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A comparison Of Some Commercial Enzymes Used in the Production of Glucose Syrup from Starch.

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Abstract

A comparison was made of two commercial enzymes (OPTIMAX® 4060 VHP and Extenda® Standard) used in the production of commercial glucose syrup or dextrose that is converted to fructose.

The comparison included the reaction mechanism for each of the enzymes and the time required to obtain the required product with fixed concentrations of the reaction material with the stability of the rest of the operating factors such as PH and temperature, and the changes were followed through the results of the HPLC device to determine the degree of polymerization obtained from each enzyme. The results showed that Extenda enzyme, which is considered a new generation of cracking enzymes, has surpassed OPTIMAX enzyme in terms of effectiveness and activity by almost two times, reducing the added quantities by half of the Extenda enzyme.

One enzyme was used in the fluidization phase, SPEZYME®POWERLIQ, for both enzymes to ensure standardization of all parameters that might affect the results.

***Keywords*:** corn starch; enzymatic hydrolysis; enzymatic deactivation; enzyme-substrate ratio.

1. INTRODUCTION

Due to the economic importance of using enzymes in the production of glucose or dextrose syrup, which is used in the production of fructose through the process of isomerization.

We have compared some of the commercial enzymes used in the production of glucose syrup in terms of the time required to obtain the desired product when the concentrations of both enzymes are equal.

The OPtimax enzyme manufactured by Genencor has been used worldwide.

The Extenda enzyme manufactured by Novozymes is a new generation enzyme used in industry.

Enzymes are very ideal catalysts for the food industry due to their high efficiency, ability to work under mild conditions, high purity, and great homogeneity.

Also, enzyme reactions can be easily controlled and stopped when the required degree of conversion is reached. Based on the enzymes used and the appropriate reaction conditions, many derivative products can be produced to suit any food and industrial requirements [1, 2]. Starch is one of the most abundant heteropolysaccharides after cellulose produced by plants in the form of granules that are insoluble in cold water. It is a polymeric carbohydrate, consisting of carbon, hydrogen and oxygen atoms in a ratio of 6: 10: 5, (C6H10O5) n.

The starch polymer consists of hundreds or thousands of glucose units, which is consistent with values of n ranging from 50 to several thousand.

The glucose units that make up the polymer are linked to each other through the C1 oxygen as a glucosidic bond.

The final glucose unit of the polymer chain contains a latent aldehyde group which is known as the reductive end group. Starch polymer consists of a mixture of two high molecular weight polymers: (1) a straight-chain molecule known as amylose, (2) and a branched-chain molecule known as amylopectin [3]. Branching regions make up about 5% of the glucose unit of amylopectin [4], and the enzymes largely used in starch processing are classified as amylases. Trade codes for these enzymes have been developed to facilitate classification globally, such as: Pullulanase with EC 3.2.1.41 or also known as α-dextrin 6-glucanohydrolase.

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Also, the Spezyme enzyme was used for the starch liquefaction process when comparing the two enzymes.

2. MATERIALS

2.1. Starch and chemicals.

The corn starch used was produced by the Al Monairy for Corn Products
, 10th of Ramadan - Industrial Zone 6A - Plot 110 - Sharkia - Egypt.
All chemicals used are laboratory grade chemicals with a high degree of purity. All chemicals used are laboratory grade chemicals with a high degree of purity.

2.2. Enzyme preparations

2.2.1. Spezyme®Powerliq Enzyme

Is a α-amylase for starch hydrolysis.When used in starch liquefaction, the enzyme preparation is of excellent stability against high temperatures and low pH as well as little dependence on calcium levels, etc. Hydrolytic properties of Spezyme® Powerliq are optimized for dextrose production and combine the best features of the thermostable bacterial amylases from *Bacillus licheniformis* and *Geobacillus stearothermophilus* along with DuPont's latest α-amylase protein engineering technologies. Activity: 30099 LU/g (the lowest) and dosage of 0.2 kg per metric ton dry starch is typical. α- amylases reduce liquefact viscosity by liquefying starch under direct steam injection heating conditions, hydrolyzing α-D-1, 4 glucosidic bonds in starch to produce soluble dextrins for saccharifcation to dextrose.
Rapid viscosity reduction ensures high quality mixing and shear in the jet cooker, enabling high-solids operation without compromising hydrolyzate quality.

2.2.2. Optimax® 4060 VHP Enzyme

Is an optimized blend of fungal glucoamylase and bacterial pullulanase.
The pullulanase, produced from a genetically modified strain of *Bacillus licheniformis*, catalyzes the hydrolysis of the (1, 6-α-D) glucosidic linkages of liquefied starch to produce linear oligosaccharides. The glucoamylase, produced from a selected strain of *Aspergillus niger*, catalyzes the hydrolysis of (1, 4-α-D) and (1, 6-α-D) glucosidic linkages of liquefied starch to produce glucose. The enzyme activities are formulated to produce preferred substrates during the course of the saccharification, which maximizes the final glucose yield. Pullulanase Activity: 390 ASPU/g (minimum) and Glucoamylase Activity: 260 GAU/g (minimum).

