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Performance of starch hydrolysis and production of corn syrup using some commercial enzymes

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Abstract

Five commercial amylolytic enzyme preparations, two liquefying (Termamyl Supra and Clarase L40, 000) and three saccharifying (AMG E, Dextrozyme DX and Optimax 4060 VHP) were used for the performance of starch hydrolysis to produce corn syrup. The operating conditions of these preparations showed that AMG E was the least effective enzyme within the saccharifying enzymes tested. Four enzyme combinations from the other four liquefying and saccharifying enzymes were tested for the starch hydrolysis. The results indicate that the liquefaction period by Termamyl S and Clarase must not exceed than 90 min whereas, the best starch concentrations were 30 gdl^{-1} for Termamyl S and 40 gdl^{-1} for Clarase. It was found that the combinations of Clarase followed by Optimax or Dextrozyme were more effective than those of Termamyl S, followed by the same two enzymes. The carbohydrate profile of the produced corn syrup showed that glucose is the main component (86.92%).The values of dextrose equivalent (DE) and the true dextrose equivalent (DX) of corn syrup were 79.587 and 85.334, respectively.

Introduction

Enzymes are ideal catalysts for food industry owing to their efficiency, ability to work under mild conditions, and their high purification and standardization. Furthermore, enzyme reactions are easily controlled and stopped when the desired degree of conversion reached. Depending on the enzymes used and the reaction conditions employed, various valuable products can be produced to suit nearly any particular requirements of the food industry (Olsen, 1995; Whitehurst and Van Oort, 2009).

The hydrolysis of starch is harmful to yeast and other organisms in subsequent fermentation processes, when using dilute acid at high temperatures accompanied by degradation of sugars to 5-hydroxymethylfurfural. Moreover, it is not possible to achieve dextrose equivalents greater than about 55 without generating off-taste (Sims and Cheryan, 1992b; Brown *et al*., 1993; Aggarwal *et al*., 2001).

Improvement in dextrose yield achieved by partial or complete replacement of acid with one enzyme or more. The processes referred to as acid-enzyme (A-E) or enzyme-enzyme (E-E), depending on whether the initial starch hydrolysis (liquefaction) is conducted with acid or bacterial α-amylase. Higher dextrose yield achieved by the E-E process, which developed in the 1960's (Hebeda, 1992). Furthermore, enzyme-catalyzed processes have fewer side reactions and by-products (Whitehurst and Van Oort, 2009).

A full enzymatic starch hydrolyzate not achieved until the high temperature stable bacterial amylases were used. Since enzymatic liquefaction and sacchrification of starch are performed at high temperatures. These enzymes enabled production of syrups containing up to 98% glucose (Olsen, 1995; Gupta *et al*., 2003; Souza and Magalhes, 2010).

Enzymatic production of glucose syrup from starch is a multistage process involving: liquefaction, saccharification, purification and concentration. Generally, the key features of the liquefaction enzymes are, 1- High dextrose yields with minimal by products formation, 2- Fast viscosity reduction-enabling high dry substance levels. 3- Low colour formation and reducing the refinery costs (Pontoh and Low, 1995). The saccharification is done by using an exo-acting glucoamylase, which specialized in cleaving α-1, 4 glucosidic bonds and slowly hydrolyzes α-1, 6 glucosidic bonds present in maltodextrins. This will result in accumulation of isomaltose. Therefore, recently pullulanase, which efficiently hydrolyzes α-1, 6 glucosidic bonds, is used (Hebeda, 1992; Van der Maarel *et al.*, 2002; Ezeji and Bahl, 2006; Brienzo *et al.*, 2008). The present work is aimed to study the operating conditions of some commercial amylolytic enzyme preparations for performance of starch hydrolysis and production corn syrup. In addition, the carbohydrate profile of the produced corn syrup was also identified.

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Materials and Methods

Starch

The corn starch used was produced by the Egyptian Starch and Glucose Manufacturing Co. Mosturod, Egypt.

Enzyme preparations

Five commercial amylolytic enzyme preparations were used throughout the present study. Three of them are products of Novozymes (Novo Nordisk Denmark). The first having the trade name, Termamyl Supra (Termamyl S), is a microbial enzyme produced from *Bacillus licheniformis*. The second, AMG E produced from Aspergillus niger. The last is Dextrozyme DX (Dextrozyme) is a mixture of fungal amyloglucosidase from *Aspergillus niger* and bacterial pullulanase from *Bacillus acidopollylyticus*.

