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The Effect of Temperature, pH, and Enzyme Activity on the Retrogradation Phenomenon in Dextrin Solution

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ABSTRACT

Low-Dextrose Equivalent dextrin solutions (DE<20), consisting of short glucose chains, are initially clear but tend to become hazy during storage due to amylose retrogradation. This study evaluated the effects of storage time, temperature, pH, and *α*-amylase enzyme type on the clarity of dextrin DE 12 solutions. Storage temperatures of 30, 50, and 70 °C were examined over 24 h, while pH levels of 4.0, 4.5, and 5.0 were applied to assess their effects on the retrogradation behavior of the solutions. As a result, samples stored at 70 °C had the highest clarity, indicating suppression of amylose reassociation despite the presence of longer glucose chains. Among the pH conditions tested, pH 4.0 resulted in the clearest solution, suggesting enhanced sensitivity of retrogradation to acidic conditions. Furthermore, the use of a newly developed high-activity enzyme significantly improved solution clarity and reduced haziness compared to the conventional enzyme. This enzyme also enabled a 50% reduction in enzyme dosage and a 40% increase in production capacity, highlighting its effectiveness in controlling retrogradation while improving process efficiency. These findings provide novel insights into the relationship between glucose chain characteristics, amylose retrogradation, and clarity stability in low-DE dextrin solutions.

Keywords: amylose, clarity, glucose chain, hazy, retrogradation

INTRODUCTION

Dextrin solution is a product of starch hydrolysis that contains glucose and complex oligosaccharides, with a low Dextrose Equivalent (DE) < 20% (Liu *et al.,* 2021). Glucose, the main component of dextrin solutions, plays an important role in the food industry, where its physicochemical properties, such as sweetness and viscosity, are influenced by chain length and DE. A lower DE value indicates longer glucose chains and gives a more viscous solution (Abduh *et al.,* 2023).

Dextrin solution is used as a raw material to produce maltodextrin powder *via* spray drying. However, a common issue that occurs in dextrin solutions is the development of haziness during storage, which is caused by the retrogradation process, the reformation of hydrogen bonds between glucose molecules, particularly within amylose chains (Ali *et al.,* 2016). The lower the DE value, the greater the potential for haziness formation. Haziness reduces product quality, especially in applications that require clarity. This condition represents a significant challenge in commercial production because hazy dextrin solutions may not meet customer quality specifications and can therefore be rejected. Product rejection due to haziness affects not only customer acceptance but also operational challenges in the production process. Internally, hazy products often require rework to restore their quality before they can be further processed or delivered to customers. This additional rework increases processing time, energy consumption, and production costs, ultimately reducing overall manufacturing efficiency. Therefore, given the negative impact of haziness on product quality, customer acceptance, and production efficiency, inhibiting retrogradation and preventing haziness formation in dextrin solutions are essential for maintaining product stability and ensuring efficient production processes.

The alpha-amylase enzyme is commonly used in hydrolysis to break glycosidic bonds. Variations in enzyme activity can influence the resulting glucose chain length, thereby affecting the solution's stability (Motta *et al.,* 2023). Differences in enzyme activity are expected to alter the pattern of glycosidic bond cleavage along the glucose chain, which in turn affects the rate and extent of haze formation in dextrin solutions (Zhao *et al.,* 2015).

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Storage temperature is one of the key factors influencing the retrogradation process in dextrin solutions. Higher temperatures tend to slow down the formation of hydrogen bonds between glucose chains, thereby delaying the onset of haziness in the solution (Caccamo and Magazù, 2023). Conversely, lower temperatures accelerate retrogradation, causing the solution to become hazy more quickly (Chen *et al.,* 2024). In this study, storage time and temperature were used as variables to evaluate the retardation of retrogradation.

pH also plays an important role in the retrogradation of dextrin solutions. Under acidic or alkaline conditions, retrogradation tends to slow down due to changes in the ionization of hydroxyl groups, which inhibit the formation of hydrogen bonds between glucose chains. Conversely, at neutral pH, the retrogradation process proceeds more rapidly, increasing the risk of haziness in the solution (Chou *et al.,* 2020).