2.2.3. Extenda® Standard Enzyme

Extenda is a unique new saccharification enzyme blend, formulated to deliver a more robust saccharification process than conventional glucoamylases through improved stability. Dosing at 0.42 & 0.53 kg/ton, respectively. Saccharification conditions at pH 4.3, 36% initial DS and 60°C.
In addition to the activity of glucoamylase enzymes, the enzyme pullulolase has a high de-branching activity in the amylopectin molecule, as well as the activity of the α-amylase enzyme to facilitate the hydrolysis of oligosaccharides, as well as the activity of the lysophospholipase enzyme to enhance the efficiency of solution filtration.

2.2.4. Clarase® L Enzyme

Is a α-amylase (EC 3.2.1.1; 1, 4-a-D-glucanohydrolase) produced by a selected strain of *Aspergillus oryzae* var. It is characterized by both dextrinizing (liquefying) and saccharifying (glucose and maltose liberating) actions on starch. The enzyme is an endoamylase capable of rapidly hydrolyzing the interior (α-1, 4 glucosidic) linkages of gelatinous starch, amylose, and amylopectin solutions yielding soluble dextrins with lesser quantities of glucose and maltose. Prolonged starch hydrolysis with CLARASE® L enzyme results in syrups with high maltose and low levels of glucose. These high maltose syrups are relatively non-hygroscopic and exhibit a decreased tendency to crystallize and exhibit a high level of ferment ability. Activity: 40,000 SKBU/g One SKB α-amylase Unit (SKBU) is that activity which will dextrinize 1.0 gram of limit-dextrin substrate per hour under the conditions of the assay.

3. METHODS

3.1. Preparation of liquefied starch

When preparing a sample of liquefied starch, the concentration of the solid in the starch milk must first be adjusted at (30 - 45%). Then the pH is set at (5.8-6.2) according to the instructions recommended for the use of the liquefaction enzyme, in order not to affect the activity of the enzyme in the conversion process. Likewise, the required temperature is set according to the reaction stages. At the beginning of the reaction, it is set at 108-110 degrees Celsius. After a period of 10-15 minutes, the temperature is reduced to 95 degrees Celsius for a period of about 100 minutes [5].

3.2. Preparation of low DE glucose syrup

After preparing the liquefied starch in the previous step, the resulting solution is cooled to a temperature of 60°C. After that, the pH of the solution is adjusted by a solution of hydrochloric acid (0.5 N) or by citric acid (0.5 M). The degree of Brix in the solution at this stage reaches from (32-34) to the solid. The specified amount of enzyme to be used is added at the zero point in the incubation process. Then the incubation period is calculated and the dextrose equivalent is measured using Fehling method or HPLC device [6].

3.3. Check for the absence of inhibitors for both enzymes

All the factors that may affect the activity of enzymes were controlled according to the recommendations of the manufacturers, and they were tested in the

laboratory to verify them first before starting the reaction.

The desired pH effect was measured according to the method described in [6]. Also, the effect of time factor on the activity of the mentioned enzymes was measured according to the method described by [7].The temperature has been set within the required limits. A uniform concentration of the liquefied enzyme was used using (0. 2 kg/ton DS starch) for spezyme without adding any enzyme inducer to ensure that the results are more accurate.Also, the concentration of the reactant was constant for the two enzymes during the saccharification phase.

3.4. Use with other enzymes to produce a customized glucose syrup

A commercial glucose syrup was produced as described in the method [8].

200 ml of the liquefied sample is incubated at 60 °C and Optimax enzyme: Clarase enzyme is added in a ratio of (2:1).

In the other enzyme, the addition is done in the same previous ratios (2:1) Extenda: Clarase.

In this method, more than one enzyme of different types is used to produce a syrup intended for certain industries, such as the bakery industry, ice cream, chemical industry, or other industries.

Where the customer determines the profile sugar required for this industry and determines the degree of dextrose equivalent as well, and accordingly the enzymes required to prepare this type of glucose syrup are determined.

Comparison of profile sugar resulting from each enzyme in terms (DE) degrees required by the customer or specified by the product specification.

3.5. Dextrose preparation

The two enzymes were used in the production of dextrose olmono glucose syrup, which is then used in the isomerization process. The quantities used of each enzyme and the time needed to reach the dextrose equivalent required for the process of producing fructose by converting dextrose were compared. All the optimal factors were provided for both enzymes, so that the results are representative of the efficiency of each enzyme in production. A commercial glucose syrup was produced as described in the method [8].

