

# Impact of Dextran Biodegradation Catalyzed by Dextranase Enzyme on the Crystallization Rate of Sucrose during Sugar Manufacturing

Mohanad Bashari<sup>1\*</sup>, Camel LAGNIKA<sup>2</sup>, Al-farga Ammar<sup>3</sup>, Mandour H. Abdalhai<sup>4</sup>, Ayman Balla Mustafa<sup>5</sup>

<sup>1</sup>A'Sharqiah University, College of Applied and Health Sciences, Dept. of Food Science and Human Nutrition, P.O.Box 42 Postal Code 400, Ibra, Sultanate of Oman.

<sup>2</sup>School of Sciences and Techniques for Preservation and Processing of Agricultural Products, National University of Agriculture, BP: 114 Sakete, Republic of Benin.

<sup>3</sup>College of Sciences, Biochemistry Department, University of Jeddah, Saudi Arabia

<sup>4</sup>Jiangsu University, School of Food and Biological Engineering, China

<sup>5</sup>Therapeutic Nutrition Department, Faculty of Nursing and Health Sciences, Misrata University, Libya

\*Corresponding author: [mohanad.bashari@asu.edu.om](mailto:mohanad.bashari@asu.edu.om)

Received April 03, 2019; Revised May 11, 2019; Accepted May 21, 2019

**Abstract Introduction:** In this research work, we investigated the influence of the biodegradation of dextran catalyzed by dextranase enzymes during sugar manufacturing on the rate of sucrose crystallization and growth rate of sucrose crystals in pure sucrose solution at different temperatures. **Methods:** To elucidate the influence of biodegradation of dextran on the growth rate of sucrose crystals, dextran of Mw 2,000,000 g/mol ( $T_{2000}$ ) was admixed in concentrations between (1000 - 10000 ppm) with (60% -75% w/w) sucrose solution.. The hydrolysis of dextran was carried out at 55.0 °C and pH 5.5 at different dextranase concentration, and then the samples were immediately subjected to the crystallization process. **Results:** The most pronounced effect of dextran on the growth rate of sucrose crystals was found with  $T_{2000}$  at concentrations more than 5000 ppm at 60°C. From the results it could be shown that an increase of crystallization rate of up to 50% after biodegradation of dextran  $T_{2000}$  using dextranase enzyme at concentration of 100 ppm, compared to crystallization rate with pure sucrose solution in the presence of dextran  $T_{2000}$ . It was obvious that after dextran hydrolyzed by dextranase, more perfect crystal surfaces are built than at 60°C. **Conclusion:** Dextran biodegradation catalyzed by dextranase enzyme has increased the crystallization rate of sucrose and more perfect crystal surfaces are built. Such a positive influence of biodegradation of dextran using dextranase enzyme decreases crystallization time in the sugar house and thus decreases the production costs of sugar manufacturing.

**Keywords:** dextranase, dextran, sugar manufacturing, sucrose crystallization, growth rate, enzymatic treatment

**Cite This Article:** Mohanad Bashari, Camel LAGNIKA, Al-farga Ammar, Mandour H. Abdalhai, and Ayman Balla Mustafa, "Impact of Dextran Biodegradation Catalyzed by Dextranase Enzyme on the Crystallization Rate of Sucrose during Sugar Manufacturing." *Journal of Food and Nutrition Research*, vol. 7, no. 5 (2019): 402-408. doi: 10.12691/jfnr-7-5-10.

## 1. Introduction

In a sugar factory, the crystallization process is an important step, which determines the yield of the sugar from sugar beet and cane. Meade and Chen reported that the rate of crystallization was affected by the degree of supersaturation, temperature, crystal surface area, and nature or concentration of impurities [1]. In fact, the crystal growth rate is also affected by high molecular mass substances and colorants in syrups, which are partially adsorbed on the crystal surface and thus impede the insertion of sucrose molecules into the lattice [2].

