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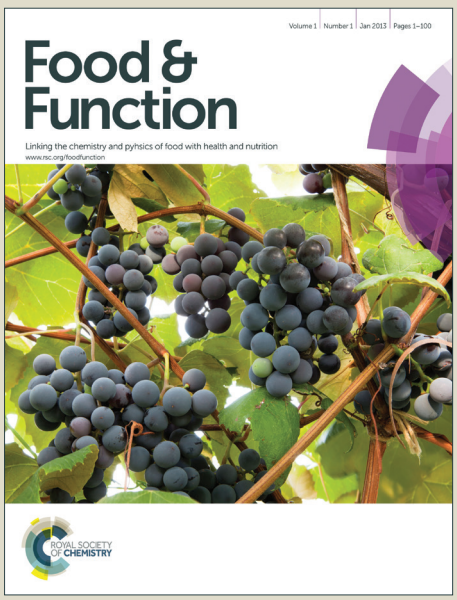

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Superior prebiotic and physicochemical properties of novel dextran from *Weissella cibaria* JAG8 for potential food applications

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Abstract

The dextran produced by dextransucrase from *Weissella cibaria* JAG8 was subjected to physicochemical characterization and assessment of prebiotic potential. Dextran displayed solubility of 24.5% and water holding capacity of 352%. The emulsion, and flocculation activity of dextran was 89% and 92%, respectively. The degradation temperature (T_d) of dextran was 353ºC. Dextran exhibited 33 and 12 fold less hydrolysis than inulin, in simulated gastric juice (pH 1.0) and α-amylase (pH 7.0), respectively. Dextran stimulated the growth of probiotic bacteria such as *Bifidobacterium animalis* sub species *lactis*, *Bifidobacterium infantis* and *Lactobacillus acidophilus* significantly and was comparable to that by commercial inulin. However, growth of *E. coli* was not enhanced by dextran or inulin. Dextran used in this study can be used as a potential prebiotic for health benefits.

Keywords: Dextran; Prebiotic; Probiotic; *Weissella cibaria* JAG8; Flocculation; Emulsion.

1.0 Introduction

Dextran, being an exo-polysaccharide (EPS) is produced by majority of lactic acid bacteria (LAB). Dextran is a homo polysaccharide comprising D-glucose units which are α-(1→6) linked in the main chain with varying percentage of α-(1→2), α-(1→3) or α-(1→4) branched linkages.[1] The EPSs have wide applications in food, cosmetics, textile, pharmaceutical and chemical industry owing to their viscous nature and stability over wide range of pH, temperature and ionic strength.[2] Microbial EPS are being exploited as bio- emulsifier, because they are biodegradable and less toxic in nature and are more efficient than chemical emulsifiers.[3] In addition, EPS can be used as bio-flocculants and bio-absorbants.[4]

Several non-digestible EPS have been reported to be excellent prebiotics.[5,6,7] However, they are not structurally or physico-chemically characterized. Any prebiotic to qualify as putative food ingredient must be resistant to hydrolysis or absorption in the upper gastrointestinal tract, stable to processing conditions, selectively metabolized by limited no of beneficial bacteria in colon.[8] Health benefits attributed to prebiotics include protection against bowel cancer, inflammatory bowel disease, pathogenic agents, coronary heart disease, obesity, low caloric content, and stimulation of growth and metabolism of specific colonic microbiota.[9] There has been a considerable increase in the demand for novel prebiotic ingredients.[10] Currently available prebiotics in the market are fructo-oligosaccharides, lactulose, inulin and galacto-oligosaccharides.[11]

It was reported that over 1.0% western population suffers from Celiac disease.[12] It is a food induced disorder caused by intolerance to wheat gluten or similar proteins from barley and rye.[13] Dextran and gluco-oligosaccharides from *Weissella* species are not digested by baker's yeast and are stable to processing conditions and improves the texture and quality of conventional and gluten free bread for patients of Celiac disease.[14] Dextran from *Weissella*

species can also replace nonbacterial hydrocolloids such as guar gum and hydroxyl propyl methyl cellulose which are used as thickening agents.[15]

In the present study a high dextran producing bacterium isolated from apple (*Malus domestica*) peel, identified by 16S rRNA sequence analysis as *Weissella cibaria* (Genbank accession no KC110687),[16] was subjected to physico-chemical characterization and analysed for its prebiotic potentials. Remaud-Simeon *et al.* reported that branched dextrans are resistant to enzyme hydrolysis by exo-dextranases and glucosidases.[17] While Johnson and Schmit reported that enzymes such as glucoamylase, sucrase and maltase present in the small intestine, hydrolyze α-(1→4) and α-(1→6) linkages of polysaccharides to yield monosaccharides.[18] Dextran from *W. cibaria* JAG8 displayed 93% of α-(1→6) linear and 7% α-(1→3) branched linkages and rheological analysis showed its non-Newtonian nature.[16] In the current study dextran was explored for resistance to enzymatic hydrolysis. Dextran from *W. cibaria* JAG8 displayed significantly lower browning than commercial prebiotic, Raftilose P-95 and *in vitro* cytotoxic studies showed its biocompatible nature.[19] The immense applications of dextran from *Weissella* species prompted to explore its prebiotic potentials as food supplement.