When dextrin solution undergoes retrogradation and becomes hazy, production must be halted to maintain product quality. The process line needs to be flushed with either product or water to obtain a clear solution. This results in partial inefficiency or start-stop production, where operations are temporarily suspended until the previous batch of solution has been processed into powder. Consequently, the glucose line's production capacity cannot be fully utilized, reducing production efficiency and negatively impacting the company's profitability.

Therefore, this study is important to evaluate the effects of temperature, pH, and enzyme activity on the clarity and stability of dextrin solutions. The objective of this research is to inhibit retrogradation by maintaining specific elevated temperatures, adjusting the pH of the dextrin solution, and using *α*-amylase with higher enzymatic activity.

MATERIALS AND METHOD

Materials

The raw materials used in this study were tapioca starch and enzymes obtained from a commercial supplier. Supporting materials used for the filtration process included filter aid, activated carbon, and resin. The equipment used consisted of an analytical balance (GF-2000, AND, Japan), hotplate (C-MAG HS 7, IKA Magnetic Stirrers, Germany), refractometer (Abbe Refractometer NAR 3T, Atago, Japan), Spectrophotometer UV-Vis (Geneys 30, ThermoFisher Scientific, China), pH meter (pH700, Eutech, India), viscometer (ROTAVISC lo-vi, IKA Viscometer, Germany), incubator (UF30, Memmert, Germany), and other supporting instruments. Glucose composition analysis was performed using a high-performance liquid chromatography (HPLC) system (HPLC 1260 Infinity 1, Agilent, Germany) available at the supplier's laboratory.

Methods

Tapioca starch was hydrolyzed to produce a 60% (w/w) dextrin solution with a dextrose equivalent (DE) of 12. Two commercial *α*-amylase enzymes were used: Type 1 enzyme with an activity of 300 KNU/g and Type 2 enzyme with an activity of 562 KNU/g. Both enzymes contained Type S (*Bacillus stearothermophilus*) and Type L (*Bacillus licheniformis*) subtypes, with Type S dominating in Type 1 and Type L dominating in Type 2.

A preliminary storage test was conducted by incubating dextrin solutions hydrolyzed with each enzyme at 30, 50, and 70 °C for 24 h. Observations were made at one-hour intervals to assess the effects of enzyme type and incubation temperature on retrogradation behavior. At each observation time, changes in solution clarity were measured using a spectrophotometer, and viscosity was determined using a viscometer as indicators of dextrin retrogradation.

A laboratory-scale experiment was conducted to compare the retrogradation behavior of dextrin solutions produced with Type 1 and Type 2 enzymes across different pH levels. Starch was accurately weighed using an analytical balance and mixed with distilled water to obtain a 20 °Bé suspension. The starch suspension was heated on a hotplate until complete gelatinization was achieved, after which the enzyme was added to initiate hydrolysis. Following hydrolysis, the mixture was filtered to obtain clear dextrin solutions, which were subsequently adjusted to pH 4.0, 4.5, and 5.0 and measured with a pH meter. After the dextrin hydrolysates reached a dextrose equivalent (DE) of 12, samples were taken to measure the sugar profile using HPLC.

Retrogradation was evaluated by measuring solution clarity at 600 nm with a UV–Vis spectrophotometer and by measuring viscosity. The data were analyzed comparatively to determine the effects of enzyme type, temperature, and pH on the retrogradation rate of dextrin solutions. The detailed experimental procedures are described in the following sections.

Differences between Type 1 and Type 2 enzymes used

The enzyme used in the first observation was Type 1, with an enzymatic activity of 300 KNU/g. Type 1 enzyme contains two subtypes: Type S, derived from *B. stearothermophilus*, and Type L, derived from *B. licheniformis*. The composition of Type 1 enzyme is dominated by Type S compared to Type L. The enzyme used in the subsequent experiment was Type 2, with an enzymatic activity of 562 KNU/g and the same enzyme subtypes, but with a higher

proportion of Type L than Type S. Type 2 enzyme exhibits higher enzymatic activity, which influences the cleavage position along the glucose chain.