Dextrans are high molecular weight polysaccharides, formed of at least 50%, by  $\alpha$ -(1-6) linked glucose units,

with  $\alpha$ -(1-3) branch linkages and may contain other branch linkages such as  $\alpha$ -(1-2) or  $\alpha$ -(1-4) [3]. Dextran is produced by micro-organisms such as *Leuconostoc mesenteroides* that contaminate sugar cane in the rainy seasons [1] and it is produced by bacterial fermentation in medium that is rich in sucrose. These dextrans are extracted in the mills along with the juices and contaminate the sugar mill flow, reaching levels in the juice exceeding 10,000 ppm (1%) in very extreme cases. In burnt sugar cane a rapid increase in the level of dextrans of almost ten times was observed from 12 to 48 h, reaching 3200 ppm [4]. The number of branches and the molecular weight of dextran are its key properties and define the behaviour of these compounds in solutions [5].

The presence of dextran in the sugar factories leads to a falsely high polarization, increased viscosity, slowing of

filtration, lower evaporation rates, elongated crystals (needle grain), longer wash and separation cycles in centrifuges and increase of sugar loss to molasses [6]. The most damaging effects of elevated dextran concentrations in a technical sucrose solution are foreseen in the crystallization process. Dextran slow down the crystallization rate or even inhibit crystallization [7]. Recently, Eggleston et al., suggested that high Mw dextran may have contributed to the hard-to-boil phenomenon which occurs in some massecuite samples [8].

Dextranase (1,6- $\alpha$ -D-glucan-6-glucanohydrolase, EC3.2.1.11), is an enzyme, which catalyzes endohydrolysis of  $\alpha$ -(1-6)-d-glycoside linkages in random sites of dextran [3]. The application of dextranases in the sugar industry was pioneered in Australia in the 1970s. In South Africa where diffusers are used, dextranase application in diffuser cane juices was deemed unsuitable mostly because of the high temperatures [9]. Recently, new methods have been reported to enhance the enzymatic hydrolysis of dextran catalyzed by dextranase using intensity ultrasonic combined with high hydrostatic pressure or microwave Irradiation which could be an effective methods for improving the industrial efficiency of dextranases in many industrial applications including sugar manufacturing processes [10,11,12].

The main focus of this research work was to investigate the effects of hydrolysis of dextran using dextranase enzyme on the crystallization process and the quality of the final sugar crystal. Moreover, the effects of addition of dextranase to sugar juices as industrially relevant method on growth rate of sucrose crystals, crystal shape and surface topography at different crystallization temperature and supersaturation were also identified.

## 2. Materials and Methods

### 2.1. Materials

Dextranase produced by *Chaetomium erraticum*, dextran T<sub>2000</sub> Mw ~ 2,000,000) used to increase the polysaccharides content of the sucrose solutions, and Sucrose crystals d= 200  $\mu$ m used as seed, were obtained from Sigma-Aldrich (Shanghai, China). Analytical grade refinery fine sucrose was obtained from Sinopharm Chemical reagent Co. (Shanghai, China). All other chemicals and solvents used were of analytical grade.

### 2.2. Preparation of Sucrose Solution and Dextran

Sucrose solutions with concentrations of 65% and 75% (w/w) were prepared by weighing the analytical grade sucrose and dissolved in the corresponding amount of distilled water. The mixture was then heated in a microwave oven (medium power) for short periods (45 sec), intercalated with agitation until to complete the sucrose dissolution [13]. The use of microwave heating, which results in high temperatures for short time periods, is based on the fact that sucrose in concentrated solutions presents a lag phase in the thermal degradation reaction [14] and will not undergo any degradation during

solutions preparation. To confirm that no sucrose degradation occurred during the preparation of the solutions, a sample of each solution was analyzed using HPLC system [15]. It was found that no significant sucrose degradation has occurred during the preparation of the samples.