2.0 Materials and Methods

2.1 Chemicals and reagents

Serine, di-sodium phosphate, citric acid, glycine, bichinconinic acid, and α-amylase (from human saliva) were procured from Sigma Chemical Co., USA, L-cysteine-HCl from Merck, Pvt. Ltd., Germany and inulin, all the media components and anaero bag system (for culturing the bacteria) from Hi Media Pvt. Ltd., India.

2.2 Microorganisms

Weissella cibaria JAG8 (Gen Bank accession no. KC110687) used in the present study was isolated from apple (*Malus domestica*) peel.[16] *Bifidobacterium animalis* sub species *lactis* NRRL B-41405, *Bifidobacterium infantis* NRRL B-41661 and *Lactobacillus acidophilus* NRRL B-4495 were maintained in MRS medium.[20]

2.3 Dextran production

Dextran was produced by separately incubating 1.0 mL of purified dextransucrase (0.44 mg mL^{-1}, 20 U mg^{-1}) from *W. cibaria* JAG8 in 10 mL of 146 mM sucrose solution at 30 °C for 24 h in 20 mM sodium acetate buffer (pH 5.6), containing 0.3 mM $CaCl_2$ and 15 mM NaN_3. The dextran produced was purified by ethanol precipitation as described by Rao and Goyal.[16]

2.4 Solubility and water holding capacity of dextran

The solubility of purified dextran of *W. cibaria* JAG8 in water was determined by the method of Ahn *et al.*[21] The water holding capacity (WHC) was determined by the method of Ahmed *et al.*[22]

2.5 Thermo-gravimetric analysis of dextran

The thermal property of dextran was determined by thermo-gravimetric analysis (TGA) using Netzsch Thermal analysis (STA 449 F3 Jupiter TGA DSC). The compound (5 mg) was subjected to a temperature range of 25-1000ºC under nitrogen atmosphere with a linear heating at rate of 10ºC min^{-1} and the corresponding weight loss was determined.

2.6 Emulsion and Flocculation activity of dextran

The emulsifying activity of dextran from *W. cibaria* JAG8 was analysed by using dextran powder (0.5 mg) dissolved in 0.5 mL deionised water by heating at 100ºC for 15 min and allowed to cool at 25ºC. The volume was then made up to 2 mL using 1x phosphate-buffered saline (PBS), pH 7.4. The sample was mixed on a vortex for 1 min after the addition of 0.5 mL n-hexadecane. The absorbance at 540 nm at 0 min (A_0) was immediately measured after mixing. The sample was incubated at 25ºC and decrease in absorbance was recorded at 60 min (A_t). A control was run simultaneously with only 2 mL of 1x PBS (pH 7.4) and 0.5 ml n-hexadecane. The emulsification activity was expressed as the percentage retention of emulsion during incubation for 60 min which was calculated by using the method described by Lim *et al.*[23]

$$\textbf{Emulsion (\%)} = (A_t/A_0) \times 100$$

where, A_0= absorbance (A_{540}) of the suspension at time t=0 and A_t= absorbance (A_{540}) of the suspension at time t= 60 min.

The flocculating activity of dextran from *W. cibaria* JAG8 was determined in presence of activated charcoal. In a test tube, 50 mg of charcoal activated carbon was mixed in 10 mL of deionised water and mixed with 0.1 mL of 6.5 mM $CaCl_2$ solution. The dextran with various concentrations ranging from 0.05 to 0.6 mg mL^{-1} was added to the suspension and mixed on a vortex for 30 s. The reaction mixture was allowed to stand at 30ºC for 10 min and the absorbance (A_{550}) at 550 nm of the upper phase (1.0 mL) was measured. The absorbance of the control (A_c) without the addition of dextran or guar gum was measured by following the method of Lim *et al.*[23]

$$\textbf{Flocculating activity (\%)} = [(A_c - A_s)/A_c] \times 100$$

where, A_s = absorbance (A_{550}) of dextran or guar gum containing suspension;

A_c = absorbance (A_{550}) of control.