3.6. Analysis of corn syrup

A Brix device was used to measure total dissolved solids in solutions, called the refractometer, as described in [9]. Dextrose equivalent (DE) was determined as the percentage of glucose from the saccharification process to the total dry matter used [10]. The true dextrose equivalent of a solution (DX) was calculated by [11] using the following equation:

DX = % glucose x 1.0

DE = % glucose × 1. 0 + % maltose × 0.58 + % maltotriose × 0.397 +

% Polysaccharides × 0.15

The resulting carbohydrate profile (glucose, maltose, maltotriose and sugars) was determined by HPLC Waters high-performance liquid chromatography apparatus, SHIMADZU [12].

4. RESULTS AND DISCUSSION

4.1. Preparation of liquefied starch

To measure the degree of enzyme activity, the amount of product formed over a specific period of time must be measured and sometimes in some cases the amount of substrate transformed, which must be the same. So ideally there should be a way to measure any product or reactant in the presence of the other. There are many different ways. In this paper I will use what are known as "stop assays". Which involves stopped tests to stop the reaction at a specified period of time, and then calculate the amount of product that was created. Any method is possible, from chemical or enzymatic biometrics, and generally the easiest method is chosen provided it is reliable. In a large number of cases, a selective method can distinguish between the reactant and the product, such that a separation step is not necessary.

These basic methods, which are used to stop enzymatic reactions, include those that denature the enzyme, such as strong acids, strong alkalis, detergents, heat, or the addition of inhibitors such as heavy metal ions. Also, in some cases, enzymes are stopped by adding some complex compounds such as ethylenediaminetetraacetic acid, known as (EDTA), which works to remove metal ions necessary for activity, and even cooling to a freezing point may be sufficient to stop the enzymes. It is very important that the stopped assays are checked at least once with varying times in the incubation period, to ensure that the rate is linear within the specified period of the standard method used [13].

The starch polymer has a DE value of zero, while dextrose, which is the final product of the hydrolysis of starch, has a DE value of 100. For laymen and non-specialists, the term dextrose equivalent or DE can be seen as an indication of the conversion process of the starch polymer into the sugar glucose. As for the specialist scientist, the DE is a measure of the total reducing sugars present in the glucose solution. It does not express the amount of glucose in the drink. Therefore, it is possible to determine the amount of dextrose with other sugars in the syrup, using the HPLC (high-pressure liquid chromatography) device (a measure of the temperature at which it freezes) [14]. The concentration of the starch solution was adjusted before the liquefaction process as in Tabal.(1)

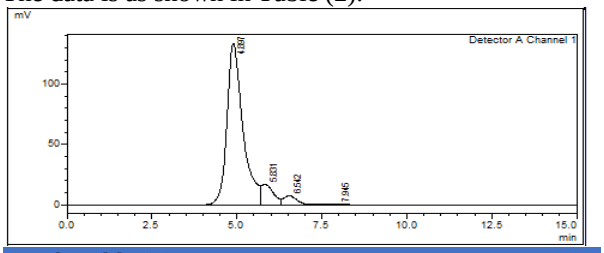
The starch milk sample was prepared according to the instructions in the enzyme use. The starch milk solid was 44 %, in order to maximize the benefit of the liquefaction process. A 1 L sample was prepared, and the pH was raised to 5.9 with a solution of sodium carbonate. As reported by other authors [15, 16, 17].

Tabal.(1) Starch milk standard

	Parameters	Specified
1	PH	5.3
2	Be	18 - 19
	Total Protein	0.3
3	Cond	120
4	Temperature	40 – 45 ºC
5	SO2	100 ≥ ppm

The specified amount of enzyme was added to the starch milk 5 µldl-1 and Activity of enzyme 30099 LU/g, Activity of the enzyme is expressed as LU. One LU unit means the time needed for color variation indicating transformation of starch to dextrin which is calibrated by Iodine solution under specific test conditions. Then the heating process started to reach 108°C for 9 minutes. Then, the temperature was reduced to 95 °C for up to 105 minutes incubation (water bath).Then the sample was left to cool down to a temperature of 60 ºC, then an iodine test was done to ascertain whether or not there was starch in the solution. A sample was then injected into an HPLC device to determine the DE obtained after completion of the fluidization process.

The data is as shown in Table (2).



Peak Table (2)				
Detector A Channel 1				
Peak	R.Time	Area %	Height %	Name
1	4.897	86.615	84.149	Polysaccharides
2	5.831	8.608	10.702	Maltotriose
3	6.542	4.269	4.704	Maltose
4	7.945	0.508	0.445	Dextrose
total		100	100	

DE = % glucose × 1. 0 + % maltose × 0.58 + % maltotriose × 0.397 +

% Polysaccharides × 0.15

= 0.508× 1. 0 + 4.269× 0.58 + 8.608 × 0.397 + 86.615 × 0.15

= 19.39

Methods are used to determine DE values in the maltodextrin range but all of them have certain advantages and disadvantages. Depending on method and columns used, the quantitative and qualitative separation of oligosaccharides up to DP = 12 is possible with HPLC techniques. Nevertheless, there is a problem in recovery and quantification of oligosaccharides of higher degree of polymerisation. Large off-scale peaks of fractions with high molecular mass create problems in their quantification as well as in their contribution as a whole to the DE value determined [18].