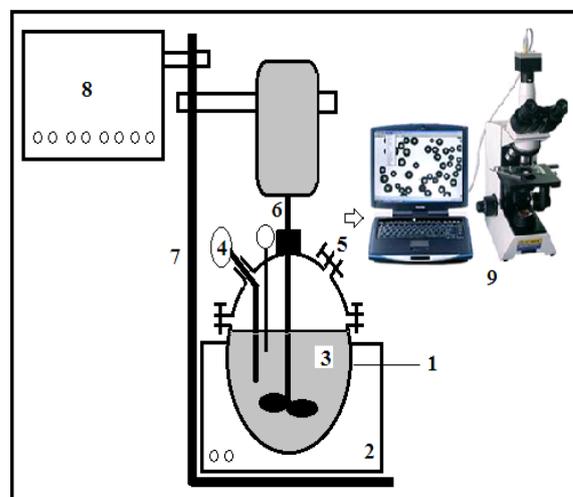
The present studies have been done by the step-wise addition of dextran at a rate of 1000 ppm (0.1%) up to a maximum of 10000 ppm (1%), as this quantity of dextran was found to exist in cane sugar process streams. In order to avoid damage or thermal degradation due to the heating process on dextran at different Mw and concentrations, initially dextran was dissolved in cold water and then the polysaccharide solutions were added to the sucrose solutions and mixed using a magnetic stirrer to give the desired concentrations.

### 2.3. Determination of Dextran

Dextran concentration for all samples was determined according to ICUMSA GS1-15 (1994) and rapid haze [16] methods with some modifications to improve the accuracy. Sucrose solution (3ml) was added to 3 ml absolute ethanol, mixed and left for 2 min. The absorbance at 720 nm was immediately read in a 1-cm cell on a UV-1800 spectrophotometer (Shanghai Mapada Instruments Co., Ltd, China). Dextran (T<sub>2000</sub>) was used to prepare the standard and dextran was precipitated with 100% absolute ethanol.

### 2.4. Enzymatic Hydrolysis of Dextran in the Sucrose Solution

Dextranase enzyme (at concentrations between 0 -100 ppm) was added to 100 mL sucrose solution containing dextran (10.000 ppm) in flask, covered with aluminum foil, and thoroughly mixed. For the control, de-ionized water was added instead of enzyme. The glasses flasks were placed in a shaking water-bath (120 rpm) at 50°C for 30 min. After incubation, the samples were immediately subjected to the crystallization process.



**Figure 1.** Pilot scheme of laboratory crystallizer equipped: 1. double wall glass crystallizer 2. water bath 3. automatic stirrer 4. refractometer 5. samples collection part 6. thermometer 7. carrier 8. control panel 9. electronic microscope

## 2.5. Crystallization Unit

The scheme of laboratory crystallization unit is given in Figure 1. The experimental apparatus used in this study consisted of a glass crystallizer equipped with automatic stirrer, Refractometer, and thermometer. The temperature in the crystallizer was controlled by means of a heating bath equipped with an external temperature sensor which was set in the solution. Dry substance and temperature were determined every 120 s.

## 2.6. Crystallization Experiments

For our research work the isothermal crystallization method was used to study the effect of hydrolysis of dextran using dextranase enzyme on the crystallization process without interference of other factors. Crystallization process was performed at constant temperatures (60, and 70°C). The agitation inside the crystallizer was maintained at a constant speed of 200 rpm. After reaching the required temperature, the solution in the crystallizer was seeded with sucrose crystals (size 200 µm) using a syringe. The amount of seed  $m_{seed}$  in g was calculated as follows [7]:

$$m_{seed} = m_{Ma} * w_{Cry} * \left( d_i / d_f \right)^3$$

Where,  $m_{Ma}$  is the massecuite mass in g,  $w_{Cry}$  is the final crystal content in massecuite,  $d_i$  is the initial size of the crystal (200 µm) and  $d_f$  is the final size of the crystal.

A sample was taken before the seeding point to carry out the first image analysis for the evaluation of the solubilization process. The kinetic measurements were obtained by periodically removing samples of the solution from the crystallizer and placing them under the microscope for image capture in order to follow the growth of crystals through the crystallization. Solution concentration was measured with the refractometer. To remove any particles sticking to the crystal surface, the crystals were washed in a slightly subsaturated solution, then dabbed with filter paper and dried in an airstream.