153 **2.7 Effect of simulated gastric juice on hydrolysis of dextran**

154 The simulated gastric juice was prepared using hydrochloric acid buffer containing (g L^{-1})
155 NaCl, 8; KCl, 0.2; $Na_2HPO_4.2H_2O$, 8.25; NaH_2PO_4, 14.35; $CaCl_2.2H_2O$, 0.1 and
156 $MgCl_2.6H_2O$, 0.18. The pH of the buffer was adjusted to 1, 2, 3 and 4 by 5 M HCl. Dextran
157 and inulin samples (1.0 mL, 10 mg mL^{-1} prepared by dissolving in milli-Q water) were mixed
158 with 1.0 mL of simulated gastric juice at the four pHs separately and the reaction mixtures
159 were incubated at 37°C for 6 h. Aliquots (100 µL) of the reaction mixture were collected
160 from each treatment at 0, 0.5, 1, 2, 4 and 6 h intervals to determine the reducing sugar and
161 total sugar content. The total sugar (expressed in glucose equivalents) and reducing sugar
162 (maltose equivalents) were determined before and after digestion by phenol sulfuric acid and
163 copper-bicinchoninate methods, respectively.[24] Percent hydrolysis of samples was calculated
164 by the equation given by Korakli *et al.*[25]
165
166 **2.8 Effect of α-amylase on digestibility of dextran**
167 Digestibility of dextran from *W. cibaria* JAG8 and inulin by α-amylase was ascertained
168 following the method of Wichienchot *et al.*[26] The dextran and inulin were dissolved in 20
169 mM sodium phosphate buffer (pH 5) to give 10 mg mL^{-1} solution and tested for digestibility
170 by α-amylase. Solution of α-amylase (2 U mL^{-1}) was prepared in 20 mM sodium phosphate
171 buffer at pH 5 and 7 containing 6.7 mM sodium chloride. Portion of 1.0 mL each from
172 solution of dextran and inulin was mixed separately with 1.0 mL α-amylase solution at pH 5
173 and 7. The reaction mixtures were incubated at 37°C and 100 µL from each reaction mixture
174 was collected at 0, 0.5, 1, 2, 4 and 6 h to determine the reducing and total sugar content to
175 calculate per cent hydrolysis as described by Korakli *et al.*[25]
176
177

2.9 Effect of dextran and inulin on the growth of gut bacteria

The growth stimulatory effects of dextran and inulin on *B. animalis* sub species *lactis*, *B. infantis*, *L. acidophilus* and *E. coli* DH5α were evaluated. The log phase cultures (1.0%, v v^{-1}) of *Bifidobacteria* and *Lactobacillus* were inoculated in 10 mL MRS medium pH 6.4, whereas, *E. coli* DH5α into 10 ml TGY medium, pH 7.0.[20, 27] Both media were supplemented with filter sterilized 0.05% cysteine-HCl as described by Vitali *et al.*[28] The cultures were treated with 1.0%, w/v of dextran and commercial inulin as carbon source (dextran and inulin were autoclaved separately and was added to the media). The respective growth media (MRS and TGY) without any carbon source were maintained as negative controls. The bacterial cultures were incubated at 37°C under anaerobic conditions in anaero bags for 48 h. The bacterial growth was monitored as absorbance at 600 nm (A_{600}) using UV visible Spectrophotometer (Varian, Cary 100). The residual carbohydrate concentration was estimated by phenol sulfuric acid method and correlated with the corresponding pH change in medium.[24] All the experiments were performed in triplicates.

3.0 Results and Discussion

3.1 Solubility and water holding capacity of dextran

The dextran from *W. cibaria* JAG8 displayed 24.5% solubility and 352% water holding capacity. The dextran from *W. cibaria* JAG8 showed porous nature as characterized by Scanning Electron Microscopy.[19] These properties are attributed to the porous matrix structure which can hold large amounts of water molecules.[29] The good solubility and water holding ability of dextran of *W. cibaria* JAG8 hold potential for the commercial food products.[30]

3.2 Thermo gravimetric analysis of dextran

Thermo gravimetric analysis (TGA) of dextran of *W. cibaria* JAG8 displayed two stages of weight loss. Dextran displayed 23% weight loss at 241ºC and the degradation temperature (T_d) was 353ºC at which 80% weight loss occurred (Fig. 1). It has been reported that degradation temperature of various polysaccharides range from 230-400ºC.[31] It was reported that the dextran and gluco-oligosaccharides produced from *W. cibaria* 10M was stable under food processing conditions.[14] *Weissella* species improve the textural, rheological and quality of conventional and gluten free bread.[15] The high thermo-stability of dextran from *W. cibaria* JAG8 indicated that it can be putative candidate for food industry.

3.3 Emulsion stability and flocculating activity of dextran

The emulsion stabilities of dextran from *W. cibaria* JAG8 displayed 79.3% of the emulsification activity after 60 min. The emulsion stability of guar gum and sodium alginate was found to be 44% and 42% after 60 min of incubation (Fig. 2A). Any compound to be a stable emulsifier it should retain at least 50% of the emulsion after its formation.[2] Emulsifying activity of EPS depends on its strength in retaining the emulsion of the hydrocarbon in water. In case of control the emulsification activity after 60 min was less than 20%, indicating that the emulsion activity of dextran, guar gum and sodium alginate was independent of phosphate buffer saline. *W. cibaria* JAG8 dextran displayed 36% and 39% higher emulsifying activity than guar gum and sodium alginate, suggesting that dextran could be potentially used as emulsifier in food industry.

