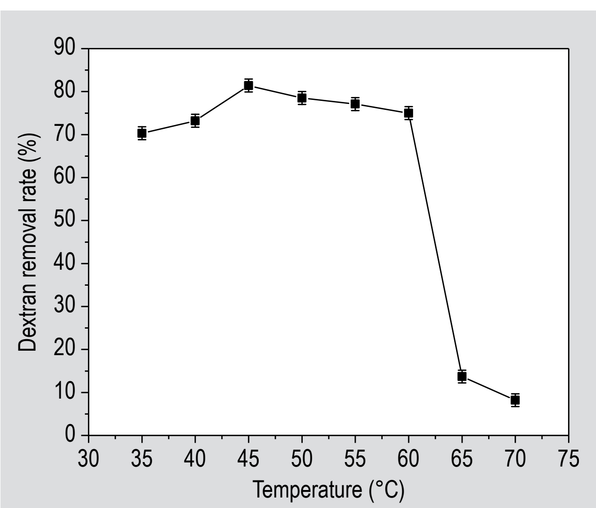

Figure 3. Influence of temperature on the rate of hydrolysis of dextran.

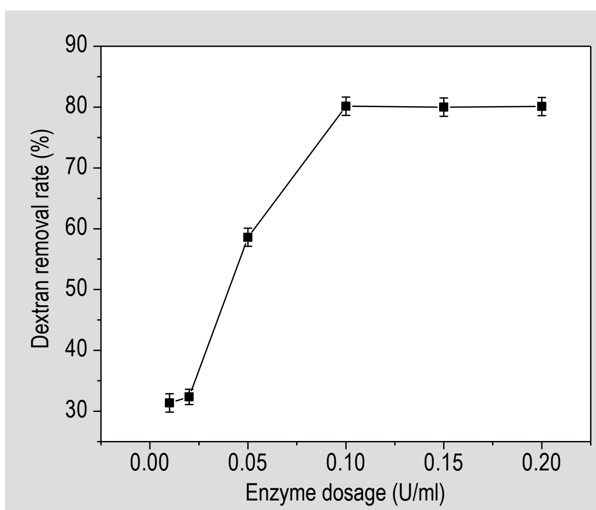

Figure 5. Influence of enzyme dosage on the rate of hydrolysis of dextran.

Orthogonal experiments

Table 2 shows that the optimal conditions for the hydrolysis of dextranase include a pH value of 6.5, a temperature of 50 °C, a reaction time of 20 min, and an enzyme concentration of 0.15 U/ml. Using these optimised conditions, 80.7% of dextrans were hydrolysed. The main factor influencing enzymatic hydrolysis was pH, followed by reaction time, temperature, and then enzyme concentration. The pH value of 6.5 was much close to fresh sugarcane juice. Thus, the dextranase from *A. oxydans* (KQ11) might be potentially used in the sugar industry.

Influence of ultrasonication

Figure 6 shows that ultrasonication for 15 min enhanced the hydrolysis rate of dextrans from 80 to 88%, however, the higher ultrasonication times could not cause more dextran removal. As shown in Figure 7, the highest rate of dextran hydrolysis was 90% with ultrasonic power of 800 W, but the ultrasonic power of 1000 W could cause less dextrans removal compared with the level of 800 W. Meanwhile, the viscosity of sugarcane juice was measured as 0.8±0.1 mpa·s. Figure 8 shows that the rate of dextran hydrolysis increased with the ultrasonic temperature ranging from 35 to 50 °C, and the highest rate of dextran hydrolysis could reach 90% at 50 °C. However, the rate of dextran hydrolysis decreased dramatically with the level of ultrasonic temperature above 60 °C.