## 2.7. Calculation of the Growth Rate and Surfaces Area of Sucrose Crystals

The growth rate of sucrose crystals ( $G$ ) in g/(m<sup>2</sup> min) was calculated later as follows:

$$G = \left( \frac{\Delta m_{s,Cry}}{t} \right) * \left( \frac{1}{A_{Cry}} \right)$$

Where  $m_{s,Cry}$  is the crystallized sucrose mass,  $t$  is the time (min), and  $A_{Cry}$  is the total crystal surfaces in m<sup>2</sup>. The crystallized sucrose mass ( $m_{s,Cry}$ ) was calculated according to this equation:

$$\Delta m_{s,Cry} = \left( \frac{w_{DS,MS,t}}{100 - w_{DS,MS,t}} .mw \right) - \left( \frac{w_{DS,MS,t+1}}{100 - w_{DS,MS,t+1}} .mw \right)$$

Where  $W_{DS}$  is dry substrate content in %,  $mw$  is the mass of water,  $M_S$  is the mother syrup.

Sucrose crystal surfaces area ( $A_{Cry}$ ) was calculated using the following equation:

$$A_{Cey} = f_A * m_{Cry}^{\frac{2}{3}} * n_{Cey}$$

Where,  $f_A$  form factor (0.0423),  $m_{Cry}$  is the crystal mass and  $n_{Cry}$  is the number of crystals [17].

## 2.8. Scanning Electron Microscopy (SEM) Analysis

A scanning electronic microscope (SEM) was used to examine the surface morphology of all the samples. A double-sided tape was first attached to specimen holder then the tape was slightly sprinkled with sample powder. Specimens were coated with a gold-palladium layer using SEM sputter coater. They were examined under the SEM (QUANTA 200F, FEI, Netherlands) at an accelerating voltage of 5.0 kV and a working distance of 8.0 or 8.2 mm.

## 2.9. Statistical Analysis

All the experiments described above, were performed in triplicate for each sample. The data was subjected to an analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ( $P < 0.05$ ) using SPSS 17 for Windows program (SPSS Inc., Chicago, USA).

## 3. Results and Discussion

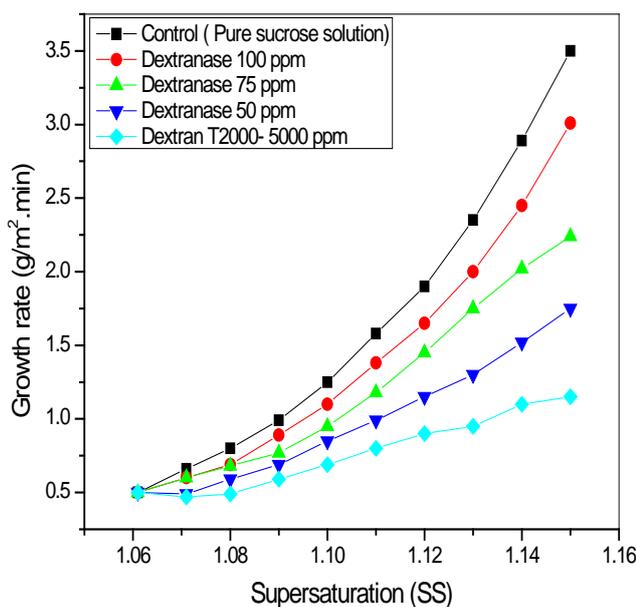
### 3.1. Enzymatic Biodegradation of Dextran

In our previous research work, we investigated the influence of dextranase enzyme on the molecular weight parameters of remaining dextran and intrinsic viscosity after different enzymatic treatments at different steps during sugar manufacturing [18]. Addition of dextranase to juice was much more efficient and economical to reduce the Mw of remaining dextran than adding it to evaporator syrups. Addition of dextranase at juice pH 5.5 showed similar minimum Mw with the lowest intrinsic viscosity, observed at 55.0°C. The highest dextran removal was observed at dextranase concentration of 100 ppm/juice which resulted in 80.29 % removal dextran in the juice. Moreover, the higher the level of concentrated dextranase applied to the juice, the more the removal of dextran occurred [19]. In addition, the longer the availability of the residence time in the factory the lower dextran Mw has been observed. Therefore, in this study the hydrolysis of dextran was carried out at 55.0°C and pH 5.5 at different dextranase concentration, and then the samples were immediately subjected to the crystallization process.

