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Comparison of Some Commercial Enzymes Used In the Production of High - Maltose Syrup From Corn Starch.

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Abstract

A comparison was made between two commercial enzymes (Optimalt BBA and Secura) which are used in the production of commercial high maltose syrup which is widely used in the food and chemical industries. The comparison included the reaction mechanism for each of the two enzymes and the time required to obtain high maltose. At constant concentrations of the reaction material 30% with the stability of the rest of the operating factors such as pH 5.3 and temperature 60, and the reaction material was free from any inhibitors or catalysts for enzymes, and the changes were followed through the results of the HPLC device by determining the percentage of high maltose syrup that is obtained It is applied to each enzyme to judge the efficiency of each enzyme in the production process, as well as the commercial advantage of each enzyme over the other. The results showed the superiority of the Secura enzyme in activity and efficiency over the Optimalt BBA enzyme, as the percentage of maltose in the syrup reached more than 80% maltose with the use of a smaller amount of enzyme and in a much less time than the time required for the Optimalt BBA enzyme.Which gives excellent superiority to the Secura enzyme in the production of very high maltose syrup. One enzyme in the fluidization phase, Spezyme Power LIQ, was used for both enzymes to ensure standardization of all parameters that might affect the results.

Keywords: high - maltose, Corn starch, Secura enzyme, Optimalt BBA enzyme.

1. INTRODUCTION

Starch polymer is the most common storage polysaccharide of glucose and maltose in plants and is a major carbohydrate in many industrial and biotechnological applications. Among the most important sources rich in starch are grains such as rice, wheat, corn, barley, and tubers such as potatoes and cassava. Starch is stored as water-soluble granules 0.1-10 mm in size in the plastids of photosynthetic plants. Starch consists of two main components:

Amylose and amylopectin, both of which are composed of α-D-glucose monomers. Amylose is a linear polymer of α-D-glucose linked by α-1, 4-glycoside bonds. Amylose makes up 15-25% of the starch polymer, while D-(+)-maltose is a glucose composed of two The glucose monomer that links with an α-1,4 glycosidic bond to form maltose, 4-O-α-D-glucopyranosyl-D-glucose ($C_{12}H_{22}O_{11}$), has a molecular weight of 342.3 D. Amylose exists in a helical form of 6 glucose residues in each turn of the helix. Amylose reacts highly with iodine which gives starch a dark blue color, and is a frequently used test for starch detection.

While amylopectin makes up 75-85% of the starch polymer, it consists of 1, 4-linked glucose monomers branched at the α-1.6 position. On average, amylopectin contains 4% of the α-1, 6 bonds at the branch points in the polymer. Therefore, the structural complexity of starch requires a combination of enzymes to break down the entire polymer into monosaccharides and disaccharides. Several known starch-olytic enzymes from plants and microorganisms are collectively called amylases, and they are of two types: α- and β-amylases.

α-Amylase is an endoglycosidase that has the ability to break α-glycosidic bonds anywhere along the starch polymer chain, whereas β-amylase

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enzymes can do so only at the non-reducing end of the starch polymer chain [1].

β-Amylases are enzymes that hydrolyze the α-bond, 1-4 glycoside in a starch polymer and in the polysaccharides attached to this bond. This enzyme is obtained from plants as well as microbes. β-Amylase is a member of the family of glycosylated hydrolases and catalyzes the hydrolysis of α-1, 4-glycosidic bonds starting at the non-reducing end of the polymer. The enzyme releases the sugar maltose as a reaction product and continues the reaction until the entire chain is broken or the enzyme encounters a blockage due to a physical or chemical irregularity in the chain.

Unlike many other enzymes, the term "β" does not refer to the bonds present in the reactant but rather to the formation reversal of the reaction product produced during β-amylase hydrolysis on the starch polymer [٢]. β-Amylase is also known to catalyze various less well-known reactions that follow other stereochemical pathways. Although the enzyme does not hydrolyze the sugar maltose [٣], it converts the β-anomer of the sugar maltose to the sugar maltotriose very little by condensation reaction, and this process reverses the hydrolysis of maltose [٤].

We also find that β-amylase catalyzes the slow, irreversible hydration of maltal, an enolic glycosyl donor that lacks the formation of either an α- or β-anomer to form β-2-deoxymaltose [٥].

The systemic name recommended by the International Union of Biochemistry and Molecular Biology (IUBMB) for β-amylase is "4-α-D-glucan maltohydrolase". Which depends on the type of reaction catalyzed by the enzyme and the type of reactant.

This enzyme also falls under a superfamily (EC number) assigned to β-amylase

Is 3.2.1.2 [3: hydrolase (class); 2: glycosylases (substrate catalysis) and 1: glycosidases (degradation of O- and S-glycosylation of compounds) and 2: serial number].

Many different types of juices containing maltose are used in many industries such as baking, fermentation, soft syrup, canning, confectionery and other industries. The most important functional properties of high maltose sugar syrup are low moisture, low viscosity in solution, crystallization resistance, low sweetness, low browning ability, as well as good thermal stability. Therefore, maltose-containing syrups can be used as moisture conditioners, anti-crystallizers, stabilizers for baits and carriers, and as fillers in some industries.

There are three important types of maltose-containing syrup:

(1) High-maltose syrup, (2) Very high-maltose syrup, and (3) Ultra high-maltose syrup over 80% [٦]. All of the above syrups also contain some maltotriose syrup.In this paper, an improved process is described for producing maltose-containing juices from starch substrates after liquefaction using high temperatures and by environmentally compatible high-temperature enzymes.