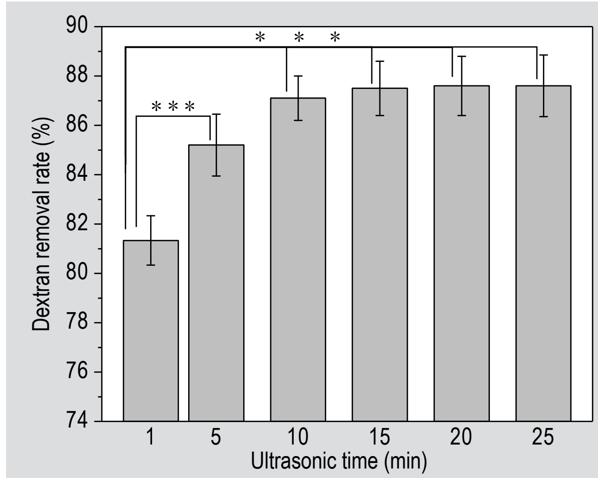

Figure 6. Influence of ultrasonic time on the hydrolysis of dextran. * = *P*<0.001.**

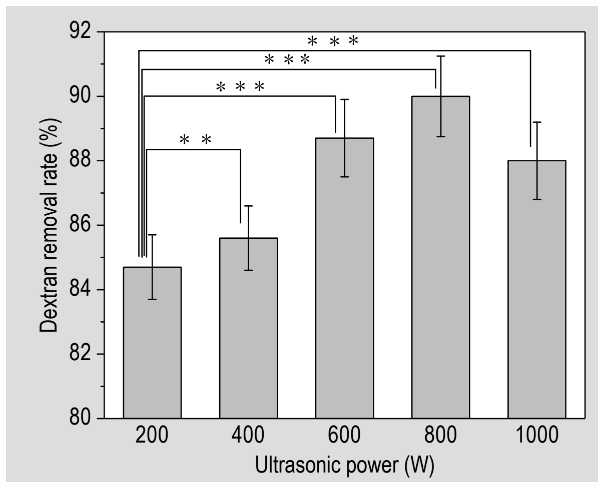

Figure 7. Influence of ultrasonic power on the hydrolysis of dextran. * = *P*<0.001; ** = *P*<0.01.**

Table 2. Analysis of orthogonal experiment.

	pH	Temperature (°C)	Time (min)	Enzyme dosage (U/ml)	Dextran removal rate (%)
1	6.0	40	10	0.05	64.2
2	6.0	45	15	0.10	71.8
3	6.0	50	20	0.15	78.4
4	6.5	40	15	0.15	80.3
5	6.5	45	20	0.05	79.6
6	6.5	50	10	0.10	77.5
7	7.0	40	20	0.10	74.6
8	7.0	45	10	0.15	75.1
9	7.0	50	15	0.05	77.3
k1	71.5	73.0	72.3	73.7	
k2	79.1	75.5	76.5	74.6	
k3	75.7	77.7	77.5	77.9	
Range	7.6	4.7	5.2	4.2	
Factors	pH>time>temperature>enzyme dosage				
Optimal level	6.5	50	20	0.15	

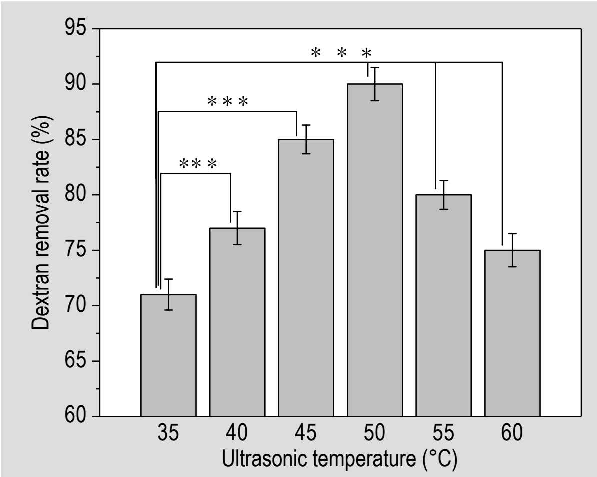

Figure 8. Influence of ultrasonic temperature on the hydrolysis of dextran. * = *P*<0.001.**

4. Discussion

Dextrans were produced by microorganisms in the environment at room temperature. Sugarcane juice was milled in the laboratory, and dextran content was detected daily, increasing by 4.3% each day. The loss of quantity and falling of quality would be dramatical for sugar industry (Jiménez, 2009). Thus, the sugarcane juice or sugarcane should be stored in an environment with controlled temperature, oxygen, and storage period. The current best method of hydrolysing dextrans involves dextranase.