### 3.2. Influence of Dextran Biodegradation by Dextranase on Growth Rate of Sucrose Crystals at a Constant Temperature

The growth rates of sucrose crystals during the crystallization process for pure sucrose solution after

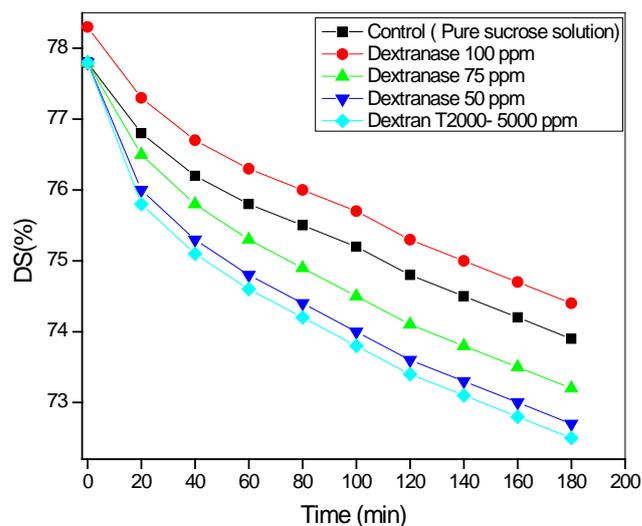
hydrolysis of dextran  $T_{2000}$  by different dextranase concentration at  $60^{\circ}\text{C}$  are shown in Figure 2. The results indicated that indicates that the crystallization rate regarding a supersaturation of 1.13 in the sugar solution without dextran (control) is the same as the crystallization rate at supersaturations of 1.135 and 1.138 in presence of 1500 and 2000 ppm of dextran  $T_{2000}$ , respectively. These results show the same tendency as reported before [1,7,20]. However, growth rates obtained in own experiments were higher than the former results of Abdel-Rahman. This is due to the use of a fast mixing system during our own experiments in contrast to the float bed (without stirrer) crystallizer that was used by Abdel-Rahman et al, [7]. Also, a stronger effect of dextranase concentration can be observed at  $60^{\circ}\text{C}$ . As it can be seen from Figure 2, the crystallization rates at  $60^{\circ}\text{C}$  at a supersaturation of 1.13 are reduced by 34% in presence 5000 ppm dextran  $T_{2000}$ . However, after hydrolysis of dextran by dextranase at 50, 75, 100 ppm concentration, the crystallization rates at  $60^{\circ}\text{C}$  at a supersaturation of 1.13 are reduced by 30.3, 21.5, 8.1%, respectively. The above observation was due to the fact that the hydrolysis of dextran reduced the viscosity of the sucrose solution. In our previous work using laboratory trials performed by adding standard dextrans to pure sucrose solutions [18], we have demonstrated that, both the apparent viscosity and dynamic modulus increased with an increase in dextran concentrations and they demonstrated strong dependence on its Mw [19]. Conversely, it can be expected that the use of dextranase will reduce the contribution from dextran concentrations and Mw, resulting in decreased viscosity and, presumably, better boiling house recoveries. However, several byproducts are associated with the formation of dextran. These include acetic acid, lactic acid, ethanol, mannitol and carbon dioxide as well as several oligosaccharides [8]. Many of these products are melassegenic and will also contribute to decreased exhaustion. Their influences will be unaffected by the dextranase treatment.