2. MATERIALS

2.1. Starch and chemicals.

The corn starch used was produced by the Al Monairy for Corn Products, 10 th 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. Optimalt BBA

Is an enzyme of 1, 4-α-Dglucan maltohydrolase (E.C.3.2.1.2) which is commonly referred to as beta-amylase. Which is extracted from soaked barley grains. Beta-amylase is an exoamylase that catalyzes the release or production of maltose sugar units by hydrolysis of the 1,4-α-D-glucosidic bond from the non-reducing end of the dextrins polymer chain. Optimalt BBA is essentially devoid of α-amylase activity which is used to produce maltose syrup from liquefied starch.

Also, this enzyme is compatible with the current purity specifications recommended for food enzymes presented by the Joint Expert Committee between the Food and Agriculture Organization and the World Health Organization regarding food additives (JECFA) as well as the Food Chemicals Codex

(FCC), which is GRAS, meaning that this enzyme is safe for food use (generally recognized as safe) in the United States.

Activity: 1230 DPº / g. Optimalt BBA barley β-amylase activity is expressed in diastolic force degrees (DP°). It is the amount of enzyme present in 0.1 mL of a 5% solution of the enzyme preparation sample that will produce sufficient reducing sugars to reduce 5 mL of Fehling's solution when the sample is incubated with 100 mL of reactant for 1 hour at 20°C (68°F).This enzyme is a proprietary product of Genencor International, USA.

2.2.3. Secura enzyme

Is a unique, microbial-sourced, thermostable beta amylase with a wide pH operating range. Secura enzyme is an exo-amylase that hydrolyzes alpha (1.4) - D-glucosidic linkages in starch polysaccharides for maltose production. This enzyme is a product of Novozymes (Novo Nordisk Denmark). Secura enzyme - Go straight to maltose syrup with the lowest risk of infection and lowest cost of conversion. Activity **5000** BAMU/g. This enzyme is a unique beta-amylase that is heat and pH tolerant in a wide range for the production of maltose sugar syrup.

It also bears a higher temperature in addition to a lower pH. All these things allow you to improve production parameters, as well as reduce the risks of bacterial infection and process costs. This enzyme is more stable during storage than beta-amylase enzymes from plant sources and thus results in smaller or more consistent and effective amounts used. It is also highly concentrated, resulting in smaller doses and smaller quantities for shipping, storage, and handling. Which provides a lot of additional commercial advantages. Gives the opportunity to work at higher temperatures (65 degrees Celsius)

As it is known, beta-amylase enzymes from plant sources lose their activity quickly when the temperature is above 60°C. However, this enzyme works well at 65 degrees Celsius and thus reduces bacterial infections during the process.

The high crushing temperature raises the yield of maltosugar in the process at the same crushing time and dosage. Also, the high cracking temperature reduces enzyme doses to achieve the same levels of maltose in the syrup.

2.2.4. Optimax® 4060 Vhp

Enzyme (Optimax) which is an enhanced combination of *fungal glucoamylase* produced from *Aspergillus niger*, and a bacteriostatic and pullulanase enzyme produced from a modified bacterial strain of *Bacillus licheniformis*.

And the enzyme Spezyme, which is α-amylase for the hydrolysis of starch.

When used to liquefy starch, the enzyme preparation has excellent stability against high temperatures and low pH and does not require the addition of catalysts such as calcium. Hydrolytic properties of Spezyme 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 were produced by Genencor International, USA.

3. METHODS

3.1. Prepare Dextrin Syrup from Corn starch

Alpha amylase (Spezyme® Powerliq) products are mainly used in the starch liquefaction process which is widely used in the dextrose industry. Starch is prepared first to which enzyme is added after solids and pH adjustments, Table (1). The warm starch slurry (near gelatinization) is then pumped under pressure into a direct injection steam jet cooker where the temperature is immediately raised to 108 ± 3 °C (226 ± 5 °F). The starches are broken down by the stove's heat and cut, and after the initial rise, the viscosity drops over the next 5-7 minutes. This first stage of cooking is necessary to remove the tough starch. The hot liquefied liquid is cooled to 96 ± 3 °C (205 ± 5 °F) and held in a second stage for an additional 120 ± 30 minutes during which DE rises above ~10. This compound formed in the second stage of cooking prevents the starch from decomposing.[ٷ]

Samples with different dextrose equivalent were prepared according to the requirements of the enzyme used in the preparation of high maltose syrup.

3.2. Preparation of Syrup High Maltose

High maltose syrup is prepared by enzymatic hydrolysis of liquefied starch (DE 10-20 may be used). After preparing the liquefied starch and converting it to dextrin and determining the dextrose equivalent of the sample, a 100 mL sample was withdrawn and placed in a 250-mL beaker. The temperature in the water bath was set at 60–65 °C, and the pH was set at 5.3–5.5 to suit both enzymes [8] . The sample was incubated for 2 hours.

The enzyme under study is added to the sample in the amount specified in accordance with the enzyme use sheet by the enzyme manufacturer.

Optimalt enzyme added 0.72 gm.

Secura enzyme added 0.144 gm.

After the end of the incubation period, the samples were filtered in a Buechner funnel on Whatman 4/6 paper, then the sample was prepared for injection in an HPLC machine to separate it and find out the shape and the percentage of sugars produced after that. Incubation for each enzyme.