Furthermore, the cleavage of glucose chains is influenced by the specific characteristics of each enzyme. Type S enzyme is derived from *B. stearothermophilus*, while Type L enzyme originates from *B. licheniformis*. The hydrolytic activity of the Type S enzyme is more moderate, with a slower cleavage process (Hu *et al.* 2023). In contrast, the Type L enzyme exhibits higher catalytic activity, enabling it to cleave more glycosidic bonds in a shorter period. This is reflected in the higher enzymatic activity of the Type 2 enzyme, which contains a higher proportion of Type L than the Type 1 enzyme.

In terms of cleavage site preference, Type S enzymes act randomly but tend to cut near the ends of linear chains, resulting in longer residual chains. Meanwhile, Type L enzymes tend to cleave closer to the middle of the chain (at random internal sites), thereby producing smaller fragments (Fan *et al.,* 2024).

Regarding the amylopectin structure, the Type S enzyme cleaves randomly, but more frequently near the ends of linear chains, thus leaving longer-chain residues (Janeček and Gabriško, 2016). In contrast, the Type L enzyme is more effective at hydrolyzing linear *α*-1,4 chains, particularly near the ends of branched chains, thereby allowing the amylopectin structure to remain relatively intact. In terms of fragment production, Type S enzyme generates a greater number of oligosaccharides with relatively longer chain lengths, whereas Type L enzyme produces more short-chain oligosaccharides while maintaining the amylopectin structure relatively intact.

Production samples with enzyme Type 1 and Type 2 and storage at different temperatures

Dextrin solution samples with a target Dextrose Equivalent (DE) of 12 were collected directly from the production process under normal operating conditions, without modifying any existing process parameters or production settings, ensuring the samples reflected actual production conditions. The experiment was conducted using a 60% (w/w) dextrin solution, measured with a refractometer, produced from tapioca starch *via* hydrolysis with two types of enzymes, namely Type 1 and Type 2. The resulting dextrin solutions were stored in an oven at 30, 50, and 70 °C. Observations were conducted at 1-hour intervals over a total storage period of 24 hours to evaluate changes in the dextrin solution. The experiment was designed to evaluate the effects of storage temperature and enzyme type on the rate of retrogradation and to compare the two enzymes' ability to maintain the stability of the dextrin solution under different storage conditions.

This experiment was conducted in the initial phase of the study, after completion of the laboratory-scale stage. All observations were carried out using the same procedures and measurement techniques.

Laboratory-scale experiment comparing Type 1 and Type 2 enzymes under different pH conditions

This laboratory-scale experiment was conducted to compare the effect of using Type 1 and Type 2 enzymes on the retrogradation process. Tapioca starch was hydrolyzed, then filtered with a filter aid to obtain the dextrin solution. The pH of the resulting dextrin solution was adjusted to 4.0, 4.5, and 5.0 by gradually adding a dilute acid or dilute base until the desired pH was reached. The pH adjustment was performed carefully to minimize changes in the characteristics of the dextrin solution. Observations were subsequently conducted under each pH condition to evaluate differences in the retrogradation rates of dextrin solutions produced by the two enzyme types. This experiment aimed to evaluate the effect of pH and enzyme type on the retrogradation behavior and stability of the dextrin solution.

Observation and data analysis

This study observed the retrogradation rate at different temperatures and pH levels and compared enzymes with varying enzymatic activities and different dominant compositions of Type S and Type L. In addition to higher enzymatic activity, both enzyme types possess specific characteristics in cleaving glycosidic bonds within glucose chains. Observations were conducted by measuring solution clarity with a spectrophotometer at 600 nm (Fukuzawa *et al.* 2016). Furthermore, differences in enzyme characteristics and retrogradation rates were also analyzed by measuring the viscosity of the dextrin solution (Nadhira dan Cahyana 2023). Evaluation of these retrogradation changes is expected to improve the efficiency and quality of production outcomes.