The flocculating activity of *W. cibaria* JAG8 dextran was measured from 0.05 to 0.6 mg mL^{-1} in presence of 5 mg mL^{-1} of activated charcoal enriched with 6.8 mM $CaCl_2$ solution and compared with guar gum under similar conditions (Fig. 2B). There was a constant decline in the flocculation activity with increase in dextran and guar gum

concentration. The maximum flocculating activity of 92% (dextran) and 89% (guar gum) was achieved at concentration of 0.05 mg mL^{-1}. The flocculation activity of xanthan gum was 94% at 0.6 mg mL^{-1} concentration as reported by Kanmani *et al.*[2] The dextran from *W. cibaria* JAG8 showed high flocculating activity at 10 fold lower concentration than that of commercial hydrocolloid xanthan gum. The above analysis indicated that dextran from *W. cibaria* JAG8 can be used as good bio-flocculent for industrial application.

3.4 Effect of simulated gastric juice on digestibility of dextran

Dextran from *W. cibaria* JAG8 was highly resistant to hydrolysis by simulated gastric juice at all pHs (Fig. 3). The percent hydrolysis of dextran was significantly lower than standard prebiotic inulin at all pHs (Fig. 3). The dextran from *W. cibaria* JAG8 showed significantly lower, 1.1, 0.9, 0.8 and 0.6% hydrolysis at pHs 1.0, 2.0, 3.0 and 4.0, respectively, after 6 h (Fig. 3A) as compared with inulin which showed 34, 28, 9, 7% hydrolysis at 1.0, 2.0, 3.0 and 4.0, respectively (Fig. 3B). Dextran from *W. cibaria* JAG8 displayed 33 fold less hydrolysis than inulin at pH 1.0. This indicated that dextran was better than inulin by having lower digestibility. The above results were in accordance with the earlier report of Hongpattarakere *et al.* where the EPS from *Weissella cibaria* A2, *Weissella confusa* A9, *Lactobacillus plantarum* A3 and *Pediococcus pentosaceus* 5S4 displayed lower levels of hydrolysis in presence of simulated gastric juice.[5] In our previous study it was reported that dextran from *W. cibaria* JAG8 comprise 93% of linear α-(1→6) glycosidic linkage and 7% of α-(1→3) branched linkages.[16] The presence of branching might be the reason for resistance to hydrolysis of dextran by simulated gastric juice as also reported earlier. [17, 18] These results are especially relevant for acidic foods such as yogurt and dairy products which may be supplemented with prebiotics as also reported by Huebner *et al.* [32]

3.5 Effect of α-amylase on digestibility of dextran

The dextran from *W. cibaria* JAG8 showed high resistance to digestion by α-amylase. However, there was no significant difference in the degree of hydrolysis at pH 5 and pH 7 as shown in Fig. 4. After 6 h at pH 5 and pH 7, the degree of hydrolysis of dextran from *W. cibaria* JAG8 was only 0.9% and 0.8% respectively, as compared to 13% and 12.8%, respectively with inulin (Fig. 4). Dextran from *W. cibaria* JAG8 displayed 12 fold less hydrolysis than inulin at pH 7.0. It has been reported that enzymes such as glucoamylase, sucrase and maltase present in the small intestine, hydrolyze α-(1→4) and α-(1→6) linkages of polysaccharides.[18] The presence of α-(1→3) linkages in dextran of *W. cibaria* JAG8, might be responsible for providing the resistance to α-amylase hydrolysis thus making it a potential prebiotic.

3.6 Effect of dextran and inulin on the growth of human gut bacteria

The growth of probiotic bacteria, *B. animalis* subspecies *lactis*, *B. infantis* and *L. acidophilus* was significantly higher in presence of dextran and commercial prebiotic inulin as compared with control without carbohydrate source (Fig. 5A-D). The growth profiles of the bacteria were correlated with carbohydrate (dextran and inulin) utilization profiles as shown in Table 1. All probiotic cultures utilized dextran and inulin as sole carbon source. *B. infantis* displayed maximum carbohydrate utilization 43% of dextran of *W. cibaria* JAG8 and 53%, of inulin, followed by *L. acidophilius* (26% of dextran and 40% of inulin) and *B. animalis lactis* (24% of dextran and 26% of inulin) (Table 1). While *E. coli* DH5α displayed very low consumption of supplemented carbohydrate (10% of dextran and 11% of inulin) after 48 h of incubation (Table 1). The carbohydrate utilization profiles clearly indicated that dextran of *W. cibaria* JAG8 effectively promotes the growth of probiotic bacteria and can be used as potential food supplement.