3.3. Preparation of Syrup Very High-Maltose

High maltose syrup is prepared by enzymatic hydrolysis of liquefied starch (DE 10-20 may be used). After preparing the liquefied starch and converting it to dextrin and determining the dextrose equivalent of the sample, a 100-mL sample was withdrawn and placed in a 250-mL beaker. The temperature in the water bath was set at 60–65 °C, and the pH was set at 5.3–5.5 to suit both enzymes [8] . The sample was incubated for 4 hours.

The enzyme under study is added to the sample in the amount specified in accordance with the enzyme use sheet by the enzyme manufacturer.

Optimalt enzyme added 0.72 gm.

Secura enzyme added 0.144 gm.

After the end of the incubation period, the samples were filtered in a Buechner funnel on Whatman 4/6 paper, then the sample was prepared for injection in an HPLC machine to separate it and find out the shape and the percentage of sugars produced after that. Incubation for each enzyme.

3.4. Preparation of Syrup Ultra High-Maltose

High maltose syrup is prepared by enzymatic hydrolysis of liquefied starch (DE 10 may be used). After preparing the liquefied starch and converting it to dextrin and determining the dextrose equivalent of the sample, a 100-mL sample was withdrawn and placed in a 250-mL beaker. The temperature in the water bath was set at 60–65 °C, and the pH was set at 5.3–5.5 to suit both enzymes [9]. The sample was incubated for ٦ hours.

The enzyme under study is added to the sample in the amount specified in accordance with the enzyme use sheet by the enzyme manufacturer.

Optimalt enzyme added 0.72 gm.

Secura enzyme added 0.144 gm.

Optimax VP 4060 enzyme added 0.07 gm.

After the end of the incubation period, the samples were filtered in a Buechner funnel on Whatman 4/6 paper, then the sample was prepared for injection in an HPLC machine to separate it and find out the shape and the percentage of sugars produced after that. Incubation for each enzyme.

3.5. Analysis of Corn Syrup

Total dissolved solids in solution are estimated by a refractometer (Brix) as described in [10]. The dextrose equivalent (DE) is also calculated as the percentage of glucose syrup formed to the total dry matter [11]. The true dextrose equivalent (DX) was also estimated as shown by [12] through the following equation:

DX = % glucose x 1.0

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

% Polysaccharides × 0.15

The percentage of each component of glucose or maltose syrup (glucose, maltose, maltotriose and sugars) was calculated by conducting sample chromatography using HPLC Waters, SHIMADZU [13].

4. RESULTS AND DISCUSSION

4.1. Prepare Dextrin Syrup from Corn starch

It is known that 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 a non-specialist person, the term dextrose equivalent or DE can be considered an indication of the extent of the conversion process from starch polymer to dextrose. For professionals, a dextrose equivalent (DE) is the total reducing sugar contained in a glucose syrup. He cannot express how much glucose is in the syrup. The amount of dextrose in syrup can be measured along with other sugars using an HPLC (high-pressure liquid chromatography) machine [14]. The concentration of the starch solution was adjusted before the liquefaction process, as shown in Table (1).

Table (1). Standardization of starch milk before liquefaction.

	Parameters	Specified
1	PH	5.9
2	SO2	100 ≥ ppm
3	Total Protein	0.3
4	Cond	150
5	Be	18
6	Temperature	40 ºC
7	DS	30

The starch milk sample was prepared according to the instructions in the enzyme use. The starch milk solid was 30 %, 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, and 17].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 of figure (1).

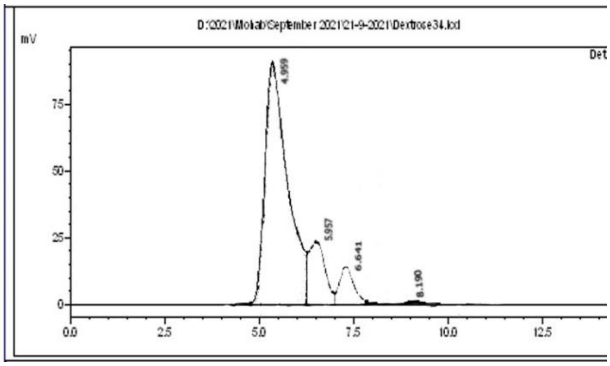

Figure (1) shows the shape of the HPLC curve at DE = 15

Peak Table of Figure (1)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	٤.٩٥٩	88.125	٨٥.٩٥١	Polysaccharides
2	٥.٩٥٧	6.806	8.147	Maltotriose
3	٦.٦٤١	٤.٤٥٠	٥.١٢١	Maltose
4	٨.١٩٠	٠.٦١٩	٠.٧٨١	Dextrose
total		100	١٠٠	

Another sample of 1 liter was prepared, and the dextrose equivalent was set at a low level of DE =12 as in figure (2), in order to prepare a sample of ultra maltose syrup. It is known that the higher the dextrose equivalent value, the lower the maltose production in the sample.

It is known that it is important to limit maltotriose formation when producing high and very high maltose hydrates.For example, [١٨,19,20] describes the use of a low DE in order to obtain maltose syrup of sufficient purity.

By using a low DE liquefaction material with a greater number of long glucose polymers, shorter glucose polymers and the consequent risk of generating odd-numbered polymers, resulting in increased maltose content, are reduced.A DES level as low as 0.5 has been described to produce very high maltose syrup.Unfortunately, working with low DE liquefaction is difficult due to its high viscosity and high risk of rollback.