RESULTS AND DISCUSSION

Observation of dextrin solutions with Type 1 and Type 2 enzymes at different temperatures

A dextrin solution sample with DE 12, obtained from production, was stored at three temperatures: 30, 50, and 70 °C, representing room, moderate, and high temperatures, to observe the effect of temperature on the rate of retrogradation. Haziness analysis was carried out by measuring the solution clarity using a spectrophotometer at a wavelength of 600 nm, which is sensitive to decreases in transmittance caused by the formation of more complex particle structures during the retrogradation process (Fukuzawa *et al.,* 2016). Observations were conducted

hourly over 24 hours to monitor changes in clarity reflecting the progression of retrogradation. The observation results are presented in Figure 1.

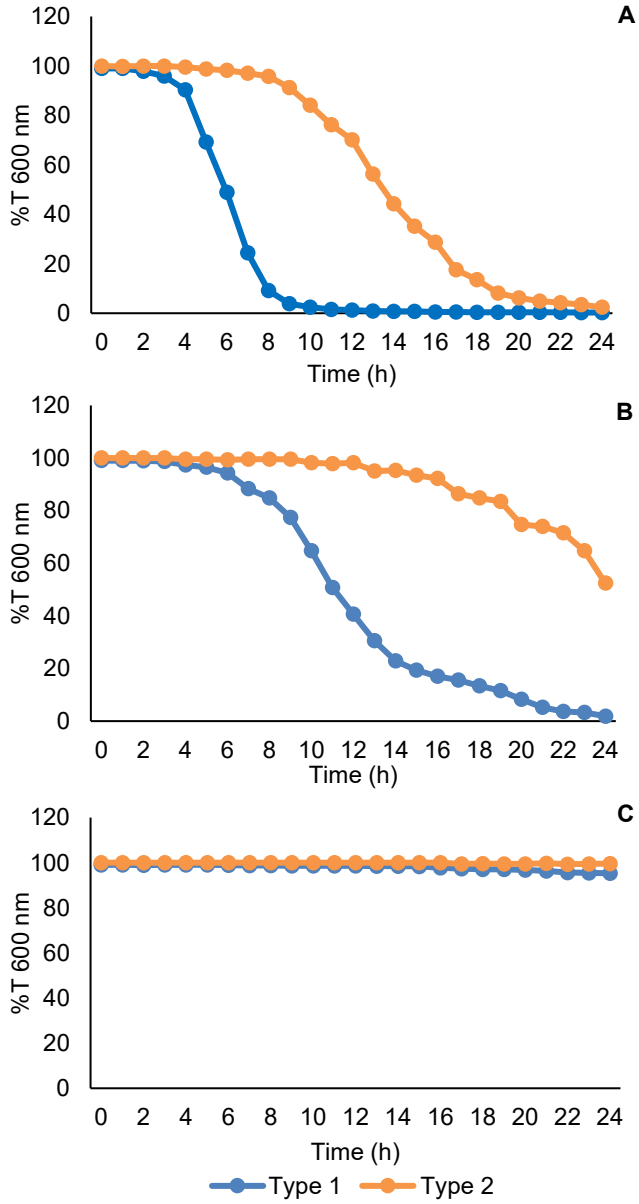

Figure 1. Differences in retrogradation of dextrin solutions at storage temperatures between Type 1 and Type 2 enzymes. A= 30 °C, B= 50 °C, C= 70 ˚C

Figure 1 illustrates the differences in retrogradation behavior of dextrin solutions hydrolyzed by enzyme Types 1 and 2 under storage conditions at 30 °C, 50 °C, and 70 °C. Changes in retrogradation were indicated by a decrease in transmittance ($\%T_{600\ nm}$), which reflects an increase in hazy solution. At a low temperature (30 °C), the dextrin solution produced by enzyme Type 2 exhibited better stability compared to that from enzyme Type 2. This was evidenced by a slower decline in transmittance values, suggesting that retrogradation occurred more gradually and was better controlled with enzyme Type 2. A similar trend was observed at the medium temperature (50 °C), where enzyme Type 2 consistently showed greater effectiveness at slowing the retrogradation process than enzyme Type 1.