In addition, in maltodextrins of lower DE value (DE=5) some insoluble carbohydrate material (dextrin fractions) might be present.

It is not recommended to inject these fractions in the HPLC system, because they would not be quantitatively recovered or even eluted. Even though this could be avoided by dilution of samples, concentrations below 10% are not recommended, since saccharides become more difficult to detect because they represent only a small fraction of the total solids [19].

When performing HPLC analysis, all of the mentioned facts should be taken into consideration.

4.2. Preparation of low DE glucose syrup

Most starches of great industrial importance consist of about 80% amylopectin. In which branching points occur on average every 20-25 D-glucose units, therefore, the amylopectin molecule contains 4%-5% of the branched bonds of the α-1, 6-glucoside type [20].

These α-1, 6-glucosidic bonds present in starch molecules act as a kind of barrier to the action of some other enzymes used in the hydrolysis of starch during the cracking or saccharification process, which comes after the gelatinization process. When the starch polymer is broken down in the process of hydrolysis by α-amylases, this endogenous enzyme is able to bypass the branching points in the polymer, but in general it cannot break the α-1, 6-glucosidic bond in the process of hydrolysis of the amylopectin polymer. Therefore, the amylopectin fraction is only partially degraded [21].

4.2.1. Optimax 4060 VHP

Enzyme is an optimized blend of fungal glucoamylase and bacterial pullulanase. The pullulanase, produced from a genetically modified strain of Bacillus licheniformis, catalyzes the hydrolysis of the (1, 6-α-D) glucosidic linkages of liquefied starch to produce linear oligosaccharides. The glucoamylase, produced from a selected strain of Aspergillus Niger, catalyzes the hydrolysis of (1, 4-α-D) and (1, 6-α-D) glucosidic linkages of liquefied starch to produce glucose. The enzyme activities are formulated to produce preferred substrates during the course of the saccharification, which maximizes the final glucose yield.

Pullulanase Activity: 390 ASPU/g. One Acid Stable Pullulanase Unit (ASPU) is the activity which liberates one equivalent reducing potential, expressed as glucose per minute, under the conditions of the ASPU assay. The enzyme contains 5-10% by weight of enzyme glucoamylase and contains less than 1 by weight of enzyme pullulanase the product is essentially free of transglucosidase activity.

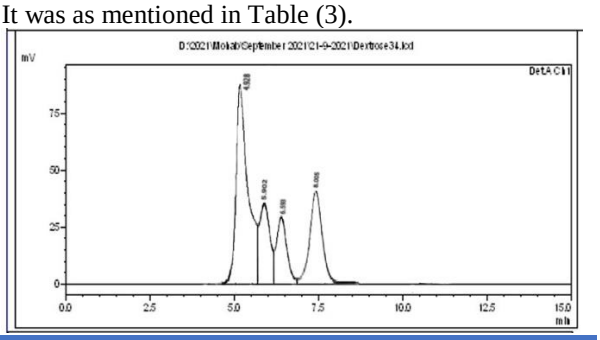
Glucoamylase Activity: 260 GAU/g. One Glucoamylase Unit (GAU) is the amount of enzyme that will liberate one gram of reducing sugar, calculated as glucose, per hour, from a soluble starch substrate under the specified conditions of the assay.

An enzyme is characterized by giving flexibility when used with other enzymes, since it contains only two enzymes, an enzyme to remove branching, and an enzyme to liberate glucose molecules with a reduced end.

After completing the liquefaction process, the solution was cooled to a temperature of 60 ºC, after which the pH was adjusted to 4.0 – 4.2 according to the enzyme's operating parameters. By that acid HCL its concentration 0.5 N. A sample of 200 ml was withdrawn from the previous liquid sample into a conical flask with a capacity of 250 ml.

Then 5 µldl-1 of an enzyme was added Optimax and incubated at 60 ºC for a 1 hour. A sample was drawn after 1 hours, and DE was determined by HPLC file.

It was as mentioned in Table (3).



Peak Table (3)

Detector A Channel 1

Peak	R.Time	Area %	Height%	Name
1	4.928	36.187	37.893	Polysaccharides
2	5.902	19.050	20.245	Maltotriose
3	6.593	14.350	15.581	Maltose
4	8.005	30.413	26.281	Dextrose
total		100	100	

DE = % glucose × 1. 0 + % maltose × 0.58 + % maltotriose × 0.397 +

% Polysaccharides × 0.15 = 51.73

DX = 30.413

The efficiency of the enzyme activity is judged by the amount of dextrose produced at a specific time at a specific concentration of the enzyme as well as a specific concentration of the reaction material. During this specified period, the dextrose production reached 30.4 % for the first enzyme.

