CHAPTER III

FRUCTOSE SYRUP

3.1 Introduction

Fructose syrup may be called in many names as high fructose syrup, isosyrup, iso-glucose syrup or glucose fructose syrup (2). The raw material for production of fructose syrup is starch which is converted into fructose syrup through 5 main stages, 3 of which are enzymatic as follows: Liquefaction, Saccharification, Pre-treatment, Isomerization and Post-treatment. The 3 enzymatic stages are shown in Figure 3-1 (15).

3.2 Starch

3.2.1 Nature of starch

Starch is a high molecular weight polymer of the hexose sugar specifically D-glucose and is the principal constituent of many foods (16). It occurs in various sites in the plant: in seeds, as in cereal grains; in the root and tuber, as in tapioca and potato; and more rarely in the stem pith, as in the sago plant (17). The character of the starch varies with the plant source from which it is derived. The counterpart of starch in the animal kingdom is glycogen, a compound stored principally in the liver (18).

Under the microscope, starch appears as minute rounded granules whose size, shape, and markings are preculiar to each variety. Starch granules contain two polymers of glucose, amylose and amylopectin,

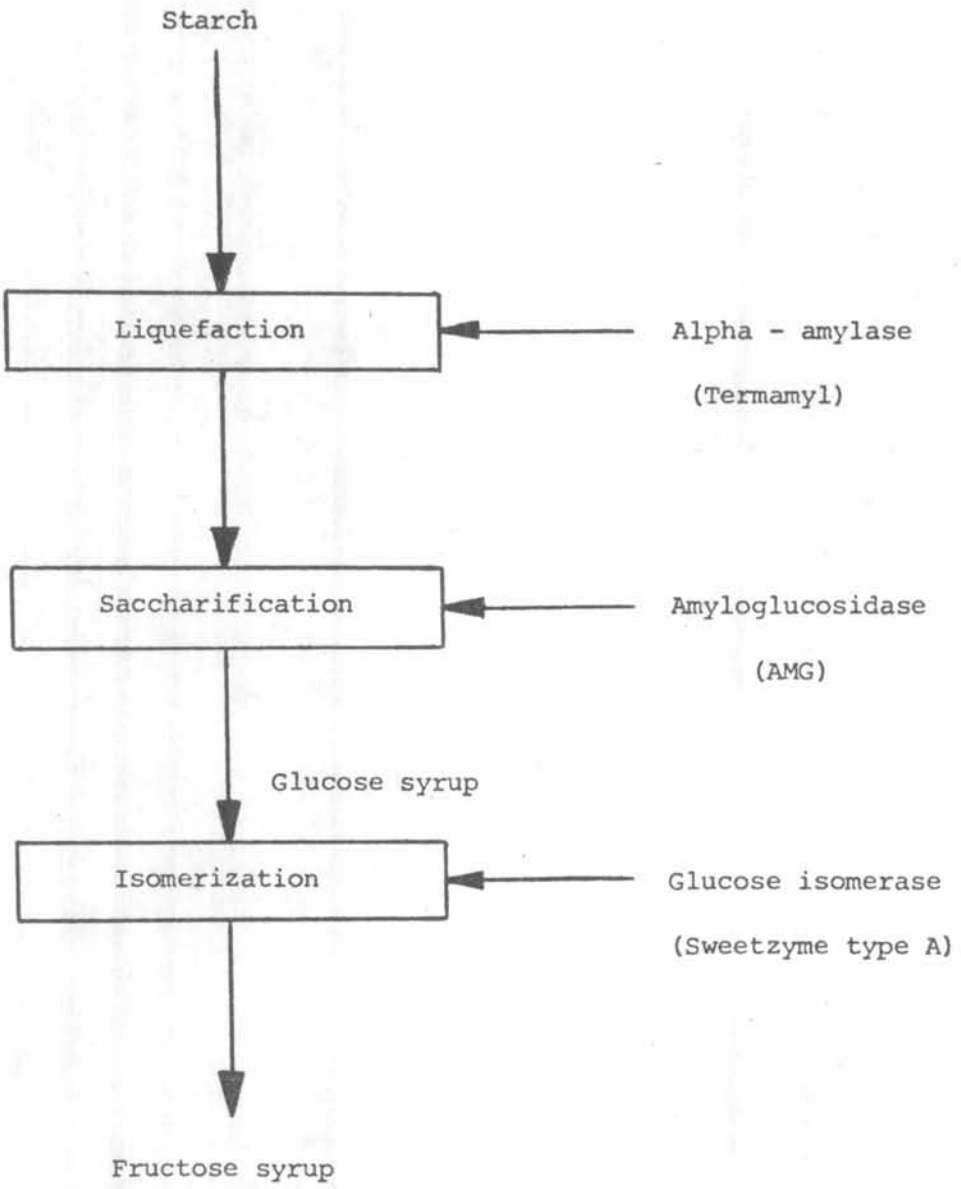


Figure 3 - 1 Production of fructose syrup (15)

which are evently distributed throughout the granules and are most probably associated with each other by hydrogen bonds. The granules are partly crystalline and partly amorphous, and exhibit birefringence in the native state. The degree of association is reflected in the physico-chemical properties of the starches. The enzymatic susceptibility of the granules also depends on the type and condition of the granules.