Regraded starch is resistant to hydrolysis by conventional malto-saccharification enzymes resulting in iodine-positive maltose syrup (commonly called “blue bag”).Iodinated maltose syrup is not widely accepted in commerce due to filtration problems associated with processing that affect the quality of the final product.To partially overcome these problems, the United States obtained a patent. [21,22,23] also describes the use of a low dry solids (DS) content. Unfortunately, low dry solid content has its own limitations. Such as large saccharification volumes required and high energy input during evaporation.Theoretically, using a liquefiant with a DE close to zero will result in the highest possible maltose content in the malto saccharification. This is because low DE limits the number of glucose polymers with an odd number of glucose residues as possible, resulting in the highest level of maltose. However, in practice, such a low level of DE is not possible with historically used conventional processes that include a liquefaction step.

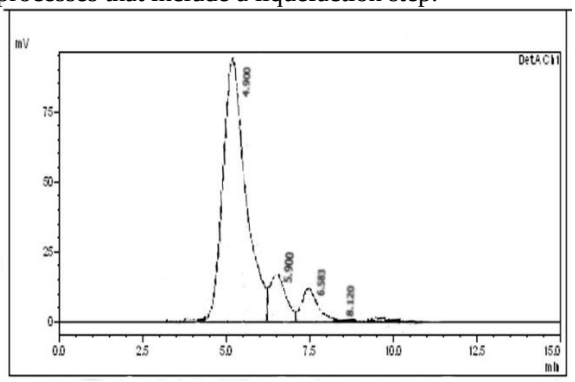

Figure (2) shows the shape of the HPLC curve at DE = 12

The methods used to determine DE dextrose equivalent values in the maltodextrin range all have certain advantages and disadvantages. Depending on the method and columns used, quantitative and qualitative separation of oligosaccharides up to DP = 12 is possible using HPLC techniques. Also, problems emerged in the recovery and quantification of oligosaccharides with a higher degree of polymerization. The large out-of-band peaks of particles with higher molecular masses of the polymer also create problems in their quantification as well as in their contribution as part of the dextrose equivalent value DE determined by [24]. In addition there are some solutions of maltodextrin with a lower DE value of dextrose equivalent (DE = 5) and some insoluble polymer carbohydrates (dextrin fractions) may be present. Injection of such fractions into the HPLC machine is not recommended because they will not be quantitatively recovered or even eliminated. Although this can be avoided by diluting the samples, concentrations of less than 10% of the injected solution are not recommended because the detection of sugars in this case becomes more difficult because they represent only a small part of the total solids in the solution [25]. When conducting an analysis on an HPLC device, it is necessary to take into account all the facts mentioned.

4.2. Preparation of Syrup High Maltose

After dextrin solution was prepared and dextrose equivalent (DE = 15) was determined, a 100 mL sample was drawn and placed into a 250 mL beaker.

The temperature in the water bath was set at 60 °C, and the pH was set at 5.3 to suit both enzymes. The sample was incubated for ٢ hours. The specified amount of enzyme was added according to the instructions of the operation sheet for each enzyme to achieve the best results.

After the end of the incubation period, samples were filtered and dextrose equivalent was measured by HPLC.

4.2.1. Optimalt BBA enzyme.

The first sample was prepared using Optimalt BBA enzyme. Where 0.72 g of enzyme was added at the time of starting the reaction. The concentration of the reactant was 30 %. I took a sample in the middle of the incubation time to follow the speed of the enzyme in the conversion process. The results were as shown in Figure (3) and Table (3). The percentage of maltose reached 21.4, while the percentage of dextrose was still high, reaching 18.26.

This enzyme was stable and stable during the incubation process, and the conversion process was rather slow from polysaccharide to maltose, as shown in the figure (3).

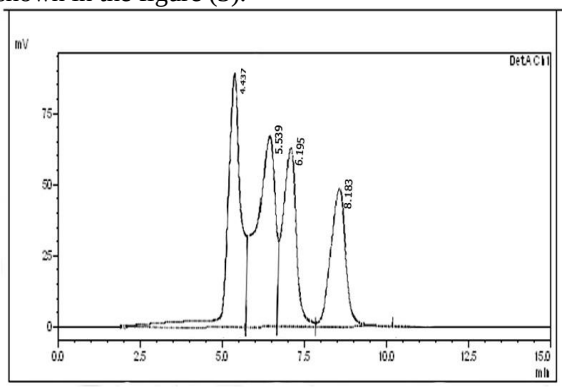

Figure (3) shows the shape of the HPLC curve at DE = 47.17

Peak Table of Figure (3)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	٤.٤٣٧	٣٠.٢٥٢	٣٣.١٨٨	Polysaccharides
2	٥.٥٣٩	٢٩.٩٩٨	٢٥.٠٨٢	Maltotriose
3	٦.١٩٥	٢١.٤٨٢	٢٣.٥٨٨	Maltose
4	٨.١٨٣	١٨.٢٦٨	١٨.١٤٢	Dextrose
total		100	١٠٠	

After the end of the incubation time, the sample was filtered and prepared for injection into the HPLC device.

The percentage of maltose was 47.7 after the end of the incubation period, as shown in the figure (4) and table (4), where it was noted that the level of maltose in the syrup was also significantly affected by the DE value of the liquefied starch.

The results indicate that this enzyme requires a time of more than two hours to raise the percentage of maltose to 50% or more, which proves that it is slow in conversion when compared to the other enzyme.

It was also found that the percentage of maltose is also affected by the percentage of solid matter in the sample, so it was set at 30% for best results. Therefore, if the DE value is reduced to 10 [26], the percentage of maltose in the sample is likely to increase because if the value of the dextrose equivalent in the sample is increased, the amount of maltose decreases to increase dextrose in the solution at the expense of the percentage of maltose.