The other two commercial preparations were produced by Genencor International, USA, namely: Clarase L-40,000 (Clarase) produced by fermentation of Aspergillus oryzae var. characterized by both dextrinzing (liquefying) and saccharifying action on starch, and Optimax 4060 VHP (Optimax) which is an optimized blend of fungal glucoamylase produced from *Aspergillus niger*, and the bacterial pullulanase produced from a modified strain of *Bacillus licheniformis*. All the enzyme preparations used were available in a liquid form.

Liquefying enzymes activity

The liquefying enzymes activity (Termamyl S and Clarase) was determined as reported by Anonymous (1978). Fifty ml of 1.0% corn starch slurry were transferred to a 250 ml conical flask, 40 ml of 0.02 M phosphate buffer pH 6.9 containing 0.006 M Na Cl were added and the mixture was equilibrated at 25ºC. At zero time the volume of the enzyme used was added to the flask, the total volume of the reaction mixture was completed to 100 ml using the phosphate buffer and incubated for 15 min. At the end of the incubation period 2 ml of the reaction mixture were withdrawn. The reducing power was determined using 3, 5-dinitrosalicylic acid method as reported by (Plummer, 1987) and calculated as mg maltose.

Saccharifying enzymes activity

The activity of the saccharifying enzymes (AMG E, Dextrozyme and Optimax) was determined according to the method described by ANONYMOUS (1978). Fifty ml of 2% gelatinized starch (heated for 15 min at 70ºC using water bath) were transferred to a 250 ml conical flask, 40 ml of 0.2 M acetate buffer pH 4.6 were added and the mixture was equilibrated at 37ºC. At zero time, the volume of the enzyme used was added to the flask and the total volume of the reaction mixture was completed to 100 ml using the acetate buffer and incubated for 15 min. The reducing power calculated as mg glucose formed. The activity of all enzymes used was calculated as micromoles of sugar formed (glucose or maltose) per minute. Whereas, the enzyme units were calculated as micromoles of sugar formed/min /ml of enzyme.

Parameters affecting the enzymes activity

The effect of time on the activity of all enzymes tested was measured according to the method described by Robyt and White (1987). The resulted progress curves were used to estimate the optimum reaction time for each enzyme. The effect of pH was determined according to the method described by Plummer (1987). For the liquefying enzymes, the universal buffer (4-10 pH values) was used, whereas for the saccharifying enzymes the pH values ranged from 3 -7 using citrate phosphate buffer. The temperatures tested for the liquefying enzymes were ranged from 50 -100ºC, whereas, those for the saccharifying enzymes were 20-80ºC.

The effect of the liquefying enzyme concentrations were studied using 25-200 μldl^{-1} for Termamyl S, and 5-40 μldl^{-1} for Clarase, whereas, for the saccharifying enzymes, AMG E, Dextrozyme, and Optimax were ranged from 5-40,1.25-15,and 0.5-10 μldl^{-1}, respectively. The substrate concentrations for the liquefying enzymes were ranged from 1 to 10 gdl^{-1}. Whereas, it ranged from 0.5 to5.0 gdl^{-1} for the three succharifying enzymes.

Measuring the effective period and starch concentration for the liquefaction step

The following experiments were carried out to estimate the maximum starch concentration, which could be used with the two liquefying enzymes tested. The concentrations of the initial starch slurry tested were ranged from 10 to 40 gdl^{-1}. The experiments were studied at the optimum operating conditions as stated before. The degree of liquefaction (measured as the amount of maltose formed) traced after the first 15 min then at 30 min intervals until 150 min.

Testing the different combinations of the liquefying and saccharifying enzymes for the performance of starch hydrolysis

Four enzyme combinations were confirmed, two of them started by the liquefying enzyme Termamyl S, while the other two started by Clarase. In each case the liquefied products were saccharified by Dextrozyme and Optimax. Three subtreatments were studied, by adding the amount of the saccharifying

enzyme used after 30, 60, and 90 min through the liquefying step.

Production of corn syrup

The production of corn syrup was carried out as described by the method of Srikanta *et al.* (1989). It started by preparation of an initial starch slurry, liquefaction, and saccharification, all steps were carried out at the optimum conditions for each enzyme. The enzymatic hydrolyzates clarified by heating at 100ºC for 15 min and filtered. The syrup was then decolourized by using activated charcoal column, demineralized by cation exchanger (Dowex 50 – X 8) followed by anion exchanger (Amberlite IRA 402 CI). Finally, the purified syrup concentrated by evaporation to 80% TSS.