The linear polymer of starch has been designated as amylose or the A-fraction. It is a flexible, linear chain molecule of 500 or more glucose units, which is capable of twisting and coiling in three-dimensional space. The glucose residues are joined by alpha 1, 4-glucosidic linkages (17, 18). Each glucose unit contains one primary and two secondary hydroxyl groups, except the terminal unit. At one end of the molecule the glucose unit contains three secondary and one primary hydroxyl groups. This is commonly called the non-reducing end. At the other end, the glucose unit contains one primary and two secondary hydroxyls, as well as an aldehydic reducing group in the form of an inner hemiacetal. This is usually called the reducing end. A segment of an amylose chain is represented diagrammatically in Figure 3-2 and structural details of a portion of this segment are also shown(7). Amyloses readily form complexes with fatty acids and organic alcohols. The complex of amylose and iodine, which produces a blue color, is the basis for the quantitative determination of the amylose content of starch.

The branched polymer of starch has been designated as amylopectin or the B-fraction. Amylopectin is usually a larger polymer than amylose and the molecular size ranges from several hundred thousand to several millions representing many thousands of D-glucose units per

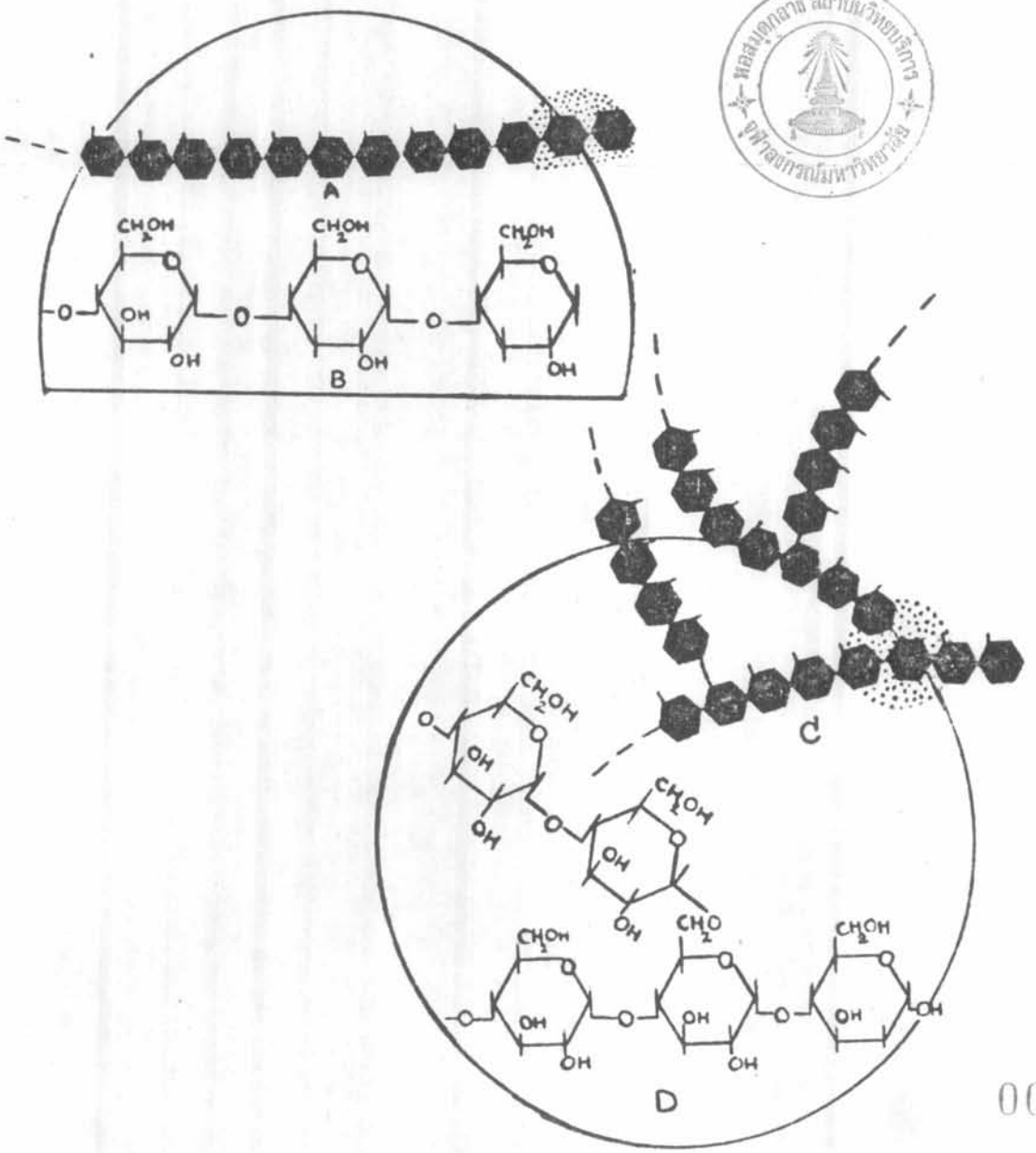


Figure 3 - 2 Structures of the amylose and amylopectin components

A: Diagram of a portion of an amylose molecule

B: Enlarged view of the shaded section showing chemical formula

C: Diagram of a portion of an amylopectin molecule

D: Enlarged view of shaded area showing chemical formula (7)

molecule. The types of linkages that have been definitely established in amylopectin are the alpha 1, 4 and the alpha 1, 6. The latter linkages give rise to the so-called branch points in the molecule (18). A representation of these structural features of the amylopectin molecule is shown in Figure 3-2. In contrast to amylose, amylopectin produces a purple to red color with iodine (6).