**Figure 2.** Growth rate of sucrose crystals at  $60^{\circ}\text{C}$  after hydrolysis of dextran  $T_{2000}$  by different dextran concentration

### 3.3. Influence of Hydrolysis of Dextran on by Dextranase on the Crystallization Time

Figure 3 illustrates the relationship between the dry substance content and the time of the crystallization process for pure sucrose solutions (control) and after addition of dextran  $T_{2000}$  (5000 ppm) at  $60^{\circ}\text{C}$ . The presence of dextran increased the crystallization time compared with the pure sucrose solution (control), however, a decrease in the crystallization time after hydrolyses of dextran by different dextranase concentration was observed. An increase of dextranase concentration caused a decrease of crystallization time.



**Figure 3.** Dry substance content at  $60^{\circ}\text{C}$  after hydrolysis of dextran  $T_{2000}$

### 3.4. Surface Topography of Sucrose Crystals

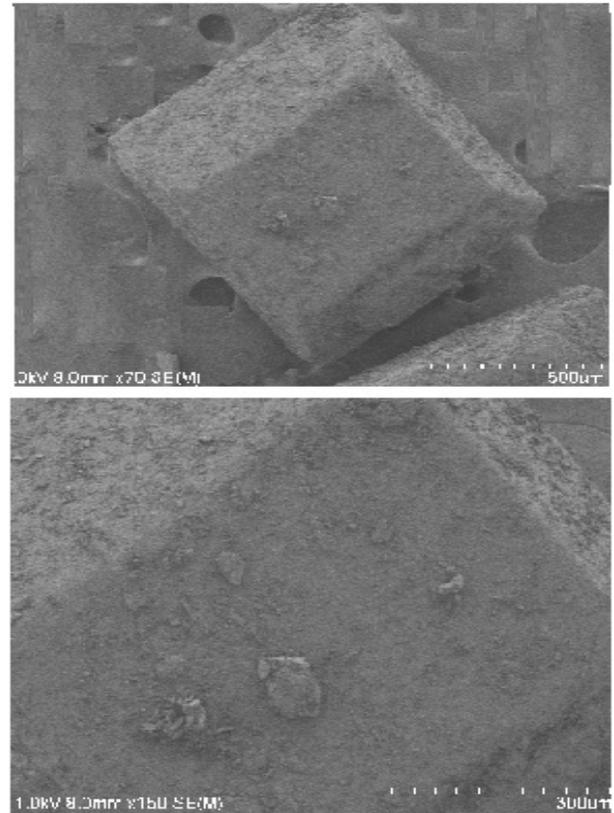
Microscopic examinations of the different faces of sucrose crystals were performed by scanning electron microscopy (SEM). Figure 4 shows the surface topography of a sucrose crystal which was grown in pure sucrose solution at  $60^{\circ}\text{C}$ . It was observed that the crystal shape has taken a normal form of a sucrose crystal. On the other hand, the microscopic examination of a crystal surface shows the fine sugar crystal on the crystal surface, which developed after the centrifugation process and during the drying operation. The influence of dextran molecules  $T_{2000}$  on sucrose crystal morphology and adsorption surface is shown in Figure 5. It was observed that in presence of this high molecular fraction of dextran the number of conglomerated crystals (multiple crystals where two or more crystals have grown together) increased. A scanning electron micrograph of a sugar crystal shows micro-particles (sucrose crystals) strongly adsorbed on the crystal surface, which cause a rough crystal surface with a number of gaps. Consequently, non-sugar and colorant particles can be adsorbed with dextran on the surface during the technical crystallization process in the sugar factories. Also in this case, the removal of impurities (non-sugars) is difficult during the washing of the crystals. An increase in washing time of the centrifugals is needed to get the required quality of sugar, increasing total centrifuging and purging time. Also, the

needle-shaped crystal reduces the purging efficiency of the massecuites in the centrifugals resulting in a poor separation of crystal and molasses, hence reducing the refined quality of the sugar [7]. Additionally, dust production was mainly due to the breaking and crushing of conglomerates and needle crystals near to the screens of centrifugals. It has been reported that the increase of sugar crystallization time causes the massecuites to become cooler than normal which increases the already abnormally high viscosity of the fluid [21,22].