The decrease in transmittance in the solution hydrolyzed by enzyme Type 1 occurred more rapidly and sharply, indicating that retrogradation was more dominant than in the solution hydrolyzed by enzyme Type 2. Meanwhile, at a high temperature (70 °C), both enzyme Types exhibited relatively stable solutions with nearly constant transmittance values throughout the storage period. Retrogradation did not occur significantly in either enzyme type, although an initial tendency toward retrogradation was observed in the enzyme Type 1 solution. Overall, these results indicate that at 30, 50, and 70 °C, enzyme Type 2 demonstrates a better ability to retard dextrin retrogradation compared to enzyme Type 1. Furthermore, it can be concluded that the higher the storage temperature, the slower the rate of dextrin retrogradation.

The retrogradation process in dextrin solutions occurs due to the reformation of hydrogen bonds between amylose and amylopectin chains after gelatinization. As shown in the graph above, temperature influences the rate of retrogradation. At lower temperatures, molecular kinetic energy is relatively low, slowing the movement of oligosaccharides and allowing the hydroxyl groups of glucose molecules to come closer and form hydrogen bonds (Nadhira dan Cahyana 2023). Conversely, as temperature increases, molecular kinetic energy increases, resulting in faster movement of amylose, amylopectin, and water molecules. This rapid movement hinders molecular interactions and disrupts hydrogen bonds. This can be observed at 50 °C and 70 °C, where the increase in temperature tends to inhibit retrogradation.

Another indication of retrogradation is a change in solution viscosity, as the formation of hydrogen bonds leads to the development of more complex structures, resulting in a thicker solution. In addition to retrogradation, viscosity is influenced by temperature; lower temperatures generally lead to higher viscosity. However, at the same temperature, retrogradation increases solution viscosity. The changes in viscosity measured using a viscometer are presented in Figure 2.

Figure 2 illustrates the changes in the viscosity of dextrin solutions stored at 30, 50, and 70 °C, hydrolyzed using enzymes Type 1 and Type 2. Viscosity was used as an indicator of retrogradation, since an increase in solution thickness is associated with the reformation of crystalline starch structures during storage. At a low temperature (30 °C),

retrogradation occurred more rapidly, resulting in a significant increase in viscosity, particularly in the solution hydrolyzed with Type 1 enzyme. The solution with enzyme Type 2 showed a higher overall viscosity than the solution with enzyme Type 1, but the increase was more gradual, indicating better stability. At the medium temperature (50 °C), enzyme Type 2 again exhibited a more favorable result. The viscosity of the enzyme Type 2 solution tended to increase at later storage periods compared to enzyme Type 1, indicating a delayed onset of retrogradation, whereas the enzyme Type 1 solution showed a faster rise in viscosity. This suggests that enzyme Type 2 was more effective in retarding the retrogradation rate. At a high temperature (70 °C), both enzyme types exhibited relatively stable solutions with low, nearly constant viscosity throughout the storage period. This indicates that retrogradation can be suppressed under high-temperature conditions, although a slight increase in viscosity in the enzyme Type 1 solution suggests that retrogradation initially occurred. Overall, these observations show that retrogradation in dextrin solutions can be characterized by changes in viscosity. The higher the storage temperature, the more inhibited the retrogradation process becomes. Moreover, under all temperature conditions, enzyme Type 2 exhibited better performance in suppressing the rate of retrogradation than enzyme Type 1.

The effect of enzyme Type 1 and Type 2 at different pH levels on a laboratory scale

In addition to temperature, pH also plays an important role in determining the rate of starch retrogradation. The differences in retrogradation due to pH variation are shown in Figure 3.