The pH values in the growth media were significantly reduced by the probiotic cultures tested, except for *E. coli*, where no significant decrease in the pH was observed. The reduction in pH by *B. infantis* and *L. acidophilus* was 5.17 and 5.65 for *W. cibaria* JAG8 dextran and 6.15 and 6.24 for control, respectively (Table 2). The pH decreased in *B. animalis* subspecies *lactis*, was 5.76 and 6.0 in dextran supplemented media and control after 48 h of incubation (Table 2). No significant change in the pH by *E. coli* DH5α was observed when dextran and inulin were used as sole carbon source (Table 2). It was inferred that *Bifidobacteria* spp. and *Lactobacillus* sp. used dextran as carbon source during fermentation and produced secondary metabolites which may include organic acids like lactic acid and acetic acid and thus bringing down the pH of growth media as also reported earlier.[6, 33] Thus, these results support the contention that the dextran from *W. cibaria* JAG8 can serve as a potential prebiotic.

4.0 Conclusion

The physicochemical characterization and prebiotic potential of dextran of *W. cibaria* JAG8 have been reported for the first time. The dextran showed excellent water holding capacity, flocculation activity and high thermal stability. Dextran was more resistant to hydrolysis by simulated gastric juice and α-amylase than commercial prebiotic inulin. The dextran supported the growth of probiotic bacteria and did not promote the growth of unwanted *E. coli*. With such desirable attributes, the dextran of *W. cibaria* JAG8 emerges as a promising ingredient for commercial applications. Further *in vivo* studies are needed to confirm the prebiotic nature of the dextran.

Acknowledgement and conflict of interest

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Legend to Figures

Figure 1. Thermogravimetric analysis (TGA) of dextran from *W. cibaria* JAG8.

Figure 2. The emulsion stability (A) and flocculating activity (B) dextran from *Weissella cibaria* JAG8. The mean value of three independent experiments is presented with ± S.D of three observations from triplicate analysis.

Figure 3. Acid hydrolysis of dextran from *W. cibaria* JAG8 (A) and inulin (B) by simulated gastric juice at pH 1, 2, 3 and 4 at 37°C for 6 h. The values are mean ± SD of three observations from triplicate analysis.

Figure 4. Enzymatic hydrolysis of dextran from *W. cibaria* JAG8 and inulin by α-amylase (A) pH 5 (B) pH 7, treatment at 37°C for 6 h. The values are mean ± SD of three observations from triplicate analysis.

Figure 5. Growth profile of *B. animalis* subspecies *lactis* (A) *B. infantis* (B) *L. acidophilus* (C) and *E. coli* (D) in the presence of dextran (1.0%, w/v) from *W. cibaria* JAG8, inulin (1.0%, w/v) and MRS medium without any carbon source (control) at 37°C. The values are mean ± SD of three observations from triplicate analysis.

Table 1. Carbohydrate utilization of bacteria in presence of dextran and inulin.

	¶Carbohydrate utilization (%)			
Prebiotic	*B. animalis* subspecies *lactis* Time (h)		*B. infantis* Time (h)	
	24	48	24	48
Dextran from *W. cibaria* JAG8	21.23±2.37	24.28±2.08	41.45±2.16	42.61±3.42
Inulin	25.38±1.55	26.34±0.57	51.93±0.24	52.82±0.99
	L. acidophilus		*E. coli*	
Dextran from *W. cibaria* JAG 8	24.91±1.32	26.19±3.83	9.77±2.37	9.33±1.561
Inulin	38.39±1.82	39.80±1.50	9.22±0.25	10.73±0.41

Note *i) Initial carbohydrate content was 10 mg mL^{-1} for both dextran and inulin.*
ii) The values are mean ± SD of three observations from triplicate analysis.

¶Carbohydrate utilization (%) = $\frac{\text{Residual carbohydrate content}}{\text{Initial carbohydrate content}}$ x 100.

443 **Table. 2** pH change during growth of probiotic bacteria and *E. coli*.

Prebiotic	*B. animalis* subspecies *lactis* Time (h)			*B. infantis* Time (h)		
	0	24	48	0	24	48
Dextran from *W. cibaria* JAG8	6.40±0.00	5.83±0.03	5.76±0.02	6.40±0.00	5.21±0.01	5.17±0.02
Inulin	6.40±0.00	5.73±0.02	5.68±0.01	6.40±0.00	4.92±0.02	4.89±0.03
Control	6.40±0.00	6.29±0.01	6.05±0.04	6.40±0.00	6.3 ± 0.04	6.15±0.12
	L. acidophilus			*E. coli*		
Dextran from *W. cibaria* JAG8	6.40±0.00	5.72±0.04	5.65±0.03	7.00±0.00	6.97±0.01	6.82±0.03
Inulin	6.40±0.00	5.85±0.01	5.85±0.03	7.0±0.00	6.88±0.01	6.85±0.03
Control	6.40±0.00	6.30±0.02	6.24±0.03	7.0±0.00	6.93±0.03	6.89±0.06

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445 Note: The values are mean ± SD of three observations from triplicate analysis.