Analysis of corn syrup

Total soluble solids (TSS) were measured by Abbé refractometer Model 2 WAJ, China, as described in the AOAC (2003). The dextrose equivalent (DE) was calculated as percentage of glucose formed to the total dry substances (Delheye and Moreels, 1988). Whereas, the true dextrose equivalent (DX) calculated as described by Whitehurst and Law (2002) using the equation:

$$DX = \%\ \text{glucose} \times 1.0 + \%\ \text{maltose} \times 0.5 + \%\ \text{maltotriose} \times 0.33$$

The carbohydrates profile (fructose, glucose, maltose, maltotriose and polysaccharides) were determined by high performance liquid chromatography HPLC using a Waters HPLC, USA (Abdel – Aal *et al*., 1993).

Results and Discussion

The optimum conditions of the enzyme preparations

The liquefying enzymes

The optimum time and temperature, which gave the maximum rate of reaction, were similar for the two liquefying enzymes, Termamyl S and Clarase, being 30 min and 80°C, respectively (Table 1) .The maximum activity of Termamyl S and Clarase was observed at pH 6.0 and 7.0, respectively. The optimum pH for different bacterial α-amylases ranged from 6.0 to 7.1 as reported by other authors (Van der Maarel *et al.*, 2002; Agrawal *et al*., 2005; Özdemir *et al*., 2011). Whereas, the optimal temperature of amylase from Bacillus subtilis varied from 57.5°C to 60ºC, and the time was 25 min (Agrawal *et al.*, 2005; Özdemir *et al*., 2011).

The optimum enzyme and substrate concentrations for Termamyl S and Clarase were 125.0 μldl^{-1}, 10.0 gdl^{-1} and 35.0 μldl^{-1}, 6.0 gdl^{-1}, respectively. In addition, the results indicate that the substrate: enzyme ratio (S: E) of Termamyl S was 80.00 which is less than half of that of Clarase (171.43). This ratio is very important industrial operating parameter. It describes well the best quantitatively balanced amount of both substrate and enzyme. Besides, this ratio indicates the maximum enzyme efficiency for the completely enzymatic process (Gorinstein, 1993).

The S: E ratio of 100 was stated by Brooks and Griffin (1987)for liquefaction of rice starch using the heat stable α-amylase, Termamyl 120 L. Higher S:E ratios were reported by other authors (Nebesny *et al*., 1998; Aggarwal *et al*., 2001).

The saccharifying enzymes

The data given in (Table 1) indicate that the optimum time of reaction for AMG E was 30 min. The optimum pH and temperature of this enzyme were noticed at pH 5 and 40°C, respectively. Other authors mentioned that glucoamylases of *Aspergillus niger* or a closely related species have an optimum pH and temperature ranged from 4.5 to 5.0 and 40 to 50°C, respectively (Pontoh and Low, 1995; Nebesny *et al*., 1998; Aggarwa *et al.*, 2001; Van der Maarel *et al.*, 2002). The results presented in (Table 1) revealed the low activity of AMG E for hydrolyzing the gelatinized starch. This may be attributed to the relatively higher AMG E optimum concentration (25 μldl^{-1}) and lower substrate concentration (3.5 gdl^{-1}) and hence low S: E ratio (140.0).

For the two other saccharifying enzymes tested, Dextrozyme and Optimax, the optimum time of the enzymatic reaction was 30 min for Dextrozyme and 15 min for Optimax. The optimum pH′s were 4.5 and 4.0 for Dextrozyme and Optimax, respectively. Other studies stated the pH of 4.5 as the optimum pH of amyloglucosidase from *Aspergillus niger* (Shiraishi *et al*., 1985, Sims and Cheryan, 1992b). The optimum temperature 60°C was noticed for the two enzymes under study. Other authors reported that the optimum temperature of amyloglucosidase and pullulanase ranged between 55 and 60°C (Guzmán–Maldonado and Paredes–lópez, 1995; Nebesny *et al*., 1998; Hossain *et al*., 2006).