In order to obtain a very high maltose syrup, the alpha amylase used in liquefaction must be inactivated in what is called the alpha killing step US Patent Pub. [27].This is generally done by passing the liquefied starch through a second jet cooker at very high temperatures.As well as changing the pH of the reaction medium and the temperature.During liquefaction, alpha-amylase hydrolyzes starch.Through each enzyme hydrolysis, one C-1,4-glycosidic bond in the starch molecule is broken resulting in the formation of 2 smaller glucose polymers, thus forming an additional reducing end and an additional non-reducing end.During malto saccharification, beta-amylase releases successive maltose molecules from the non-reducing end of glucose polymers formed during hydration.

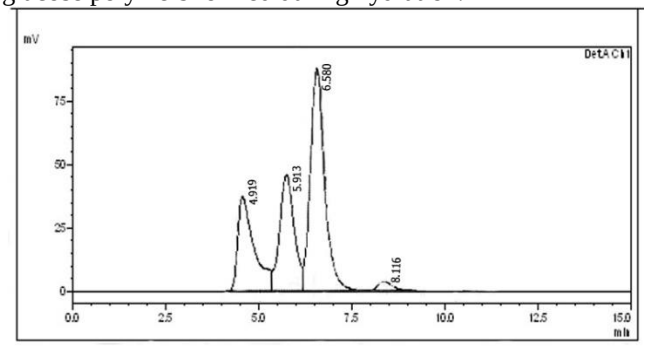

Figure (4) shows the shape of the HPLC curve at DE = 43.7

ak Table of Figure (4)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	4.919	24.027	٢١.٦٥٣	Polysaccharide
2	5.913	٢٦.١٤٨	٢٦.٤٨٨	Maltotriose
3	6.580	٤٧.٧١٤	٤٩.٧٢٦	Maltose
4	8.116	٢.١١٠	٢.١٣٣	Dextrose
total		100	١٠٠	

4.2.2. Secura enzyme

The second sample is prepared with a Secura enzyme. Where 0.144 g of enzyme was added at the time of starting the reaction. The concentration of the reactant was 30%. I took a sample in the middle of the incubation time to follow the speed of the enzyme in the conversion process. The results were as shown in Figure (5) and Table (5). Where the percentage of maltose reached 35.8, while the percentage of dextrose was low compared to the sample of the Optimalt enzyme, which reached 1.5.

This enzyme was characterized by high conversion efficiency during the incubation process, as shown in the figure (5). Which predicts the advantage of this enzyme in the preparation process in terms of reaction speed, efficiency, and stability.

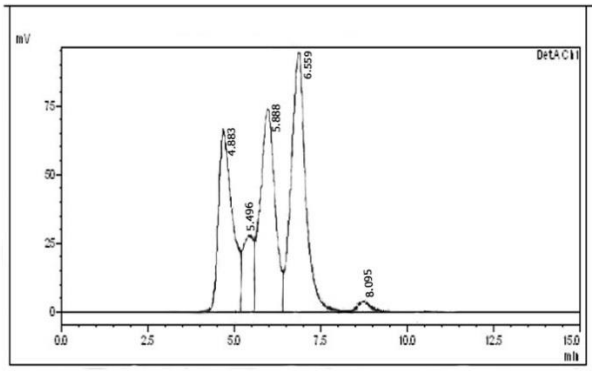

Figure (5) shows the shape of the HPLC curve at DE = ٣٨.٩٨

Peak Table of Figure (5)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	٤.٨٨٣	٢٥.١٠٩	٢٤.٩٧٨	Polysaccharides
2	٥.٤٩٦	٨.٠٧٥	١٠.٦١٠	
3	٥.٨٨٨	٢٩.٤٣٧	٢٧.٧٤٥	Maltotriose
4	٦.٥٥٩	٣٥.٨٦٤	٣٥.٢١٢	Maltose
٥	٨.٠٩٥	١.٥١٥	١.٤٥٥	Dextrose
total		100	١٠٠	

After the end of the incubation time, the sample was filtered and prepared for injection into the HPLC device.

The use of Secura enzyme is required to completely stop the activity of the alpha-amylase enzyme used in the starch liquefaction process.

This is because not stopping the alpha-amylase enzyme will lead to the production of more glucose at the expense of maltose [28]. The results confirm this, as in figure (6) and table (6) there is an absence of dextrose, whose percentage in the sample was about 0.6, while the percentage of maltose reached 53.7, which is a clear difference in the conversion efficiency between both enzymes, despite the provision of the same working conditions for both.

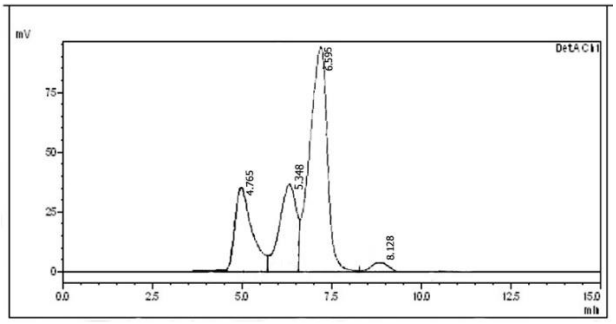

Figure (6) shows the shape of the HPLC curve at DE = ٤٨.٤

Peak Table of Figure (6)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	٤.٧٦٥	٣٣.٤١٨	٣٤.٦٠١	Polysaccharides
2	٥.٣٤٨	١١.٢٤٨	١١.١٦٧	Maltotriose
3	٦.٥٩٥	٥٣.٧١٢	٥٣.٤٧٣	Maltose
4	٨.١٢٨	٠.٦٢١	٠.٧٥٩	Dextrose
total		100	١٠٠	

4.3. Preparation of Syrup Very High Maltose

For the preparation of high maltose syrup in which the percentage of maltose is more than 60%.