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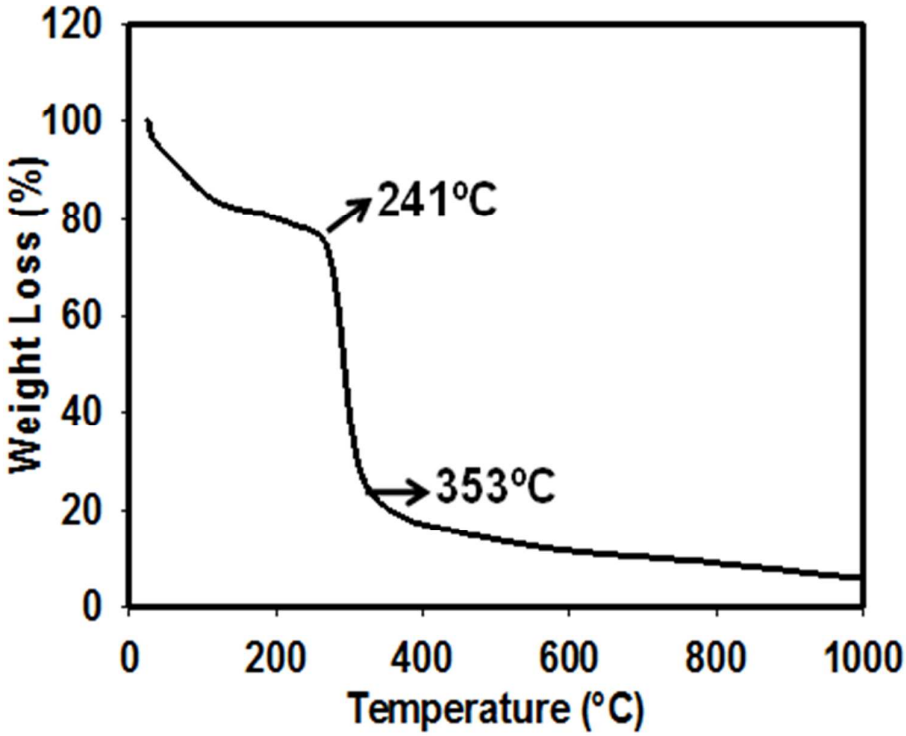
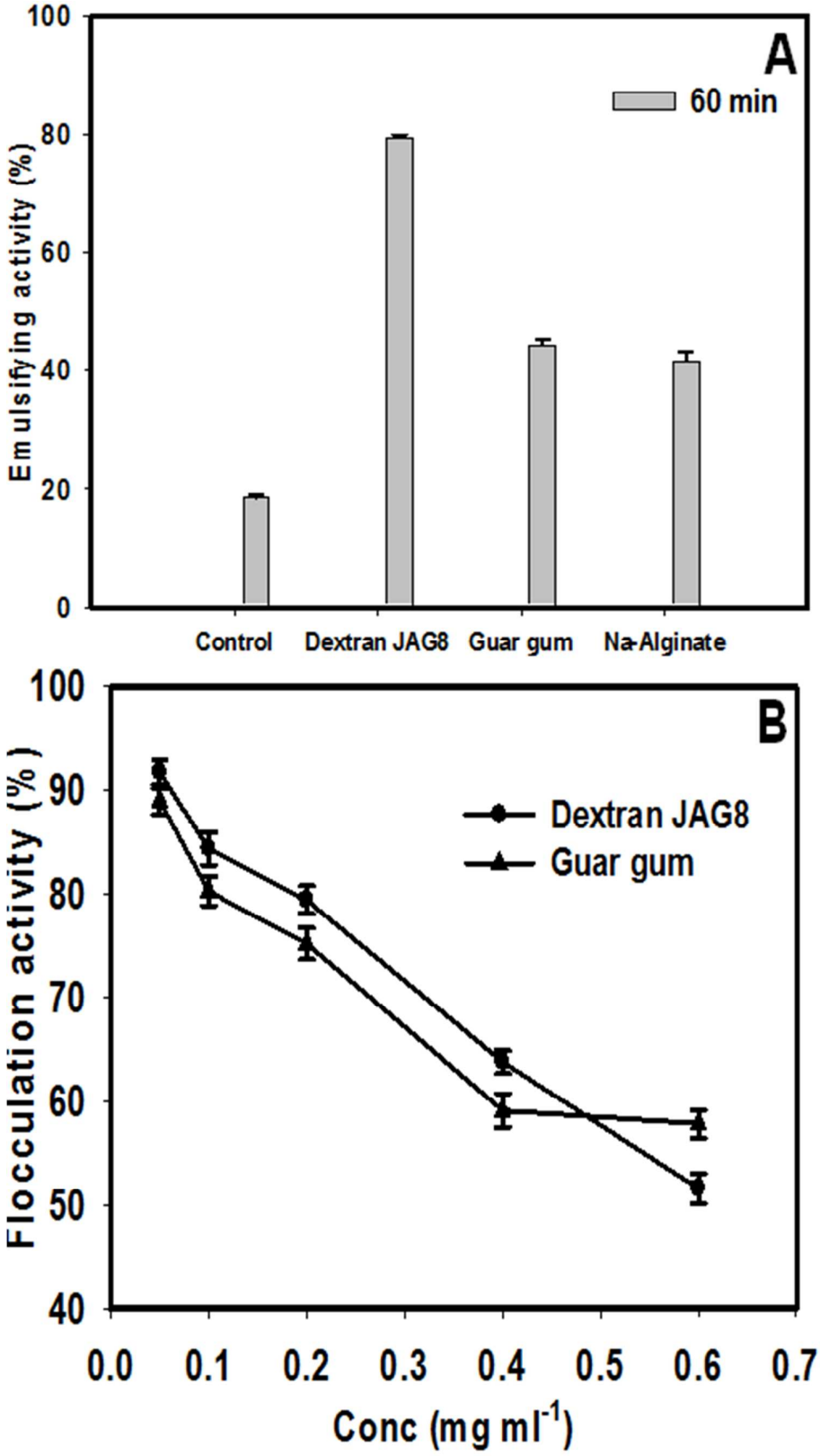
Fig. 1