The results shown in Figure 3 indicate differences in the retrogradation rates of dextrin solutions hydrolyzed by enzyme Types 1 and 2 under storage conditions at pH 4.0, 4.5, and 5.0. The solution hydrolyzed with enzyme Type 1 exhibited a faster decrease in transmittance (%$T_{600\ nm}$), indicating that retrogradation occurred at a higher rate and with greater intensity. In contrast, enzyme Type 2 maintained solution stability for a longer period, as evidenced by a slower, more stable decline in %$T_{600\ nm}$ values, suggesting that the retrogradation process proceeded more gradually. In addition, the effect of pH on the rate of retrogradation was also clearly observed. Retrogradation of the dextrin solution occurred more rapidly at pH 5 (near-neutral conditions) than at pH 4 or 4.5. This finding indicates that pH values between 4 and 4.5 slowed the rate of retrogradation, thereby more effectively maintaining the stability of the dextrin solution than at pH 5.

At near-neutral pH, the formation of hydrogen bonds between glucan chains (both amylose and amylopectin branches) occurs more effectively, thereby accelerating the association of starch chains into crystalline structures. Under acidic conditions, however, the cleavage of glycosidic bonds tends to occur more readily at specific sites, yielding shorter, less stable fragments that are unable to form large crystalline structures. Therefore, retrogradation proceeds more rapidly at near-neutral pH, whereas at pH 4–4.5, retrogradation can be slowed, leading to greater stability of the dextrin solution (Fang *et al.,* 2020).

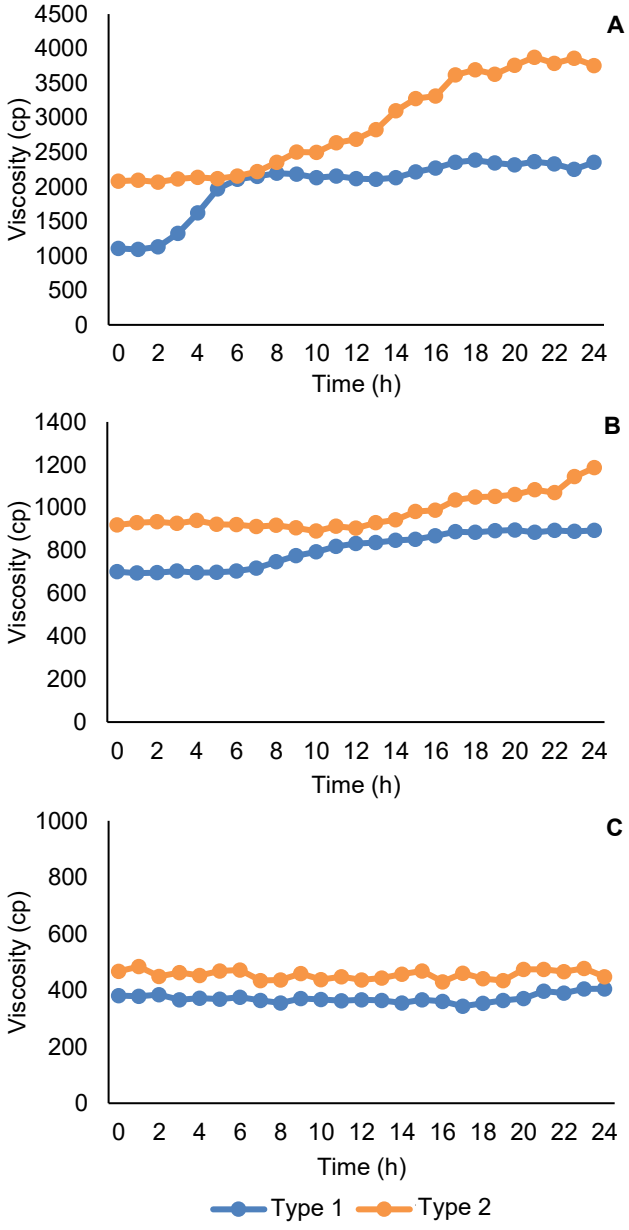

Figure 2. Differences in the viscosity of dextrin solutions during storage at 30 (A), 50 (B), and 70 °C (C) between enzyme Type 1 and enzyme Type 2