The pH value of fresh sugarcane juice was neutral, however, when the juice was derived from the deteriorated sugarcane, its pH was lower and thus more acidic (Jiménez, 2009), which in turn further increased dextrans content. The results showed that approximately 80.3% of the dextrans were hydrolysed (pH 6.0), and the activity of dextranase was rapidly decreased when the value of pH was above 6.5, which indicated that the acidic environment was beneficial for the hydrolysis of dextrans. In sugarcane processing, the pH of sugarcane juice was 5.0-6.0 before the clarification section of process (Eggleston *et al.*, 2009).

Dextranase was denatured at temperatures >65 °C. Generally, the temperature of storage and early processing stages of sugarcane juice (milling process) was within the range of 20-40 °C (Zhang *et al.*, 2017). Dextranase thus meets the requirements of large-scale sugar milling. In the earlier stages of sugar mill, dextrans could be removed and the viscosity of juice was reduced by dextranase. Resulting reduction of energy consumption, formation of better sugar crystals, the yield and quality were improved in the later stages of sugar mill (Jiménez, 2009). In addition, addition of dextranase to sugarcane juice in milling process was much more efficient and economical than adding them to evaporator syrups. (Bashari *et al.*, 2013).

Compared to dextranase of *Paecilomyces lilacinus,* which could remove 70% of dextrans after 24 h at 30 °C (Bhatia *et al.*, 2010). The pioneer studies performed with the enzymatic preparation Glucanase D-1, which did not specify the dose used, reported the hydrolysis of 70% of dextrans present in the juice with the lowest dose (Eggleston *et al.*, 2009). A study showed that the use of dextranase from *Chaetomimu* sp. could remove 29.6% of dextrans at 32.2 °C for 10 min, and 66.6% of dextrans in sugarcane juice could be removed at 50 °C for 10 min with concentrated dextranase which was a considered over-dose (Eggleston *et al.*, 2005). Another study showed that 80.29% of dextrans could be removed by dextranase from *C. erraticum* at pH 5.5 and 55 °C (Bashari *et al.*, 2013). The dextranase used in this study was highly efficient in removing dextrans in sugarcane juice. Specially, the time of process was relative short (<6 min), which was more suitable than other dextranases for the sugar industry.

Optimal enzyme concentration could accelerate enzymatic reactions. The hydrolysis rate did not further increase with more enzyme added when the enzyme dosage was above 0.1 U/ml. The dosage of dextranase in this study was lower than dextranase from *Chaetomimu* sp. and efficiency of hydrolysis of dextrans was higher (Eggleston and Monge, 2005). The viscosity of sugarcane juice might have prevented dextranase from interacting with its substrate.

T-test showed that the effects of ultrasonication on dextran hydrolysis rate were significant. Ultrasonication could stimulate dextranase to interact with the substrate without impairing its activity at appropriate levels. Exorbitant ultrasonic levels might change the conformation of the enzyme and denature it. (Eggleston *et al.*, 2009; Yachmenev *et al.*, 2004). In this study, ultrasonication improved the rate of dextrans removal by 10% as compared to the control (with no ultrasonic treatment). Ultrasonication probes are suggested to be utilised in sugarcane mills.

5. Conclusions

Dextranase from *A. oxydans* (KQ11) could efficiently hydrolyse dextrans in sugarcane juice. The optimal conditions for dextran hydrolysis were as follows: a pH of 6.5, a temperature of 50 °C, a reaction time of 20 min, and an enzyme concentration of 0.15 U/ml. These conditions resulted in 80.7% of the dextrans removal in sugarcane juice. Ultrasonication enhanced dextrans-removal rates. The rate of dextran hydrolysis was further increased by 10% and to 90%, and the viscosity of sugarcane juice decreased by 20% with ultrasonic power of 800 W for 15 min. Ultrasonic probes could be installed in certain processing sections of sugarcane mills.

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Conflict of interest

The authors confirm no conflicts of interest.

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