16 **Fig. 2**



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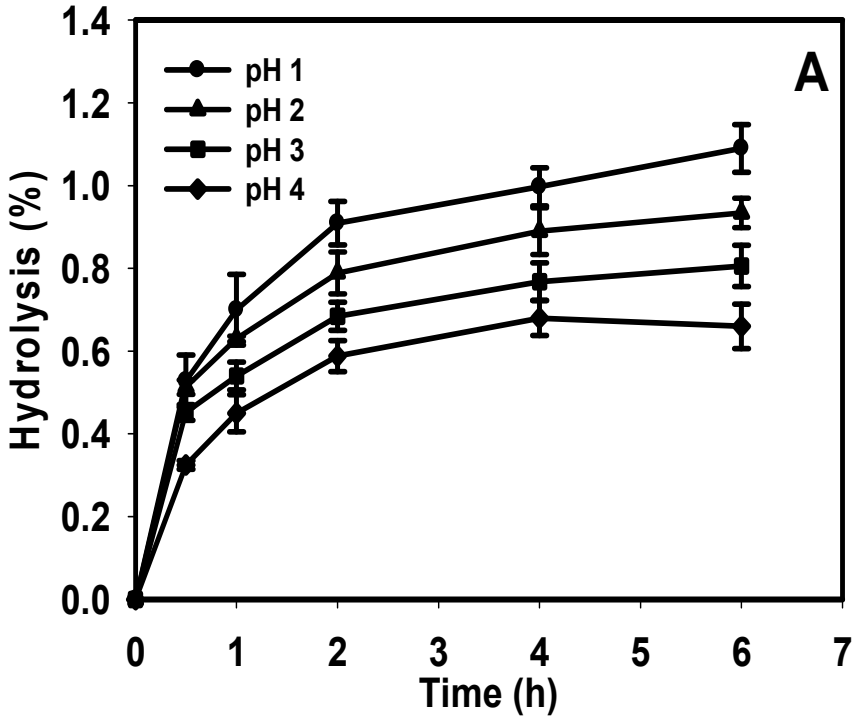
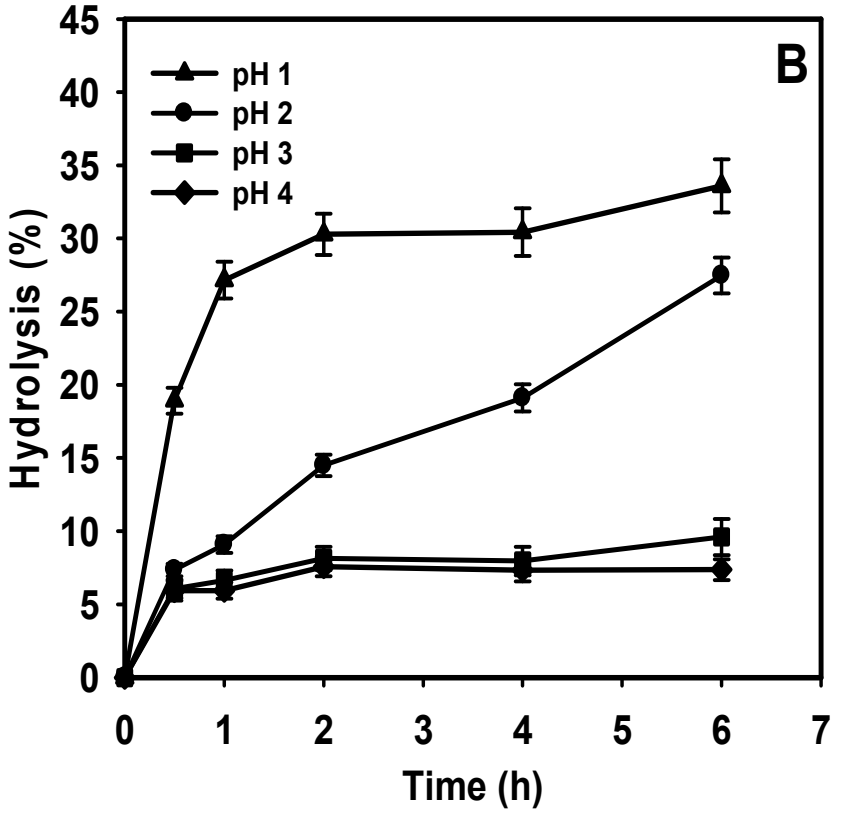
18 **Fig. 3**