The optimum enzyme concentrations for Dextrozyme and Optimax were practically closed to each other being 10 and 9 μldl^{-1}, respectively. This similar concentration of the two enzymes showed maximum activity in presence of 4 gdl^{-1} of substrate for Dextrozyme and only 2 gdl^{-1} when Optimax was used. This indicates that Optimax have a higher

Table 1. Optimum conditions of the enzyme preparations

Parameters	Liquefying enzymes		Saccharifying enzymes		
	Termamyl S	Clarase	AMG E	Dextrozyme	Optimax
Optimum conditions					
- Time (min)	30.00	30.00	30.00	30.00	15.00
- pH	6.00	7.00	5.00	4.50	4.00
- Temp. (ºC)	80.00	80.00	40.00	60.00	60.00
- Enzyme concentration (ul dl^{-1})	125.00	35.00	25.00	10.00	9.00
- Substrate concentration (g dl^{-1})	10.00	6.00	3.50	4.00	2.00
- Substrate : Enzyme ratio	80.00	171.43	140.00	400.00	222.22

Table 2. Comparison between the best four enzyme combinations tested for the performance of starch hydrolysis

Liquefaction		Saccharification		Percentage of Hydrolysis	Amount of glucose formed (mg)	Total process time (min)
Enzymes (Initial starch conc.)	Time (min)	Enzymes	Time (min)			
Termamyl S (30%)	30	Dextrozyme	90	95.00	27384.03	120
	30	Optimax	45	97.40	28075.16	75
Clarase (40%)	60	Dextrozyme	60	85.85	32994.44	120
	60	Optimax	30	91.80	35281.69	90

affinity to liquefied starch than that of Dextrozyme. The same observation declared from the S: E ratio of the two enzymes, being 222.22 for Optimax which was about half of that of Dextrozyme reaching, 400.0. Nebesny *et al.* (1998) used an S: E ratio of 333.33 for Spezyme GA 300 wy 553. Whereas, the S: E ratio of 400 described by Aggarwal *et al.* (2001) using Amylo 300.

In conclusion, AMG E was the least effective enzyme within the three saccharifying enzymes tested. This is mainly because AMG E contains amyloglucosidase, which hydrolyses only the α–1,4 glucosidic linkages. Whereas the two other enzymes (Dextrozyme and Optimax), which contain both amyloglucosidase and pullulanase are capable to hydrolyze the α–1,4 as well as α–1,6 linkages and hence more effective for saccharifying the liquefied starch. Therefore, Dextrozyme and Optimax will be the only saccharifying enzymes used for the performance of starch hydrolysis in the following experiments.

Measuring the effective period and starch concentration for the liquefaction step

The following experiments were carried out to find out the effective conditions for the liquefaction step i.e., the shorter period and the maximum starch concentration, which could be used .The operating conditions including S: E ratio were those stated before for each enzyme. The progress curve of Termamyl S using starch concentrations ranged between 10 to 40 gdl^{-1} was shown in(Figure 1a). In practice, it was necessary to add the amount of the liquefying enzyme to the reaction medium before the gradual addition of starch, especially at the higher concentrations of starch. It was noticed that the most amount of maltose was formed through the first fifteen minutes mainly for the lower concentrations used (20 gdl^{-1}). When the starch concentration increased to 40 gdl^{-1} a gradual increase in the amount of maltose was noticed through the first 90 min of the liquefaction. When the reaction period prolonged to 150 min no increase was observed. So, the liquefaction period by Termamyl S must not exceed than 90 min under the conditions used.

In order to define the suitable starch concentration within this period, the velocity of Termamyl S was calculated (μ mol maltose formed / min). It was found that the reaction velocity increased by increasing the starch concentration to 30 gdl^{-1}, and it decreased when the higher concentrations were used. So, the best starch concentration is 30 gdl^{-1} for Termamyl S. Saha and Zeikus (1989) used Termamyl 120 L to liquefy 35% starch slurry, the liquefication time was 5-15 min. Sims and Cheryan (1992a,b) stated that 30% corn starch was liquefied after 30 min using heat stable α-amylase isolated from *Bacillus licheniarmis* (Termamyl). On the other hand, Pontoh and Low (1995) used 30% (w/w) starch slurry to produce glucose syrup. They mentioned that the liquefaction time for corn and cassava starch was 30 min whereas, it varied from 30 to 90 min for palm starch.

For the second liquefying enzyme (Clarase), it was observed that there was a slight gradual increase in the Clarase activity until 90 min of the reaction (Fig.1b). Then being nearly constant with slightly decrease at the end of the reaction period. The maximum enzyme activity was observed when 40% of the starch was used after 90 min. Therefore, the liquefaction period by Clarase must not exceed than 90 min and the starch concentration will be 40% under the conditions used.