Although the alpha 1, 3-linkage has been found in amylopectin from a waxy maize, the presence of such a linkage in other types of amylopectins has not been demonstrated. In the amylopectin molecule, several types of linkages, namely the alpha 1, 4, the alpha 1, 6, and perhaps the alpha 1, 3 must be considered in relation to their synthesis or hydrolysis by enzymes. Also, different types of segments of alpha 1,4-linked glucose units are present in the amylopectin molecule, and these segments are quite similar to those available in amylose. Other segments ranging in size from five to ten D-glucose units are available in the inner portions of the amylopectin molecule, and these may differ markedly in their susceptibility to enzymes. The location of the alpha 1, 6 or the alpha 1, 3-bonds near the alpha 1, 4-bond may have a significant effect on the rate of the enzyme action on the alpha 1, 4-bond.

3.2.2 Swelling and gelatinization of starch

Starch granules are insoluble in cold water. When wetted or exposed to high humidities they will absorb water and swell slightly. The swelling is reversible, however, and on drying the granules shrink. The abundance of hydroxyl groups in the starch granules is a primary factor in their tendency to absorb moisture (18).

When starch granules are progressively heated in water, they do not change in appearance until a certain critical temperature or gelatinization temperature is reached (17, 19). At that point, some of the granules swell irreversibly and the birefringent properties of the granules are lost. This process is referred to as gelatinization which is due to an irreversible breakdown in the order and crystallinity of the molecules within the granule (18). During this process the starch suspension changes into a paste and the viscosity increases with rising temperature. The granules in their native state are resistant to the action of chemicals and enzymes and have a low water-binding capacity; however, when swollen, they become susceptible to chemical, mechanical, and enzymatic action and imbibe water many times their weight. Some shorter linear molecules dissolve and diffuse out of the swollen granules; the longer linear amylose chains reinforce the structure of the granules (17). In any given species of starch, not all the granules will gelatinize or swell at the same time and temperature. Some are substantially more resistant than others and will differ by as much as 10°C or so in gelatinization temperature (18). Gelatinization occurs during food processing and effects the susceptibility of the starch component to an enzymatic attack.

Tapioca flour, which is produced by extraction from cassava roots, can be used as the raw material for the production of syrups. The words flour and starch are almost used in the same meaning but in fact starch is the main composition of flour. Flour is composed of water, starch, protein, fat, pulp, ash, etc. and the quality of flour is usually designated to these certain properties. The compositions of cassava

roots are shown in Table 3-1 (7) and the characteristic requirements for tapioca flour grading are shown in Table 3-2 (20).

3.3 Enzyme

3.3.1 Nature of enzyme

Enzymes belong to the broad class of substances which the chemist calls catalysts. A catalyst is a substance which influences the velocity of a reaction without being used up in the reaction. The catalysts take part in reactions, but reappear in their orginal form, describing a cycle. Enzymes are a very special kind of catalysts with very distinctive properties (18).

An enzyme is a soluble, colloidal, organic catalyst produced by a living cell, even more simply, an enzyme is a biocatalyst produced by a living cell. Since all enzymes are either simple or conjugated proteins, another definition quite widely used is that an enzyme is a protein with catalytic properties due to its power of specific activation (18, 21). The high specificity of the catalytic function of an enzyme is due to its protein nature; that is, the highly complex structure of the enzyme protein, can provide both the environment for a particular reaction mechanism and the template function to recognize a limited set of substrates. That region of the protein which participates directly in the conversion of substrate to product is called the active site (6).

Enzymes, like all catalysts, affect the rate of chemical reactions, but not the extent of the chemical change concerned. The enzyme action can be described by stating that the enzyme lowers the

Table 3-1 The compositions of cassava roots (7)

Composition	Weight percent (wet basis)
Edible part	81.4
Moisture	63.8
Ash	1.44
Protein	0.96
Starch	27.65
Fat	0.26
Fiber	0.85
HCN or prussic acid	0.02
Kcal/kg	1,403
Others	5.04

Table 3-2 Characteristic requirements for tapioca flour grading (20)

Characteristic		Requirements		
		Grade 1	Grade 2	Grade 3
% Moisture content	Max	13	14	14
% Ash (on dry basis)	Max	0.15	0.3	0.5
% Acid insoluble ash (on dry basis)	Max	0.05	0.10	0.15
% Protein (on dry basis)	Max	0.3	0.3	0.3
% Starch (on dry basis)	Min	97.5	96	94
Pulp, ml/50 gm of flour before drying	Max	0.2	0.5	1.0
pH of aqueous extract		4.5-7	3.5-7	3.0-7
% Fineness of starch left on 150 µm	Max	1	3	5

amount of activation energy required by the reaction (18). However, enzymes differ from other catalysts in several respects. These differences are, of course, due to the protein nature of the enzymes. Being proteins, enzymes are denatured and inactivated when subject to nonphysiological conditions. Hence, one of the distinctive properties of enzymes, in contrast with ordinary chemical catalysts, is their thermal lability, and sensivity to acids and bases. But the difference between enzymes and other catalysts is most clearly displayed in one respect: enzymes generally have extremely specific actions. Whereas acids, that is hydrogen or hydronium ions, may catalyze hydrolysis of many kinds of substances such as esters, acetals, glycosides or peptides, separate and different enzymes are necessary for the hydrolysis of each of these, and even of specific members of each class (18).