After preparing the dextrin solution and determining the dextrose equivalent (DE = 12), the second sample of dextrin prepared with reduced dextrose equivalent will be used.

A 100 mL sample is withdrawn and placed in a 250 mL beaker. The temperature in the water bath was set at 60 °C, and the pH was set at 5.3 to suit both enzymes.

In this experiment, the time factor will be doubled, i.e. the incubation time will be incubated for 4 hours.

Each enzyme in the sample was added in the quantities previously determined at the beginning of the reaction. After the end of the incubation period, samples were filtered and dextrose equivalent was measured by HPLC.

4.3.1. Optimalt BBA enzyme

Optimalt enzyme was used with the second sample of dextrin, and the time was doubled to 4 hours to obtain a higher percentage of maltose syrup in the sample under the same operating conditions described previously.

Samples were drawn at regular intervals to monitor the percentage of maltose in the sample by injecting it into the liquid chromatography apparatus.

After the end of the incubation period, the percentage of maltose was 62.4 % as shown in the figure (7) and table (7) and what was confirmed in this process is that with the increase in the percentage of maltose in the syrup, the enzyme activity began to decrease at

the same concentration of the enzyme and the reaction product.

This indicates that this enzyme is affected by the concentration of the product (maltose) in the syrup, and thus the conversion rate decreases at 50% of the maltose, after which the reaction curve decreases to the bottom as a result of the increase in the percentage of maltose [29]. Rather, a decrease in the amount of maltose may occur at a certain limit so that the resulting maltose units are linked with glucose to be maltotriose.

Therefore, the alpha amylase enzyme must be stopped before the reaction begins, as mentioned earlier.

The effect of the dose of OPTIMALT BBA on the dissolution of granulated starch in addition to the effect on the composition of the sugar resulting from hydrolysis at 60°C with doubling time.It was found that the degree of solubility of starch increased significantly compared to the first sample, as the filtration efficiency of the solution was better and at a higher rate. The degree of solubility ranged from 87.6 to 89.1%.The DP2 content increased at the same dose and the DP3 content decreased with increasing incubation time DP3+ is particularly reduced early in the reaction and DP1 is practically unaffected.This rapid decrease in DP3+ content demonstrates that beta-amylase rapidly hydrolyzes DP3+ released from granulated starch by the action of alpha-amylase.

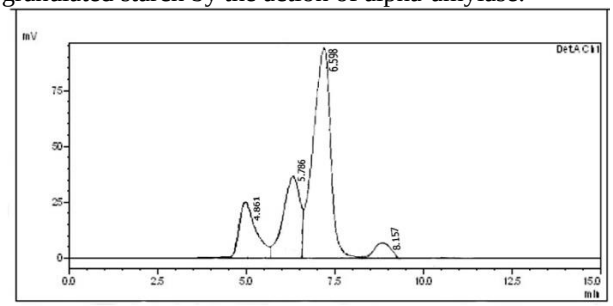

Figure (7) shows the shape of the HPLC curve at DE = ٤٨.٥

Peak Table of Figure (7)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	٤.٨٦١	13.627	14.753	Polysaccharides
2	٥.٧٨٦	٢٢.٨٧٩	٢٢.٤٧١	Maltotriose
3	٦.٥٩٨	٦٢.٤٧٨	٥٨.٤٦٣	Maltose
4	٨.١٥٧	1.094	١.٣١٣	Dextrose
total		100	١٠٠	

4.3.2. Secura enzyme

The second sample was prepared using Secura enzyme, where the incubation time was doubled for the previous sample to become 4 hours with the same amount of enzyme and reaction material added. The process was carried out under the same previous conditions of temperature and pH. They were incubated in a water bath at 60 °C. Samples were drawn at regular intervals to monitor the rate of conversion from dextrin to maltose. The conversion rate was increasing and stabilizing during the incubation period. The required percentage of maltose was reached 3:30 an hour, as the percentage of maltose was 66.9% as shown in the figure and table (8), it was also noted that the degree of enzyme activity decreased by increasing the resulting substance (maltose) was less than the decrease in Optimalt enzyme activity. Which suggests that the enzyme extracted from microbiological origin is more stable than the enzyme extracted from plant origin. Instead, when the temperature was raised to 65 °C, it was observed that the Optimalt enzyme began to stop the conversion process, while the Secura enzyme continued to be active in the conversion rates. This may be an advantage of increasing enzyme activity, or it may be a disadvantage, as this may force us to raise the temperature above 80°C to inactivate the enzyme in production lines.

It is also noted that the optimum pH of this enzyme ranges in a wider range than that of Optimalt, which makes it better in terms of operating conditions, from liquefaction to full production.