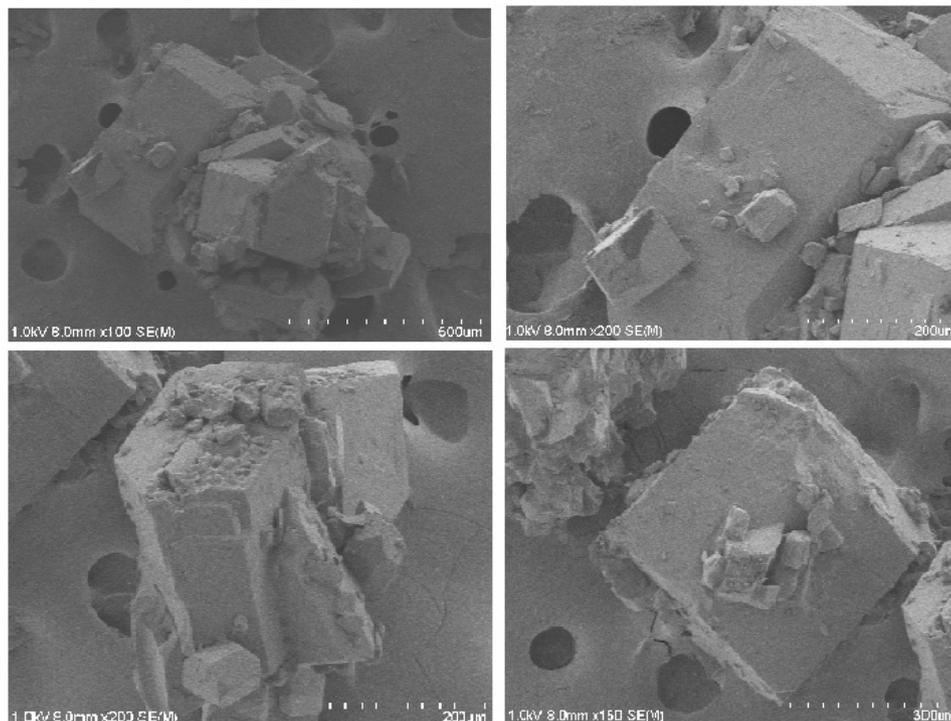
Consequently, sugar fine corn in the final product is increased. Such micro-particles may be seen on the surface of a sugar crystal in Figure 5. The conglomerates contain higher ash than unconglomerated sugar. The higher ash is contained in trapped mother liquor, either partially dried in the re-entrant angles, or in the inclusions in those angles.

The effect of crystallization temperature on the surface topography after hydrolysis of dextran by dextranase, at different temperatures was studied (Figure 6). It is obvious that after dextran is hydrolysed by dextranase, more perfect crystal surfaces are built than at 60°C. As a possible reason, the increase of viscosity caused by dextran and the effects of hydrolysis of high molecular weight dextran on the viscosity of dextran- sucrose solutions can be suggested. Also in crystal topography, the influence of dextran seems to be diminished by the application of higher concentrations of dextran as it could be observed for crystal growth rates. This effect can also be correlated to the increase of colorant and turbidity components of sugar produced at low temperatures (after product). Additionally, the presence of dextran can assist the adsorption of these particles on the crystal surface. Rogé et al., reported that there is a good correlation between white sugar turbidity and calcium ion concentration in stored syrups [2]. As well they reported that macromolecular organic substances (as for example