Enzymes can be produced only by living cells to accomplish specific metabolic needs. The enzymes can be separated readily from the cells which produce them and perform their catalytic activities entirely apart from the cells, and hence are available for the production of industrial enzymes for useful applications. Industrial enzymes are produced from animal tissues, plant tissues and microorganisms. A wide range of enzymes of all classes is obtained from a variety of species and strains of molds, yeasts and bacteria. Microbial production processes afford a degree of control of efficacy, quality and quantity which is difficult to achieve with plant or animal sources. For this reason, together with the greater number of enzymes readily available from microorganisms, microbial enzymes are assuming increasingly predominate roles as industrial enzyme products (17, 18).

3.3.2 Naming and classification of enzymes

Enzymes bring about changes in specific compounds. The compound upon which an enzyme acts is known as its substrate. Up until recently there have been no completely systematic methods for naming and classifying enzymes. Later it became customary, where possible, to name an enzyme after the substrate upon which it acts, with the ending "-ase". In other cases, enzymes were named from the reactions they catalysed (18).

In 1961, and modified slightly in 1964, the commission on Enzymes of the International Union of Biochemistry published an entirely systematic method for classifying and naming enzymes, based on substrate and type of reaction. The enzymes are divided into six main classes, oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Each class is further divided into a number of sub-classes and sub-sub-classes, according to the nature of the chemical reaction catalyzed, and is coded on a four number system intimately connected with this system of classification. Oxidoreductases catalyze oxidations or reductions. Transferases catalyze the shift of a chemical group from one donor substrate to another acceptor substrate. Hydrolases catalyze hydrolytic splitting of substrates. Lyases remove groups or add groups to their substrate (not by hydrolysis). Isomerases catalyze intramolecular rearrangements. Ligase (synthetases) catalyze the joining together of two substrate molecules. Both systematic and trivial names are recommended for the enzymes. In general the systematic name consists of two parts; the first part names the substrate, and the second, ending in "-ase", indicates the nature of the process. The trival name is sufficiently short for

general use, and in the majority of cases is the name already commonly employed. To illustrate, the familiar beta-amylase (trival name) has the systematic name: beta-1,4-glucan maltohydrolase. The name indicates the substrate is a glucan (starch), a glucose polymer in which the glucose molecules are joined by beta 1,4-linkages, and the reaction is hydrolytic splitting of maltose (6, 18, 21).

3.3.3 Factors affecting enzyme action

There are numerous factors which affect enzyme activity, and these must be taken into account in the use of the enzyme. Among the most important are (1) concentration of enzyme, (2) concentration of substrate, (3) time, (4) temperature, (5) pH, and (6) presence or absence of activators or inhibitors (6, 18).

3.3.3.1 Concentration of enzyme

For most enzymatic reactions the rate of the reaction is directly proportional to the concentration of enzyme, at least during the early stages of the reaction. Figure 3-3 depicts the relation between the rate of a reaction and increasing enzyme concentration in the presence of an excess of substrate (6).

3.3.3.2 Concentration of substrate

With a fixed concentration of enzyme and with increasing substrate concentration, a second important relationship is observed and a typical curve is shown in Figure 3-4 (6). An increase of substrate will result at first in a very rapid rise in velocity or reaction rate. As the substrate concentration continues to increase, however, the increase in the rate of reaction begins to slow down until, with a large substrate concentration, no further change in velocity is observed.

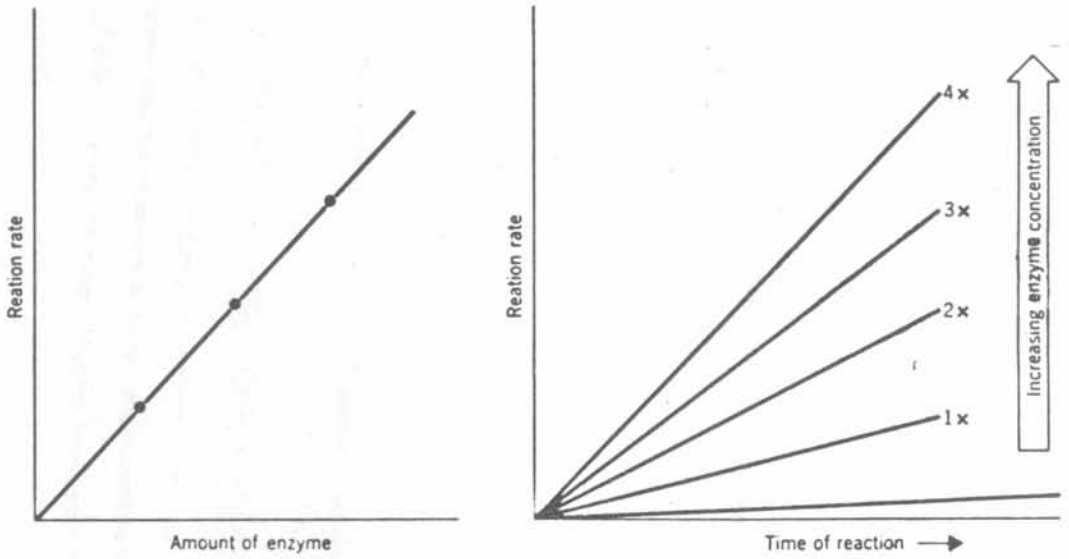


Figure 3 - 3 Effect of enzyme concentration on reaction rate, assuming that substrate concentration is in saturating amounts (6)