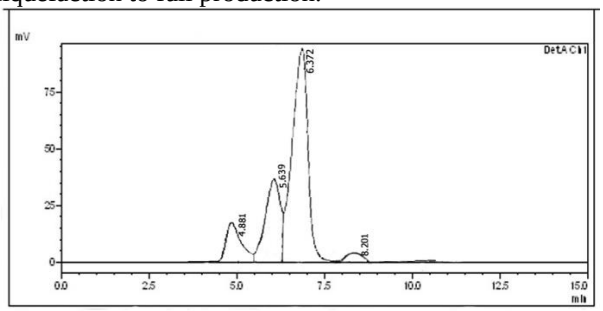

Figure (8) shows the shape of the HPLC curve at DE = ٤٩.٩

Peak Table of Figure (8)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	4.881	٩.٧٠٣	١٠.٣٨٦	Polysaccharides
2	٥.٦٣٩	٢٢.٥٩٧	٢٢.٥٥٥	Maltotriose
3	٦.٣٧٢	٦٦.٩٨١	٦٦.٢١٦	Maltose
4	٨.٢٠١	٠.٧١٩	٠.٨٤٣	Dextrose
total		100	١٠٠	

4.4. Preparation of Syrup Ultra High Maltose

To prepare a high-maltose syrup containing 80% or more maltose, another enzyme must be added to de-branch the alpha 1-6 glucose bond. This is because

the beta-amylase enzyme hydrolyzes the alpha 1-4 glucose bond.

During malto saccharification, beta-amylase releases successive maltose molecules from the non-reducing end of glucose polymers formed during hydration.The action of alpha-amylase during liquefaction creates many sites for beta-amylase to attack hydrolyzed starch.Beta-amylase cannot hydrolyze C-1,6' glycosidic bonds and its action will stop at or near branch points.The branching enzyme (such as pullulanase) hydrolyzes the C-1,6'-glycosidic bonds, thus facilitating a more complete hydrolysis of liquefied starch by beta-amylase, an effect well known in industry.When the branch-bond-breaking enzyme hydrolyzes the C-1,6-glycosidic bond, it creates only one additional reducing end, not a non-reducing end.

There may be more than one theory to explain this process, the most important of which are the following:

The final production of β-amylase is good, but there is considerable debate as to whether the enzyme hydrolyzes single-chain or multi-chain linear starch molecules (amylose) [30]. The enzyme completely hydrolyzes one reactant molecule before it attacks the second molecule, in the single chain mechanism. In the multi-chain mechanism, the enzyme, after removing the maltose unit from the substrate, attacks the second-chain molecule, causing all polymer molecules to break down one way or another.

In general, validating or against this theory has led to the conclusion of some methods for estimating the change in the mean DP (degree of polymerization) of the reactant. A narrow DP score distribution with no change in the overall DP score of the substrate indicates that the mechanism is either single-chain or multi-chain. Instead, the multichain mechanism proceeds through the decrease in the average degree of DP polymerization of the substrate during the reaction. A method for estimating the degree of DP polymerization of a substrate was generally derived from iodine spectrometry or from physical methods [31].

In this sample, dextrin solution was prepared with dextrose equivalent less than the previous samples, DE = 12.

This is due to the fact that the dextrose equivalent negatively affects the maltose content at the end of the reaction, so a sample was prepared with a low dextrose equivalent as the higher the maltotriose content. Another type of enzymes was added to remove the branches in the amylopectin polymer, to break down maltotriose into maltose and glucose, and to accelerate and facilitate the conversion of enzymes to produce maltose syrup.

4.4.1. Optimalt enzyme

The first sample was prepared using Optimalt enzyme with the addition of Optimax enzyme. The sample was incubated at a temperature of 60 ° C and pH = 5.3, the same amount of Optimalt enzyme was added as before with the addition of 0.07 gm. of Optimax enzyme. The sample was incubated under the aforementioned conditions for 6 hours, with samples being drawn continuously at regular intervals to follow up the conversion process.

After the end of the incubation period, the sample was filtered and prepared for injection into the HPLC device. After 6 hours, the percentage of maltose in the sample reached ٧١.67 % maltose Figure (9) and Table (9). This confirms that despite the lengthening of the incubation period and the addition of an enzyme to remove the branches, the occurrence of inhibition due to the increase in the reaction product affects the efficiency and activity of this enzyme despite its stability at the beginning of the reaction [32].

It was also noted that the percentage of dextrose in the previous samples was higher than in the samples prepared with Secura enzyme.

There is a clear relationship between the dose of alpha amylase and DP2, DP3, and DP3+.For solubility, there is a trend toward decreased solubility at a higher dose of alpha-amylase.The relationship between sugar composition and alpha-amylase dose is not affected by the pullulanase dose in the range of 0.5-3.0.The data also shows that the ratio of beta-amylase over alpha-amylase units is important.

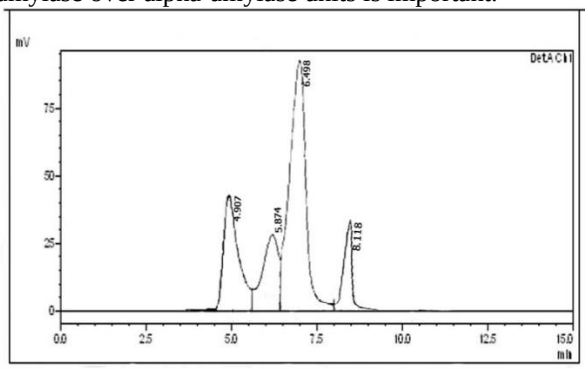

Figure (9) shows the shape of the HPLC curve at DE = ٥٤.٩٩

Peak Table of Figure (9)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	٤.٩٠٧	١٢.٠٠٥	١١.٩٤٥	Polysaccharides
2	٥.٨٧٤	٧.٧٨٤	٨.١٩٧	Maltotriose
3	٦.٤٩٨	٧١.٦٧٥	٧٠.٦٨٩	Maltose
4	٨.١١٨	٨.٥٣٦	٩.١٦٩	Dextrose
total		100	١٠٠	

4.4.2. Secura enzyme

In the second sample, the Secura enzyme was used to prepare it, with the addition of Optimax enzyme to remove the branches from amylopectin, where the same previous quantities of each enzyme 0.144 gm of Secura enzyme were added with the addition of 0.07 gm of Optimax enzyme. It was incubated for 6 hours. The temperature was set at 60 °C and the pH at 5.3. Samples were incubated and drawn at regular intervals to follow the transformation process.