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21 **Fig. 4**

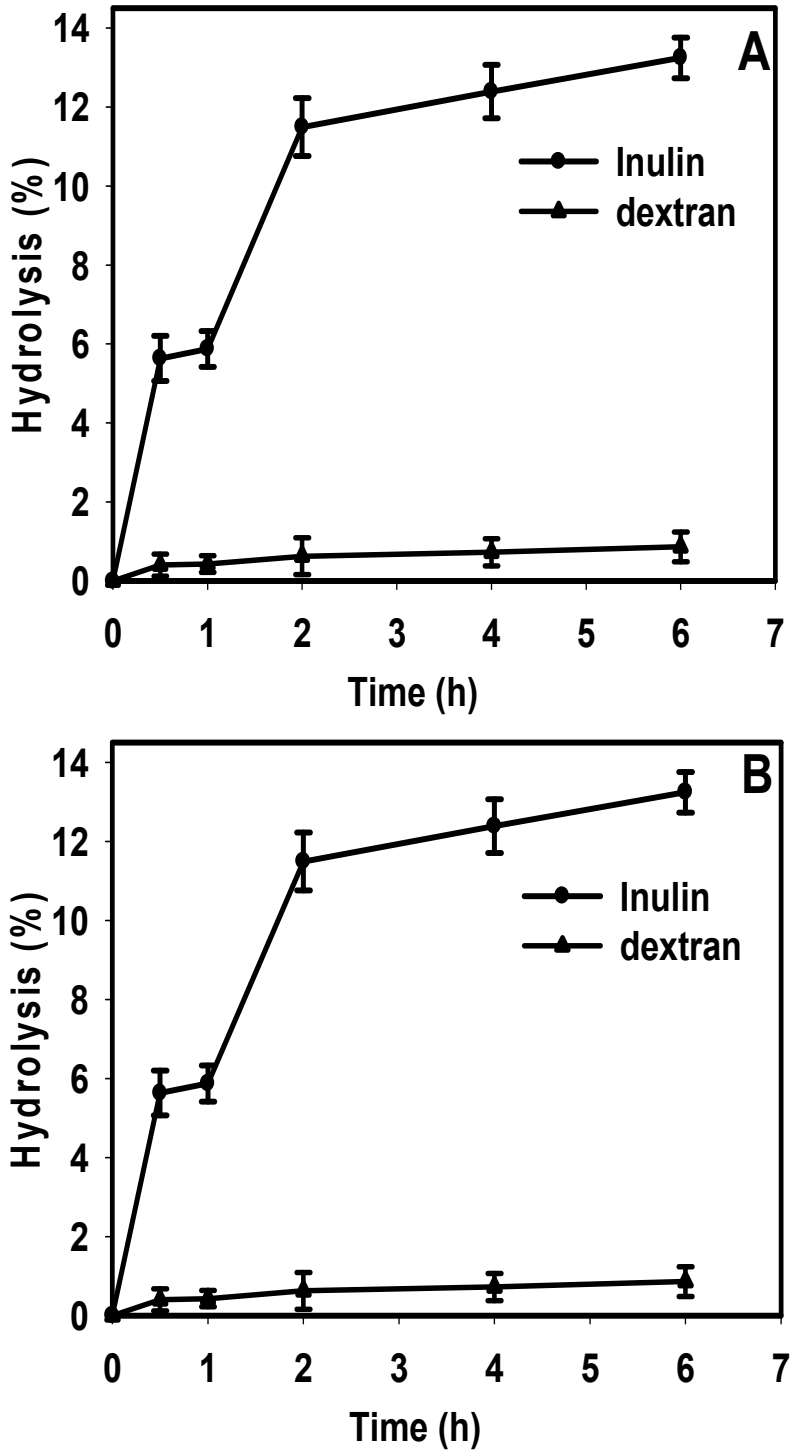

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Fig. 5