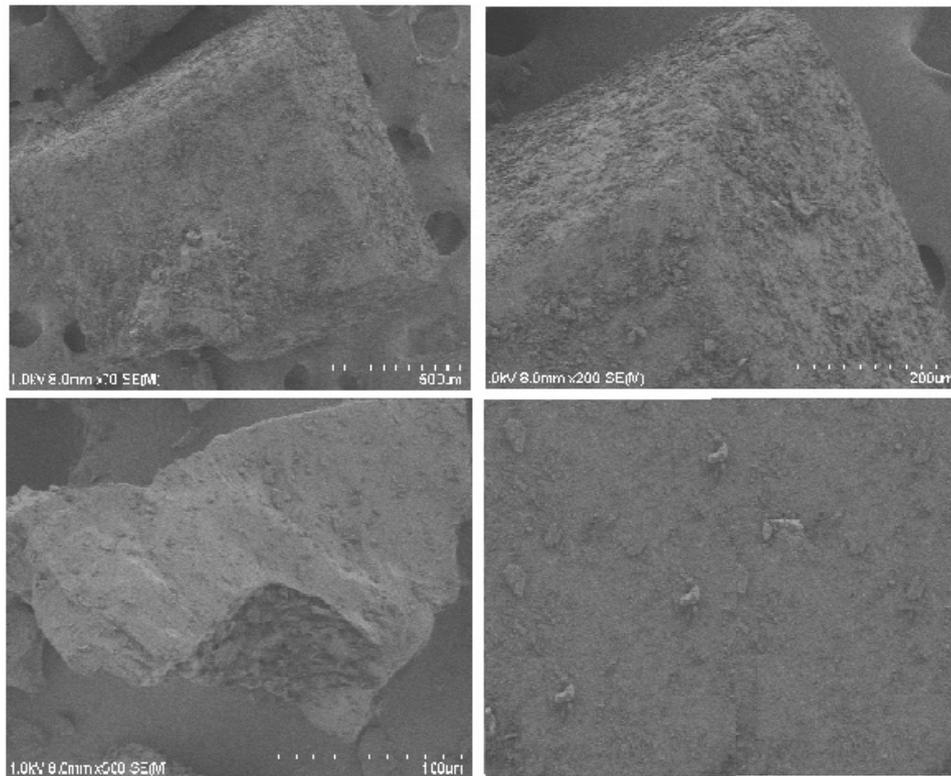
dextran) may act as a carrier for components causing turbidity. Therefore, hydrolysis of dextran before the crystallization process may lead to enhance the crystallization rate and decrease the fine sugar crystal on the crystal surface. On the other hand, the number of conglomerated crystals (multiple crystals where two or more crystals have grown together) decreased as can be seen in Figure 6.



**Figure 4.** Surface topography of a sucrose crystal grown in the pure sucrose solution



**Figure 5.** Surface topography of a sucrose crystal grown in the presence of 5000 mg dextran  $T_{2000}$  at 60°C



**Figure 6.** Surface topography of a sucrose crystal grown after hydrolysis of dextran T<sub>2000</sub> by dextranase enzyme at 60°C

In many factories, despite the use of ion exchange to reduce calcium ion, turbidity phenomena in the produced sugar were observed. In addition, a decrease of elongated crystals in the after product crystallization step was observed after hydrolysis of dextran. It has been reported that the elongation of face *c* is significant and such a phenomenon increases with syrup turbidity. From the technical sucrose crystallization point of view, the three stage crystallization scheme is regarded as the standard scheme. The process of producing sugar is through several stages, because the high concentration of non-sugar and the increase of solution viscosity hinder the sugar separation from the mother liquor [2].

#### 4. Conclusions

In this work, we investigated the influence of the biodegradation of dextran catalyzed by dextranase enzymes during sugar manufacturing on the rate of sucrose crystallization and growth rate of sucrose crystals in pure sucrose solution at different temperatures. To elucidate the influence of biodegradation of dextran on the growth rate of sucrose crystals, dextran of Mw 2,000,000 g/mol (T<sub>2000</sub>), were admixed in concentrations between (1000 - 10000 ppm) with (60% - 75% w/w) sucrose solution. The most pronounced effect of dextran on the growth rate of sucrose crystals was found with T<sub>2000</sub> at concentrations more than 5000 ppm at 60°C. From the results it could be shown that an increase of crystallization rate of up to 50% after biodegradation of dextran using dextranase enzyme at 100 ppm, compared to crystallization rate with pure sucrose solution in the presence of dextran T<sub>2000</sub>. Such a positive influence of biodegradation of dextran using dextranase enzyme decreases crystallization time in the

sugar house and thus decreases the production costs of sugar manufacturing.

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