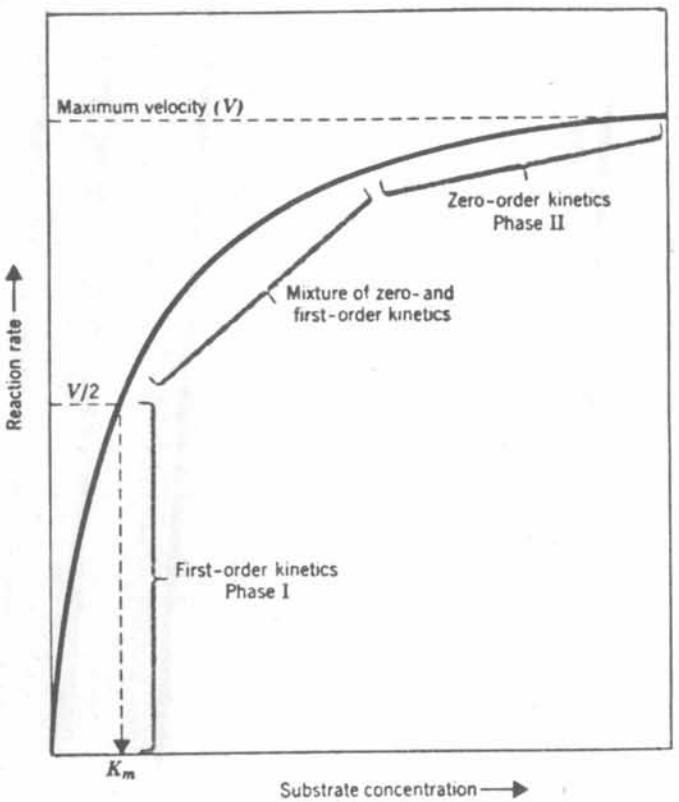


Figure 3 - 4 Effect of substrate concentration on reaction rate, assuming that enzyme concentration is constant (6)

Michaelis and others in the early part of this century reasoned correctly that an enzyme - catalyzed reaction at varying substrate concentration is diphasic, that is, at low substrate concentrations the active sites on the enzyme molecules are not saturated by substrate and thus the enzyme rate varies with substrate concentration (phase I). As the number of substrate molecules increases, the sites are covered to a greater degree until at saturation no more sites are available, the enzyme is working at full capacity and now the rate is independent of substrate concentration (phase II). This relationship is shown in Figure 3-5 (6).

3.3.3.3 Time

Time is a very important factor in practical enzymatic applications. During the early stages where substrate is present in considerable excess, the reaction follows zero-order kinetics, and the amount of product formed (P) is proportional to time (T):

$$\frac{dP}{dT} = k_0$$

where k_0 is the zero-order reaction constant. For such reactions the amount of end product is doubled if the reaction time is doubled.

However, during the course of an enzymatic reaction there is a continuing decrease in substrate concentration which results in slowing down of the reaction with time as shown in Figure 3-6 (18). Most enzymatic reactions follow the kinetics of a first-order reaction:

$$\frac{dP}{dT} = k_1 \times (S - P)$$

where k_1 is the first-order reaction constant and (S - P) is the concentration of substrate remaining at any given time. The rate of the reaction

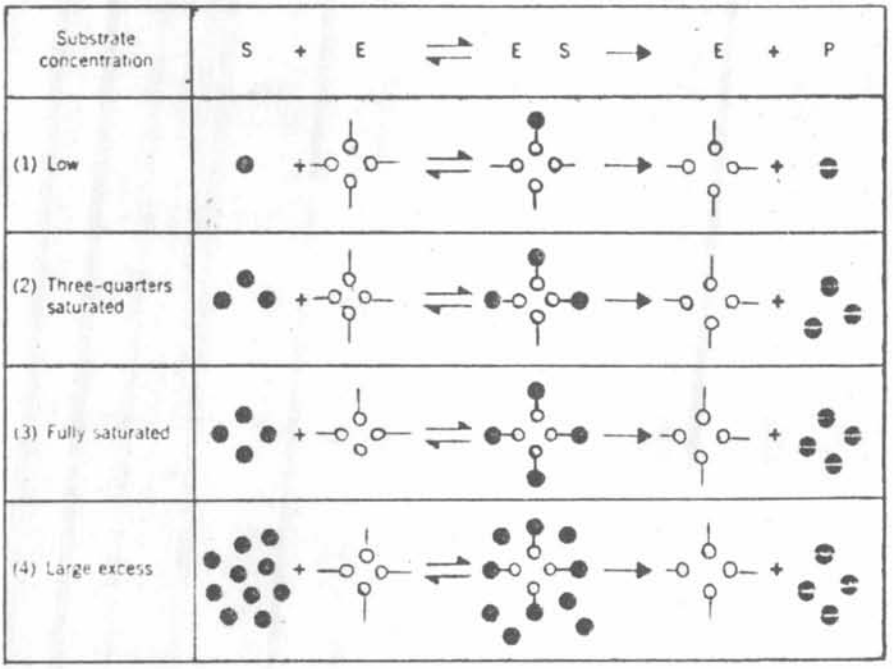


Figure 3 - 5 Diagrammatic demonstration of effect of substrate concentration on saturation of active sites of enzyme molecules (6)