Hebeda (1992) reported that starch slurry of 30-40% is thinned at 103-107ºC for 5-10 min. Guzmάn-Maldonado and Paredes-López (1995) used a starch slurry of 30-40% for liquefaction at 90-95ºC for 90-120 min using thermostable α-amylase from *Bacillus licheniformis*. Aggarwal *et al.* (2001) reported that

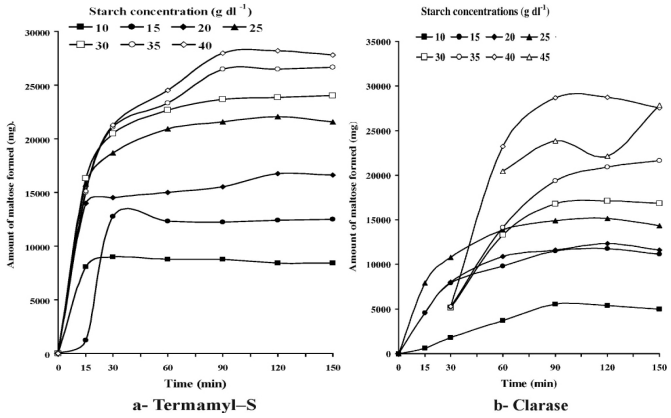

Figure 1: Enzyme progress curves using different substrate concentrations

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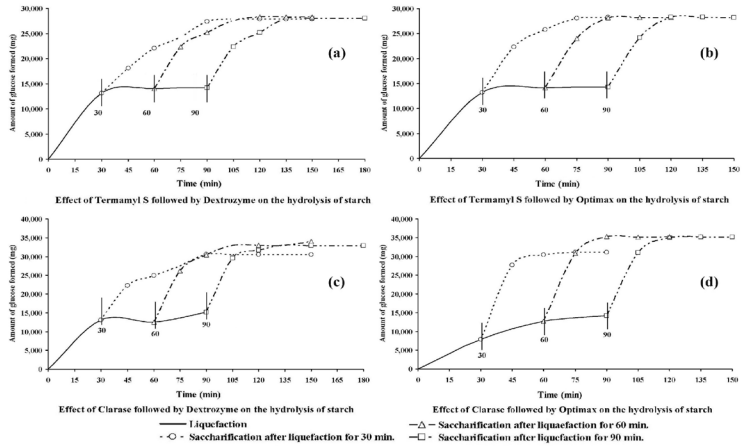

Figure 2: The different combinations of the liquefying and saccharifying enzymes for the performance of search hydrolysis

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the liquefaction of high concentrated slurry (25% w/w) needs 60 min in a water bath at 95°C.

The different combinations of the liquefying and saccharifying enzymes for the performance of starch hydrolysis

Four enzyme combinations from the more effective liquefying and saccharifying enzymes used were tested for the performance of starch hydrolysis. The first was Termamyl S followed by Dextrozyme. As shown from (Figure 2a), most liquefying effect of Termamyl S was practically done through the first 30 min. No noticeable increase was observed when the liquefaction period extended to 60 or 90 min followed by the same period of saccharification. The data given in (Figure 2b) show the hydrolysis of starch when Optimax followed Termamyl S. The maximum hydrolysis effect was obtained after only 30 min of liquefaction followed by saccharification for 45 min. Very slight increase was noticed if the liquefaction period extended to 60 or 90 min (Figure 2b).

Clarase was the liquefyine enzyme used in the other two combinations. When Clarase followed by Dextrozyme, it was found that the maximum hydrolysis was obtained after only 60 min for both the liquefaction and saccharification steps (Figure 2c). The last combination was that of Clarase and Optimax. The most effective hydrolysis process was that started with 60 min of liquefaction and followed by 30 min for saccharification steps. Extending the period of the both steps did not markedly increase the final percentage of starch hydrolysis (Figure 2d).

The aforementioned results are in agreement with other authors who reported that the liquefying step is very important because it prepares the starch molecules or "liquefying" it and increasing its susceptibility to the saccharifying enzymes (Guzmán- Maldonado and Paredes-López, 1995; Van der Maarel *et al*., 2002). Brooks and Griffin (1987) found that the lower liquefaction temperature should lead to decrease process costs and elimination of Millard reaction products, resulting higher product quality. In addition, the short time process for starch hydrolysis can be used to reduce labor requirements and energy consumption (Slominska 1989, Lee and Kim 1990, Paredes-López *et al.*, 1990, Sims and Cheryan, 1992b, Aggarwal *et al*., 2001).