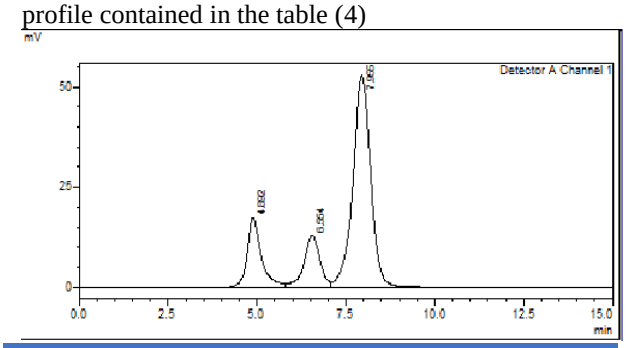
4.2.2. Extenda Standard

In addition to unique glucoamylase activity, contains pullulanase activity for debranching, acid alpha-amylase activity to facilitate oligosaccharide hydrolysis, and lysophospholipase activity to enhance syrup filtration efficiency.

By comparing the components of both enzymes, we find that an enzyme Extenda has more components than an enzyme Optimax with new enzymes that are acid alpha-amylase and lysophospholipase. It will be seen through the results whether these added enzymes have new effects on the old enzymes, or whether they will be of the same efficiency.

After completing the liquefaction process, the solution was cooled to a temperature of 60 ºC, after which the pH was adjusted to 4.1 – 4.5 according to the enzyme's operating parameters. By that acid HCL its concentration 0.5 N. A sample of 200 ml was withdrawn from the previous liquid sample into a conical flask with a capacity of 250 ml.

Then 5 µldl-1 of an enzyme was added Extenda and incubated at 60 ºC for a 1 hour. A sample was withdrawn 1 hour later, and DE determined by HPLC profile contained in the table (4)



Peak Table (4)

Detector A Channel 1

Peak	R.Time	Area %	Height%	Name
1	4.892	17.514	20.893	Polysaccharides
2	0	0	0	Maltotriose
3	6.554	14.757	15.581	Maltose
4	7.955	67.729	63.527	Dextrose
total		100	100	

DE = % glucose × 1. 0 + % maltose × 0.58 + % maltotriose × 0.397 + % Polysaccharides × 0.15 = 78.915

DX = 67.729

Through the results of the Profile Sugar device HPLC, it turns out that the amount of dextrose produced is almost twice the amount of dextrose produced from the use of an enzyme Optimax. At the same amount of enzyme used and under the same operating conditions. It was also noted the absence of Maltotriose (DP3) completely in the sugar profile. The dextrose equivalent (DE) was calculated and it was double the degree (DE) for the enzyme Optimax. From here it is clear that the activity and efficiency of an enzyme Extenda is superior to the activity and efficiency of an enzyme Optimax by almost double.

4.3. Check for the absence of inhibitors for both enzymes

Enzyme activity is affected by factors such as temperature, enzyme concentration, pH, and substrate concentration.

4.3.1. Temperature effect

When testing the effect of temperature on enzyme activity, the results showed that Optimax and Extenda worked best at a temperature of 60°C, and when the temperature dropped to 50°C, enzyme activity decreased. At 35 °C, the enzyme activity decreased sharply. The flexibility of this enzyme can be affected due to the effect of temperature on covalent and hydrogen bonds.

Chemical bonds shape the protein's shape and the function of the protein is related to its shape, so the activity will reduce or affect the enzyme that catalyzes the reaction of [22].When the temperature is high, the rate of enzyme activity is high because the substrates collide with the active sites on the enzyme more frequently as the molecules move quickly. [22].Therefore, the temperature was set at 60 °C according to the instructions for using the enzyme in the cracking stage.

The saccharification enzymes are inactivated when the temperature is raised to 80°C or more. But through experiments, it was found that the Extenda enzyme to this degree reduces its activity a lot, but does not stop it.

4.3.2. Effect of enzyme concentration

Next, testing the effect of enzyme concentration on enzyme activity showed some correlation between enzyme concentration and activity. The enzyme activity was higher with double the specified amount of enzyme concentration. Enzyme activity decreased by reducing the amount by half.

Therefore, the same amount of both enzymes was used to see which one was more efficient and active.

4.3.3. PH effect

The importance of adjusting the pH of the reaction medium is due to the fact that pH affects ionic and hydrogen bonds which are important for enzyme formation and thus enzyme activity [22].

PH also plays a role in enzyme activity. For example, the highest activity was at pH = 4.2. But at pH = 9, the activity was very low and there was no activity at pH = 1. Through experience, it was found that the best way to stop the activity of the Extenda enzyme is to reduce the pH to 2 or less. Although this method is effective in stopping the activity of the enzyme, it has a negative effect on the stage of ion exchanges specialized in removing salts and yellowing from glucose syrup.

4.3.4. The effect of reactant concentration

The concentration of the reactant has a significant effect on the performance and efficiency of the enzyme. The more water present in the reaction medium, the higher the enzyme activity, and thus the lower the concentration of the reactant.

Therefore, the enzyme industry determines the maximum concentration of the reactant, which is proportional to the activity of the enzyme in the desired catalysis. The concentration of the reactant in this experiment was adjusted according to the enzyme manufacturers' instructions, (30-45%).