It was observed that the increase in conversion rates was more efficient and active with Secura enzyme also in this experiment. After the end of the incubation period, the sample was filtered and prepared for injection into the HPLC device, where the proportion of maltose in the sample reached 82.9. Figure (10) and table (10) show these results. As the percentage of maltose in the syrup is related to the percentage of solids in the sample from the beginning of preparation. The higher the percentage of water in the sample, the greater the potential for converting sugars into maltose. In this case, the sugars have been converted to maltose, and the maltotriose has been converted to maltose, as the percentage of maltose in the syrup increases at the expense of both the maltotriose and the sugars. Because an enzyme was used to remove the branches, the dextrose content in the experiment was also increased to 6% [33].

When comparing the efficiency of two enzymes when consuming the same quantities of enzymes in the preparation processes, the efficiency of the Secura enzyme in conversion and reaction speed was demonstrated on the Optimalt enzyme. Even when an equivalent concentration of both enzymes was used when other factors such as temperature, pH, and reactant concentration were stabilized, the Secura enzyme was favored in terms of stability in a wide range of temperature and pH.

This may be due to the difference in the source of both enzymes, as it was found that the Optimalt enzyme is clearly affected in the degree of its activity when the pH or even the temperature changes, which requires strict control of these parameters. It is also noted that the Secura enzyme is more pure and higher in concentration, and this may be due to modern technologies in its production from bacteria.

The Secura enzyme can withstand operation at temperatures higher than 65 degrees Celsius, while beta-amylase products from plant sources quickly lose their activity when the temperature is higher than 60 degrees Celsius.

This reduces bacterial contamination during production and can reduce CIP cycles. Also, a higher saccharification temperature results in a higher maltose yield, with the same time and dose [34]A higher cracking temperature reduces enzyme doses to achieve the same maltose levels.

Beta-amylase products from plant sources perform poorly at pH below 5.0.

While the Secura enzyme maintains its activity at a pH of (4.5 - 6.0). Plant amylase enzymes have an optimum temperature of 65°C maximum but microbial enzyme can exceed 75°C.

The range is mostly between 50-65°C for both plant and bacterial enzyme.

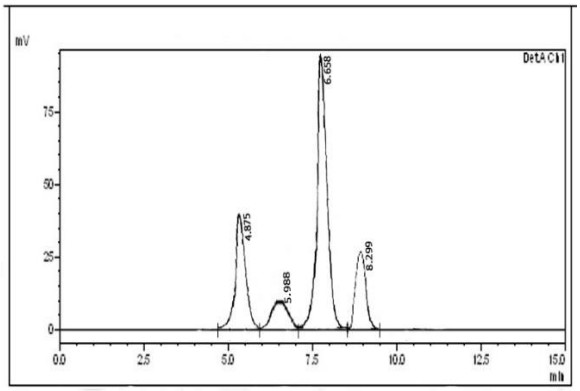

Figure (10) shows the shape of the HPLC curve at DE = ٥٦.٦

Peak Table of Figure (10)				
Detector A Channel 1				
Peak	R.Time	Area %	Height%	Name
1	4.875	٨.٣٠١	٨.٩٧٦	Polysaccharides
2	٥.٩٨٨	٢.٥٧٣	٢.٤١٢	Maltotriose
3	٦.٦٥٨	٨٢.٩٠٨	٨٣.٠٠١	Maltose
4	٨.٢٩٩	٦.٢١٨	٥.٦١١	Dextrose
total		100	١٠٠	

Table (12) .The most important aspects of the difference between the two enzymes.

		Secura®	Optimalt® BBA
1	Temperatures	55 - 65<	55 - 60
2	PH	4.5 - 6	5.2-5.5
3	D.S	35 – 40 %	30 – 40 %
4	Glucose %	1>	2 >
5	Maltose	85<	70<
6	Concentration	5000 u/g	1230 DP°/g
7	Dosing/ton metric	0.07- 0.9 kg	0.12 -0.36 kg
8	DE Starting	11	11- 20
9	Source From	microbial sources	plant sources

*The enzyme alpha amylase produced by liquefaction is completely inactivated.

5. CONCLUSIONS

Through the results of the research, the superiority of the secura enzyme in performance, stability and speed of conversion from the optimalt enzyme was shown, as the percentage of maltose in the first sample of the secura enzyme reached 47.7%, while it reached more than 50% in the sample of the secura enzyme at the same operating conditions and in a time less than about 20 % of the estimated time.

The maximum percentage of maltose when adding the branching enzyme to the optimalt enzyme sample reached 71.6% after the end of the incubation period, while the other sample of Lysecura enzyme reached more than 80% maltose.

It is clear that this enzyme was designed to get rid of the defects of enzymes extracted from plant origin, as we find that this enzyme works in a wider range of temperatures 55 - more than 65, as well as in a wider range of pH, as it works efficiently at 4.5 - 6, which allows greater flexibility in operations.

It was also shown a significant decrease in the activity of the Optimalt enzyme when the percentage of maltose reached more than 50%, while the decrease in activity at the same level of maltose was much less, and the enzyme retained its stability and activity to some extent.

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