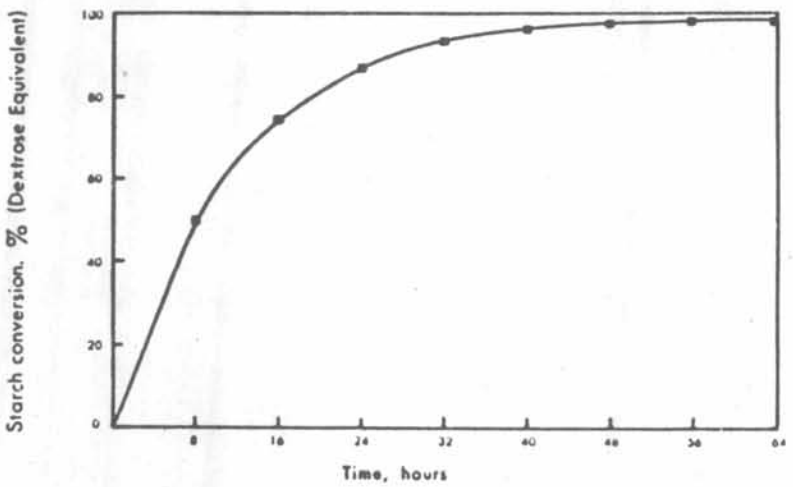


Figure 3 - 6 Effect of time on starch conversion (18)

is directly proportional to the remaining substrate concentration. The decreased velocity of the reaction rate may be due to many reasons. Most important usually are exhaustion of the substrate and inhibition of the reaction by its end products. For practical uses, because of this inherent behavior of enzymes, sufficient time must be allowed for enzyme reactions to approach completion. This may involve reaction periods of several hours or even days, for example 48 to 96 hours in the industrial enzymatic process for glucose production from starch.

3.3.3.4 Temperature

Heat may affect enzymes in two ways. One effect is inactivation since high temperatures cause denaturation of the enzyme protein resulting in a loss of catalytic properties. The actual temperatures at which heat inactivation is substantial, vary a great deal upon the particular enzyme. The second effect of temperature on enzymatic reactions is in their rate. Like most chemical reactions, enzyme-catalyzed changes are increased in rate by raising the temperature. A rough rule for chemical reactions, including those catalyzed by enzymes, is that every 10°C increase in temperature doubles the rate. Raising the temperature of an enzyme reaction, however, may thermally inactivate the enzyme so rapidly that it offsets increased rate of reaction due to the higher temperature. The so-called optimum temperature is that point of maximum activity above which the rate of reaction decreases because of thermal inactivation. Optimum temperature values must be interpreted with caution, since such factors as substrate concentration and particularly time of an enzymatic reaction have considerable effect on optimum temperature (18). The dual effects of a temperature-enzyme reaction relationship are depicted in

Figure 3-7 (6).

3.3.3.5 pH

Since enzymes are protein, pH changes will profoundly affect the ionic character of the amino and carboxylic acid groups on the protein and will therefore markedly affect the catalytic site and conformation of an enzyme. Low or high pH values can cause considerable denaturation and hence inactivation of the enzyme protein. Moreover, since many substrates are ionic in character, the active site of an enzyme may require particular ionic species for optimum activity. These effects are probably the main determinants of typical enzyme activity-pH relation. Thus a bell-shaped curve obtains with a relatively small plateau and with sharply decreasing rates on either side as indicated in Figure 3-8 (6). The plateau is usually called the optimum pH point. There is also a pH range for best enzyme stability, which is not necessarily the same as for optimum activity (18). In enzyme studies it becomes extremely important to determine early in the investigation the optimal pH and its plateau range. The reaction mixture must then be carefully controlled with buffers of suitable buffering capacity.

3.3.3.6 Presence or absence of activators or inhibitors

Some enzymes need cofactors or activators for maximum effectiveness. Frequently metal ions, such as those of calcium, magnesium, or manganese, are necessary enzyme activators, either for maximum activity or stability or both (18).