4.3.5. Effect of protein concentration

As well as ensuring that the reaction material is free of any enzyme inhibitors, as corn protein is also one of the inhibitors, so the amount that does not affect the activity of enzymes was determined to be less than 0.3% of milk starch, so it must first be ensured that the protein percentage in starch milk is within the limits allowed as shown in Table (1).

Each enzyme has different preferences for these factors. Therefore, every experiment needs to provide the right conditions for all of its enzymes to function efficiently.

4.4. Use with other enzymes to produce a customized glucose syrup

Glucose syrup, in commercial practice called glucose, is obtained by hydrolysis of starch mainly from wheat, corn and potatoes.

The method and extent of hydrolysis (conversion) influence the final structure of carbohydrates and thus many functional properties. The degree of hydrolysis is generally defined as dextrose equivalent (DE), expressing the reducing strength as a percentage of pure dextrose, calculated on a dry weight basis. Originally, acid conversion was used to produce glucose syrup. Today, because of their specificity, enzymes are frequently used to predetermine how hydrolysis will occur.

In this way, it is possible to manufacture specially designed glucose syrup with specific sugar spectra. Sugar spectra can be analyzed by various techniques, such as high-performance liquid chromatography (HPLC).

Glucose syrup is produced with customized specifications according to the customer's request or the general specifications of the country.

Therefore, more than one enzyme must be included in the production of glucose syrup to meet these requirements. The enzyme used for Clarase is a-amylase (EC 3.2.1.1; 1, 4-α-D-glucanohydrolase) produced by a selected strain of Aspergillus oryzae var. It is characterized by both dextrin (liquefaction) and glycosylation (release of glucose and maltose) on starch, the enzyme is an endoamylase capable of rapidly hydrolyzing the internal bonds (β-1, 4-glucosidic) of gelatinous starch solutions, amylose and amylopectin, yielding soluble dextrin less glucose and maltose. High levels of maltose and low levels of glucose.

4.4.1. Using the Optimax enzyme

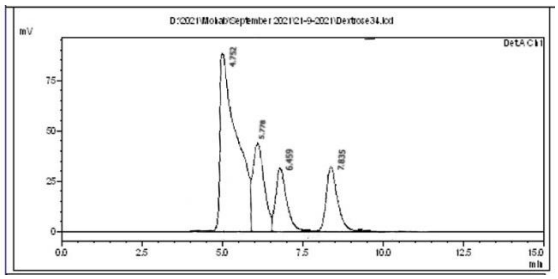
Specialized in de-branching the amylopectin molecule and glucose production, Clarase enzyme was added: Optimax enzyme in a ratio of 1: 2 in a row of these enzymes after adjusting the pH to 4.3 and adjusting the temperature of the solution to 60 ° C and incubating for an hour. The enzyme was stopped by heat and

injected. A sample in the HPLC device to find out the resulting profile sugar and its compliance with the general specifications for glucose syrup as shown in the table (5). The Optimax enzyme with Clarase enzyme was stable during the reaction, even during the heating phase to inactivate the enzymes. Another sample was injected to ensure that the profile sugar image did not change after the heating process to stop the enzyme activity, and its accuracy did not change, where the results were as shown in table (6). Which indicates the stability of the enzyme activity during the reaction with other enzymes. Therefore, this is considered one of the distinguishing properties of the enzyme Optimax.

Table (5)

Standard Glucose	
Area %	Name
55 ±2	Polysaccharides
12 ±2	Maltotriose
15 ±2	Maltose
18 ±2	Dextrose
100	

Average dextrose equivalent (DE) = 38 – 40



Dextrose equivalent % (DE) = 38.369

Peak Table (6)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	4.752	54.922	51.275	Polysaccharides
2	5.778	17.129	14.811	Maltotriose
3	6.459	10.994	16.293	Maltose
4	7.835	16.955	17.621	Dextrose
total		100	100	

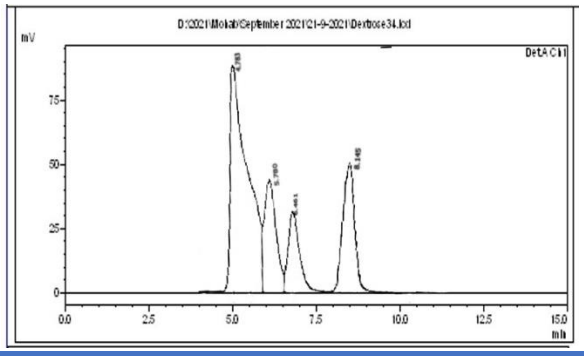
4.4.2. Using the Extenda enzyme

Another sample was drawn from the previously prepared solution. Where the pH was adjusted to 4.2 and the temperature set at 60 °C, with the same concentration of the solid in the reactant.