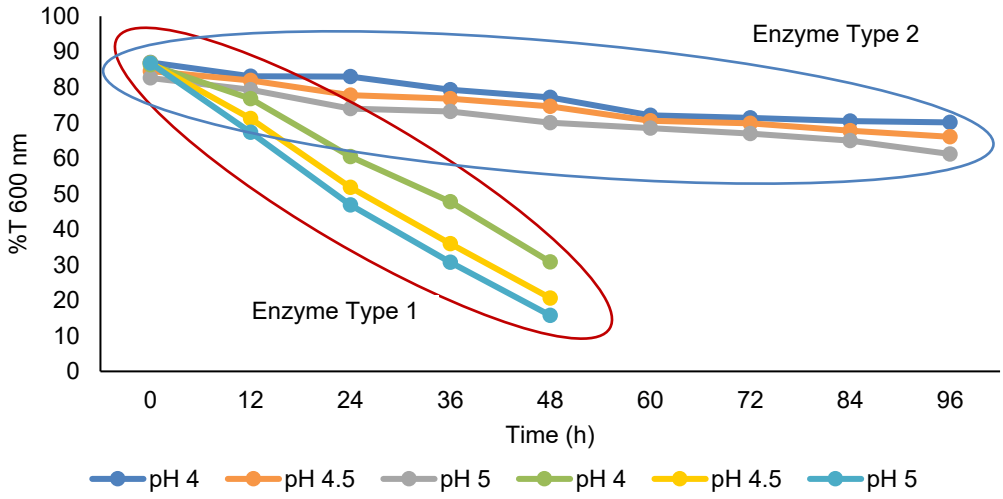

Figure 3. Differences in the retrogradation time of dextrin solutions stored at pH 4.0, 4.5, and 5.0 between enzyme Type 1 and enzyme Type 2

The concentration of dextrin chains was analyzed by High-Performance Liquid Chromatography (HPLC) to determine the Degree of Polymerization (DP), as shown in Table 1. Degree of Polymerization (DP) refers to the number of glucose units linked together within a carbohydrate molecule and indicates the relative length of the molecular chain. Therefore, DP1 represents a single glucose unit, while higher DP values indicate longer carbohydrate chains with more glucose units. The results showed that enzyme Type 2 produced a higher concentration of DP1 (2.66%) compared with enzyme Type 1 (1.46%). The higher DP1 concentration observed in the enzyme Type 2 treatment indicates greater hydrolysis, resulting in a higher proportion of shorter carbohydrate chains than in the enzyme Type 1 treatment. In addition, the concentration of long-chain oligosaccharides (DP10+) generated by enzyme Type 2 was lower (55.07%) than that of enzyme Type 1 (67.93%). This indicates that enzyme Type 2 exhibits greater depolymerization capability, producing shorter glucose chain fragments. This condition plays an important role in the retrogradation process during storage, as retrogradation generally occurs more intensively in long-chain molecules such as amylose and high-molecular-weight oligosaccharides (DP10+), which tend to form crystalline structures through inter-molecular hydrogen bonding. With a decrease in DP10+ concentration and an increase in DP1 and DP2–DP6 fractions, the potential for retrogradation can be reduced, since shorter fragments exhibit higher mobility and are less likely to form stable crystalline structures. The chain-length distribution of amylose and amylopectin significantly influences starch retrogradation and recrystallization behavior. A higher proportion of long glucose chains (high degree of polymerization, DP) contributes more substantially to the formation of stable retrograded structures (Li *et al.,* 2021).