Comparison between the best four enzyme combinations tested for the performance of starch hydrolysis

The results of the parameters which considered for evaluating the four enzyme combinations are summarized in (Table 2). The two couples started by the liquefying enzyme Termamyl S have the higher percentage of hydrolysis (95.00 and 97.40) than the other couples started by Clarase (85.85 and 91.80). Whereas, it is the opposite if the treatments were ranked with respect to the amount of glucose formed. The treatments started by Clarase have the higher amount of glucose than those started by Termamyl S. For example, Clarase-Optimax (started with 40% starch slurry) produced 35281.69 mg glucose as compared with 28075.16 mg glucose presents in the same total volume, for Termanyl S-Optimax treatment (started with 30% starch slurry). The amount of glucose formed is more important than the percentage of hydrolysis. Therefore, the treatment, which has higher glucose content, in the same volume, is commercially viable due to the decrease in the processing cost (Aggarwal *et al*., 2001). In addition, the total process period is an important parameter, the lower the process period the lower the cost. In conclusion, it is clear that Optimax was superior to Dextrozyme if it is used after Termamyl S or Clarase. In addition, the total process period was lower for Optimax treatments than that of Dextrozyme.

Table 3. TSS, carbohydrate profile and dextrose equivalent of the prepared glucose syrup

Glucose syrup samples (Enzy-combinations)	TSS	DP–1		DP–2	DP–3	DP>3		
		Fructose	Glucose	Maltose	Matotriose	Polysaccharides	DE	DX
Temamyl S- Dextrozyme	80.6	0.995	67.829	13.618	7.274	10.284	67.859	78.033
Termamyl S– Optimax	80.5	2.624	85.270	4.000	2.915	5.191	85.270	90.856
Clarase– Dextrozyme	80.9	0.673	78.322	0.496	8.500	12.009	78.322	82.048
Clarase– Optimax	80.6	1.877	86.928	0.636	3.961	6.698	86.928	90.397

Dp is the degree of glucose polymerization.

TSS, Carbohydrate profile and dextrose equivalent of corn syrups

The four enzyme combinations selected before were utilized to produce batches of corn syrup. The TSS of the samples ranged between 80.5 and 80.9% (Table 3). The value of 80% TSS or 80 Brix was to be the final concentration of commercial glucose syrup as reported by others (Hebeda, 1992; Wilson *et al.*, 1995). The carbohydrate profile showed low amounts of fructose ranged between 0.673 and 2.624% of the total carbohydrates (Table 3). Wilson *et al.* (1995) stated that fructose might occasionally present in glucose syrups. Glucose is the main component of the samples of corn syrup. The lowest glucose percentage was that obtained from Termamyl S-Dextrozyme treatment (67.829). On the other hand, the maximum glucose content was that obtained from the treatments, which used Optimax as a saccharifying enzyme. These results indicate that Optimax was more active than Dextrozyme as revealed before. The maltose and maltotriose content varied within the samples tested. The highest values obtained mainly when Dextrozyme was the saccharifying enzyme used. The polysaccharides fraction represents the molecules contained more than three units of glucose. The treatments, which contained Optimax, have the lowest percentage of polysaccharides.

The most common expressions have been used to monitor the rate of acid or enzyme hydrolysis of starch for the production of corn syrup are dextrose equivalent (DE) and the "true dextrose equivalent" (DX) as termed by Whitehurst and Law (2002). The mean value of DE and DX using the analytical data obtained from HPLC method were79.587 and 85.334, respectively (Table 3). It is clear that DX is more realized parameter since it used the three carbohydrate fractions (Dp-1, Dp-2, Dp-3) whereas, DE used only the Dp-1 fraction. According to the Corn Refiners Association, Inc (Anonymous, 2002) corn syrup could be classified into four types based on DE, type 1: 20-38DE, type 2:38-58DE, type 3:58-73 DE and type 4: 73DE and above.

Wilson *et al.* (1995) stated that knowledge of saccharide distribution in glucose syrups is of prime importance, due to the ability to "tailor – make" glucose syrups. Recently numerous corn syrups are available started from low conversion corn syrup of about 32-42 DE and reached to extra high conversion corn syrup of 95 DE.

Conclusion

Commercially amylolytic, liquefying and saccharifying enzymes, are ideal tools for production of glucose syrup. These enzymes are available from different producers. Different combinations of these enzymes were tested for performance of glucose syrup. It was noticed that, a combination of enzymes from different sources might be more effective than that produced by one source.

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