Enzyme inhibition is a very important area of enzymology. Enzymes are inhibited by a number of conditions. However, the word enzyme inhibitor usually refers to a substance or chemical which

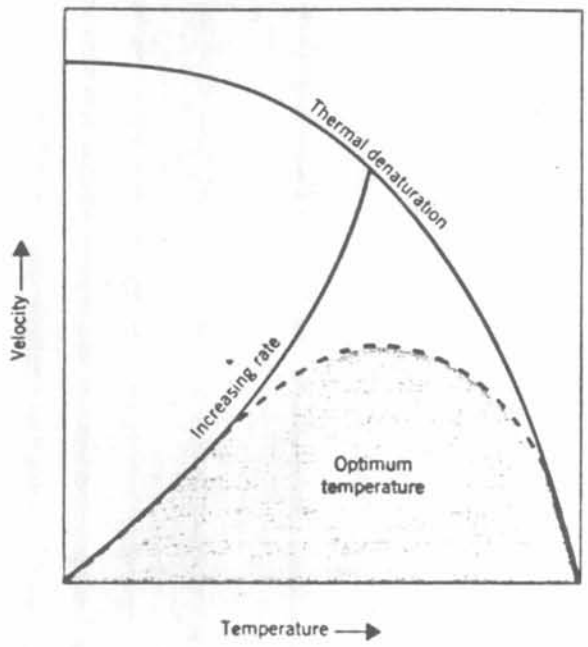


Figure 3 - 7 Effect of temperature on reaction rate of an enzyme - catalyzed reaction (6)

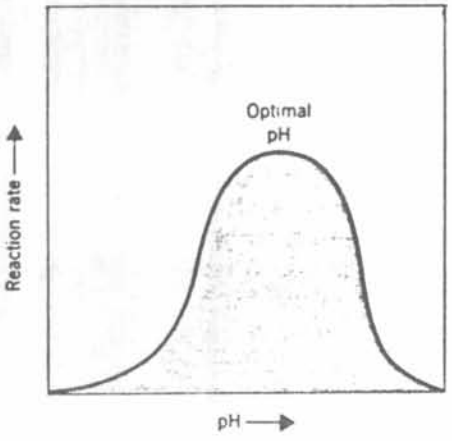


Figure 3 - 8 Effect of pH on an enzyme - catalyzed reaction (6)

causes inhibition of enzyme reaction. There are reversible and irreversible, specific and non-specific inhibitors.

Since enzymes are proteins, groups such as free carboxyl, amino, and sulfhydryl groups will be common to many enzymes and the blocking of these groups will, of course, give rise to a rather non-specific inhibition. If the enzyme activity is restored by removal of the inhibitor, as by dialysis, the inhibition is reversible. Irreversible inhibition is produced by some poisons which cause irreversible denaturation or destruction of the enzyme.

Specific inhibitors are those substances which block groups conferring specificity on an enzyme; that is to say, which react with combining sites of the active centers. A study of these reversible and specific inhibitions shows that two types may be distinguished, the competitive and the non-competitive.

Competitive inhibition is shown by substances with structural similarity to the normal enzyme substrate, including frequently products of the enzyme action. The enzyme is capable of combining with such substances, but cannot activate them, and inhibition of the enzyme reaction results from hindered access of the normal substrate to the active centers. In non-competitive inhibition, there is no competition between substrate and inhibitor. Instead, the inhibitor combines with groupings not essential for the formation of the enzyme-substrate complex, but necessary for the substrate activation (6, 18).

3.3.4 Starch-splitting enzymes

Enzymes responsible for the breakdown of starch are widely distributed in nature. Among these are the amylases, which act on starch, glycogen, and derived polysaccharides to hydrolyze the alpha 1, 4 - glucosidic linkages. The amylases may be divided into three groups: the alpha-amylases, which split the bonds in the interior of the substrate (endoamylases); the beta-amylases, which hydrolyze maltose units from the non-reducing terminal end of the substrate (exoamylases); and the gluco-amylase, which split off glucose units from the non-reducing terminal end of the substrate (17,21).

3.3.4.1 Alpha amylases

Alpha-amylase (alpha 1, 4-glucan 4-glucanohydrolase, EC 3.2.1.1) is an endo-splitting enzyme which hydrolyzes the alpha 1,4 - glucosidic bonds in both amylose and amylopectin at different places in the interior of the molecules. Alpha-amylase is also called "liquefying" or "dextrinizing" or "saccharifying" amylase from its action on starches(7). Its action leads to a rapid decomposition of gelatinized starch to dextrins with an average chain length of 6 to 10 glucose units. The visible result is a very pronounced thinning of the initially highly viscous starch paste. If alpha-amylase action is continued for a prolonged period, a further decomposition into sugars, particularly maltose and maltotriose, takes place (1). Since alpha-amylase cannot hydrolyze alpha 1,6 - branching linkages in starch, the ultimate products from high saccharifying alpha-amylase are maltose, small quantities of other malto-oligosaccharides, a small amount of the trisaccharide panose which contains the original alpha 1,6-linkages of the branched starch fraction, and a little glucose.

Considerable amounts of low molecular weight dextrins are generally formed by the low saccharifying or dextrinizing types of alpha-amylase (18).

Koshland proposed the mechanism for the action of alpha-amylase as shown in Figure 3-9 (21). The enzyme contains a carboxyl and a nitrogen group in the transforming component of the active site. The substrate forms an adsorptive complex with the enzyme which positions the susceptible glucosidic bond in juxtaposition with the carboxyl anion and imidazolium group. In the proposed scheme the carboxyl anion serves as an attacking nucleophile on the C(1) position of the substrate and this attack is aided by protonation of the linkage by the general acid (imidazolium ion). A glucosyl-enzyme intermediate involving covalent bonds is formed as a result. In the deglucosylation reaction, the imidazole group (unprotonated) assists as a general base to remove a proton from water leaving the $\dot{O}H^-$ to attack at the C(1) position of the glucosyl-enzyme. The configuration about C(1) is not changed by this double displacement mechanism so the product of the reaction is of alpha-configuration (17,21).