Clarase enzyme was used: Extenda enzyme at the same concentration as the previous enzyme 1: 2 .The sample was incubated in a water bath at 60°. The sample is then withdrawn and injected. The HPLC device matches the resulting glucose profile with the control sample as shown in Table 5.

When calculating the dextrose equivalent for this sample, it was much higher than would be expected for the standard sample (DE = 52). This resulted in the inability to adjust profile of the glucose syrup produced under the same incubation period and the same operating parameters.

The experiment was repeated again with the same previous parameters during the incubation period. The time has been reduced by half. The incubation period was only 30 minutes, then his sample was withdrawn and injected into the HPLC device, and it was close to the control sample, as shown in the data of Table (7). However, dextrose increased by 3% compared to the control sample. Which indicates that the speed of dextrose production by the Extenda enzyme is faster than that of the Optimax enzyme, and also that the Optimax enzyme is more stable than when mixed with other enzymes.



Peak Table (7)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	4.783	47.661	40.003	Polysaccharides
2	5.780	18.799	18.504	Maltotriose
3	6.461	11.994	17.329	Maltose
4	8.145	21.546	24.164	Dextrose
total		100	100	

Dextrose equivalent % (DE) = 43.114

It was found that the reason for the increase in the percentage of dextrose equivalent of the sample after raising the temperature to the highest 80 degrees Celsius. This is the temperature at which the activity of glycoenzymes stops [23, 24, and 25].But the Extenda enzyme continued to function, resulting in an elevated dextrose equivalent. Which then led to an attempt to inactivate it by lowering the pH ≥ 2.

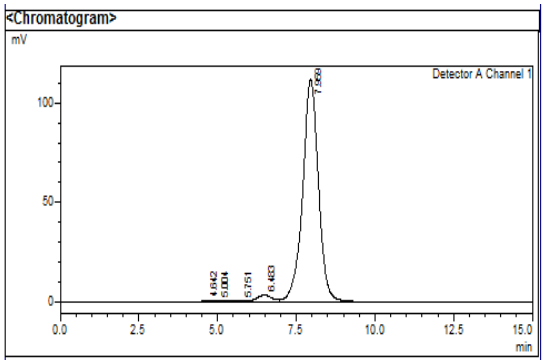
4.5. Dextrose preparation

4.5.1. Dextrose preparation by Optimax enzyme

Dextrose syrup has a very great commercial importance, since it is involved in many industries, whether food or chemical, so it has gained great commercial importance worldwide.

To measure the efficiency of the optimal enzyme in the production of dextrose syrup, a sample was drawn from 200 ml of the previously prepared solution, and the temperature was set at 60 °C. The pH was adjusted

to 4.3, and the solids concentration in the sample under study was kept constant. 50 µL of Optimax enzyme was added and incubated for 24 hours. Then the enzyme activity was stopped, and a sample was injected into the HPLC to see what degree of dextrose was reached. It is as shown in the table (8).



Peak Table (8)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	4.642	0.695	1.012	Polysaccharides
2	5.751	0.551	0.716	Maltotriose
3	6.483	2.790	2.873	Maltose
4	7.959	95.963	95.399	Dextrose
total		100	100	

DX = 95.963

Through the presented and previous results of this study from other studies, a higher glucose or dextrose sugar percentage was not obtained than that, and some of the reason for this is that the amyloglucosidase enzyme hydrolyzes the alpha-l, 4 bonds quickly, but during the cracking process, the alpha- bonds are formed. 1,6-amylopectin is highly branched and hydrolyzes more slowly than the other two. Therefore, the dose of Optimax enzyme is usually adjusted to obtain the highest percentage of sugar, cracking, or dextrose DX production, and this is done within approximately 24 to 48 hours. At the beginning of the cracking process, we find that the rate of dextrose formation is high, but it gradually decreases over time at the end of the reaction or process. This may be due in part to an increase in the production of branched dextrins in the solution and may also be because an increase in the dextrose concentration in the solution increases the inversion (repolymerization of the dextrose into isomaltose and other sugars). At a certain point of the reaction, the rate of inversion outperforms the rate of glucose formation and therefore if the cracking is not stopped at this point (maximum production of DX), the level of glucose or dextrose will begin to decrease gradually until it reaches the chemical equilibrium stage (which is about 85 % of glucose at 40% DS, 60°C).

The pullulanase enzyme was used with an amyloglucosidase enzyme such as Optimax at the beginning of saccharification or cracking, and thus the alpha-1, 6 bonds of branched dextrins are hydrolyzed more quickly than before. As a result, fewer sugars accumulate in the solution, especially branched oligosaccharides, after the end of the cracking process [26]. Thus the time required to obtain the maximum amount of dextrose DX depends on the dose of enzyme added. Also, the maximum amount of dextrose DX that can be obtained from this process is affected by the dry matter level of the reactant as well as the nature of the enzymes used. During the saccharification process, the amylose chain molecules and the partially hydrolyzed amylopectin chain are depolymerized by the enzyme glucoamylase which liberates the glucose units from the non-reducing chain end of the polymer [27].