Benefits and potential applications of enzyme Type 2

The application of enzyme Type 2 provides significant cost-efficiency advantages compared to the previously used enzyme. Enzyme Type 1 costs $4 per kilogram, with an activity of approximately 300 KNU, resulting in a monthly enzyme requirement of 1,200 kg at a total cost of $4,800 per month. In contrast, enzyme Type 2 costs $6 per kilogram but has a higher activity of around 600 KNU, reducing the monthly enzyme requirement by half to 600 kg, for a total cost of $3,600 per month. Based on this comparison, the use of enzyme Type 2 yields a cost efficiency of $1,200 per month. This finding indicates that higher enzymatic activity can reduce the required enzyme amount without compromising hydrolysis effectiveness.

In addition to reducing operational costs, the use of enzyme Type 2 also increases production capacity. Prior to the implementation of the new enzyme, the production process for the dextrin solution required 5 days and had a production capacity of 120 tons per day. After the new enzyme was applied, the production time was reduced to 3 days, with a maximum capacity of 200 tons per day. This acceleration of processing time provides additional benefits, including improved production efficiency and optimized facility utilization. With a shorter production period, the fourth and fifth days can be allocated to producing other glucose types, thereby increasing total plant output without requiring additional resources or equipment capacity.

Table 1. HPLC analysis results of sugar concentration from enzyme Type 1 and enzyme Type 2

Sample Dextrin	DP10+	DP9	DP8	DP7	DP6	DP5	DP4	DP3	DP2	DP1
Enzyme Type 1 (%)	67.93	2.48	3.17	4.25	6.25	5.56	2.89	2.92	3.10	1.46
Enzyme Type 2 (%)	55.07	1.99	2.81	4.77	8.99	9.55	4.36	4.12	5.68	2.66

Study contribution of science

This observation demonstrates that retrogradation in low-DE dextrin solutions can be effectively controlled through the combined regulation of temperature, pH, and *α*-amylase activity. The results show that storage at elevated temperature (70 °C) and acidic conditions (pH 4.0) significantly improves solution clarity by inhibiting amylose reassociation and delaying haze formation during storage.

In addition, the use of *α*-amylase with higher enzymatic activity plays a critical role in modifying glucose chain-length distribution, particularly by reducing long-chain fractions that are prone to retrogradation. This enzymatic effect strengthens the stabilizing influence of temperature and pH, indicating that enzyme selection is an integral factor in controlling retrogradation behavior, rather than merely a hydrolysis parameter.

Overall, this research confirms that the retrogradation of dextrin solutions is not governed solely by storage conditions. It can be systematically suppressed through an integrated approach that combines control of enzyme characteristics, temperature, and pH. Several sectors could apply these findings directly. Starch hydrolysate and maltodextrin manufacturers could choose the enzyme and holding conditions for liquid dextrin before refining or spray drying, which would reduce haze, filter clogging, and reprocessing. Beverage producers could add low-DE dextrins to clear products such as sports drinks, ready-to-drink teas, and flavored waters to prevent cloudiness during shelf life. The confectionery, brewing, and fermentation industries could maintain consistent dextrin syrups and feedstocks during storage and handling. In each case, controlling enzyme activity, storage temperature, and pH is a low-cost way to keep products clear, maintain the stability of intermediate solutions during storage, and reduce production losses. This approach provides a practical framework for improving clarity, stability, process efficiency, and production performance in industrial dextrin processing

CONCLUSION

In dextrin solutions, product clarity was observed under variations of temperature, pH, and *α*-amylase enzymes with different activity levels over a 24-hour period. The results showed that a temperature of 70 °C produced the clearest solution compared to 50 °C and 30 °C. Variation in pH indicated that a pH of 4.0 yielded the clearest solution compared to pH 4.5 and 5.0. Meanwhile, the enzyme activity factor indicated that enzymes with higher activity and specific catalytic characteristics produced fewer high-molecular-weight glucose chains, resulting in a clearer product than those produced by lower enzyme activity. This high-activity enzyme also improved cost efficiency by reducing the required enzyme quantity and increased production capacity by up to 40%.

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