It was found that the rate of hydrolysis by glucoamylase depends on the specific binding, as well as the size of the molecule that the enzyme hydrolyzes, as well as the order in which the α-1,4 and α-1,6 bonds are arranged. Glucoamylase hydrolyzes α-1,4 bonds very efficiently but at a much slower rate than α-1,6 bonds. An example is the rates of hydrolysis of 1,4-α, 1,6-α and 1,3-α bonds into tetrasaccharides in a ratio of 300:6:1. The enzyme glucoamylase also catalyzes the reverse reaction (reversion) whereby this enzyme fuses Dextrose or glucose molecules to form maltose sugar and isomaltose sugar. When the enzyme α-amylase and glucoamylase enzyme are used in sequence together, when heating sago starch, isomaltose is produced [28]. dextrose, with no isomaltose present in solution. Dextrose reflux specifically requires condensation of the β-anomer of Dglucopyranose with an α- or β-D-glucose molecule in the presence of glucoamylase. When increasing the incubation time, we find that the percentage of dextrose decreases, while the percentage of maltose and isomaltose increases, as indicated by the references previously.

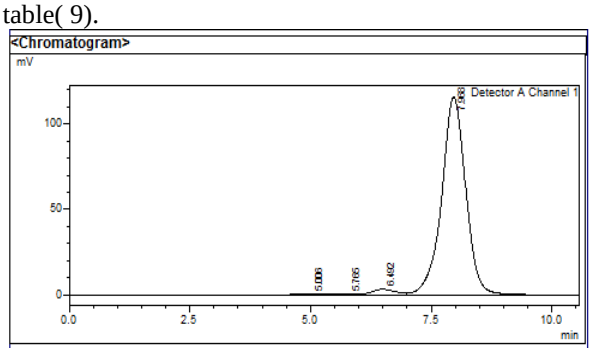
4.5.2. Preparation of dextrose by Extenda enzyme

When using Extenda enzyme to prepare dextrose syrup, all the previous parameters were provided so that the concentration of the enzyme was the only variable. Due to the activity of the Extenda enzyme, which is superior to the activity and efficiency of the Optimax enzyme, half of the added amount was added, 25 microliters of Extenda enzyme, and it was incubated for 24 hours as well.

After the expiration of the period, the activity of the enzyme was stopped by reducing the pH to less than 2, because during previous experiments it was found that this enzyme does not stop its activity by raising the temperature. And filtration, in which the glucose syrup must be higher than 50 ºC.

Therefore, its activity was stopped by reducing the pH to less than 2.

After that, a sample was injected into a device HPLC to know the amount of dextrose produced by this enzyme, and it was as in the following table(9).



Peak Table (9)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	5.006	0.740	0.805	Polysaccharides
2	5.765	0.522	0.677	Maltotriose
3	6.492	2.808	2.772	Maltose
4	7.986	95.929	95.746	Dextrose
total		100	100	

DX = 95.929

All references confirmed that the enzymes specialized in the production of dextrose syrup, which contain the enzyme glycoamylase, are characterized by complex action, as they reach the maximum value of conversion, and then collect dextrose molecules in the form of maltose or isomaltose, which is considered undesirable in the fructose industry or other industries It is based on dextrose syrup.

We previously referred to the Extenda Standard enzyme consisting of glucoamylase, pullulanase, alpha-amylase, and lysophospholipase.

Therefore, we find that the extenda enzyme, after reaching the maximum degree of conversion, also combined the dextrose molecules into isomaltose and thus reduced the total dextrose percentage.

Therefore, in both enzymes, the time at which the reaction is stopped must be determined in order to overcome this reaction problem and to reduce the production of isomaltose.

5. CONCLUSIONS

Through the results, it is clear that the Extenda enzyme produced by Novozymes. A promising enzyme, as we find that it has a high degree of efficiency and activity when compared to the Optimax enzyme, which is widely spread all over the world, especially in the production of specialized glucose syrup and the production of dextrose syrup, where we find that the Optimax enzyme takes longer to produce the same amount of dextose at the same time, operating conditions.

The ratio of dextrose when use the enzyme Optimax (95.963) was when incubated for 24 hours, while a similar amount of dextrose was obtained when using half the amount of Extenda and at the same incubation period for 24 hours.

The efficiency and activity of the Extenda enzyme was twice that of the Optimax enzyme, with almost half the amount being consumed to get the same results.

However, the Extenda enzyme needs further study and experimentation, because when it is deactivated in the traditional way, raising the temperature to more than 80 degrees Celsius, its activity does not stop, and this is not desirable in specialized glucose synthesis as the enzyme continued the cracking process. And glucose production, where the dextrose equivalent of the sample reached 52 when using the same amount of enzyme after raising the temperature to stop its activity. And when it is inactivated by lowering the pH below 2, this is also undesirable because it is a burden on the ion exchange phase, because the pH is lowered by hydrochloric acid.

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