Alpha-amylase occurs commonly in plants, mammalian tissues, and microorganisms. In 1833, Payen and Persoz succeeded in isolating the active component from malt which was found later to be consisted of alpha-and beta-amylase. From microorganisms, bacterial alpha-amylase is more heat-stable than fungal alpha-amylase (19). The majority of commercial preparations of bacterial alpha-amylase are produced from Bacillus subtilis or related species. The action of the enzymes on the starch is rather similar to that of malt alpha-amylase, but the bacterial enzymes are considerably more heat-stable. As the enzyme action on raw starch is very slow, practical use of enzymes for starch degradation

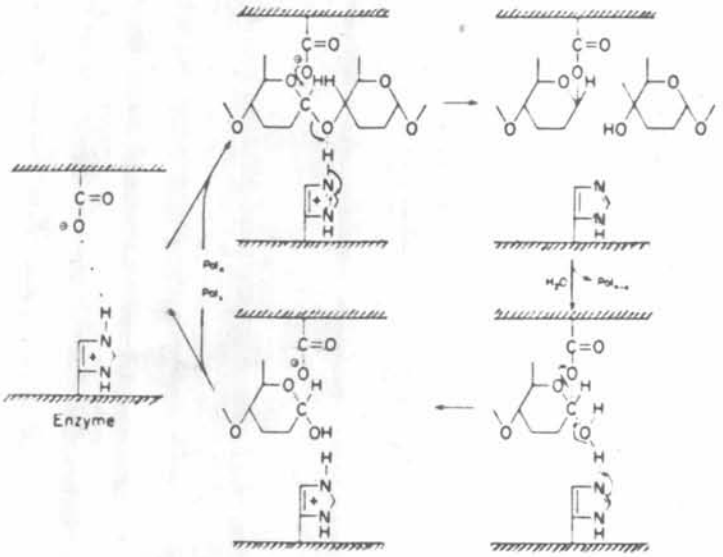


Figure 3 - 9 A proposed mechanism for alpha - amylase (21)

requires gelatinization of the starch prior to enzyme action. For many common starches, heating to temperatures above 80°C is necessary to achieve a complete gelatinization. Thus, the heat stability of bacterial alpha-amylases is very important for their practical use (1).

Despite the success of bacterial alpha-amylasses produced from Bacillus subtilis, modern technology involving the use of jet or high temperature continuous starch cookers has led to the development of alpha-amylases capable of performing at temperatures in excess of 100°C. In 1973, Slott et al. described a new enzyme which is useful at temperature as high as 115°C. The optimum temperature for this enzyme is over 90°C, and it is therefore possible to carry out the liquefaction at gelatinization temperatures (17). It is perhaps more important to compare this new enzyme, prepared from strains of Bacillus licheniformis with enzyme from Bacillus subtilis. Bacillus subtilis alpha-amylase can be used up to 85°- 90°C in starch slurries, whereas the Bacillus licheniformis amylase can be used in starch slurries up to 110°-115°C. The pH optimum for the new enzyme is 6 at low temperatures and moves to 7 with increasing temperatures. A comparison of the pH versus activity at varying temperatures for both enzymes is given in Figure 3-10 and the influence of temperature on the activity of both enzymes is shown in Figure 3-11 (17).

The presence of calcium in the alpha-amylase molecule appears to be 1 gram-atom per each molecule. On complete removal of calcium the enzymes become essentially inactive and less stable to denaturation by heat, acid or urea (21). The calcium does not participate directly in the formation of the enzyme-substrate complex, but maintains the enzyme molecule in the optimum conformation for maximum activity and

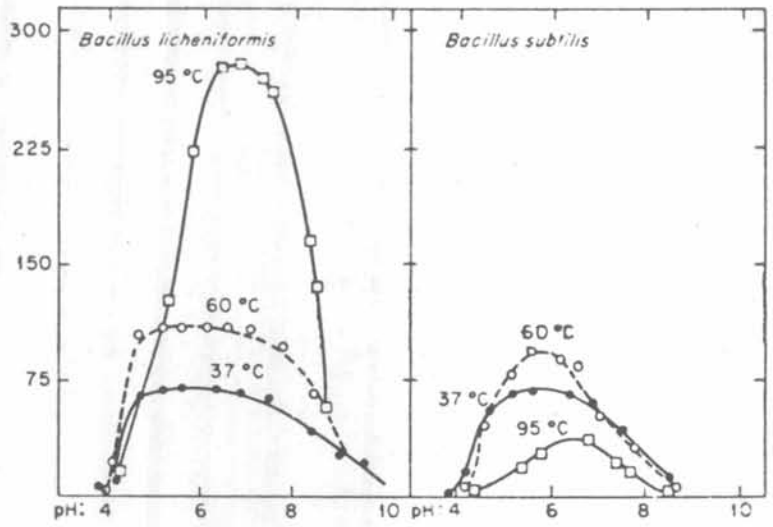


Figure 3 - 10 Effect of pH and temperature on amylase activity (17)

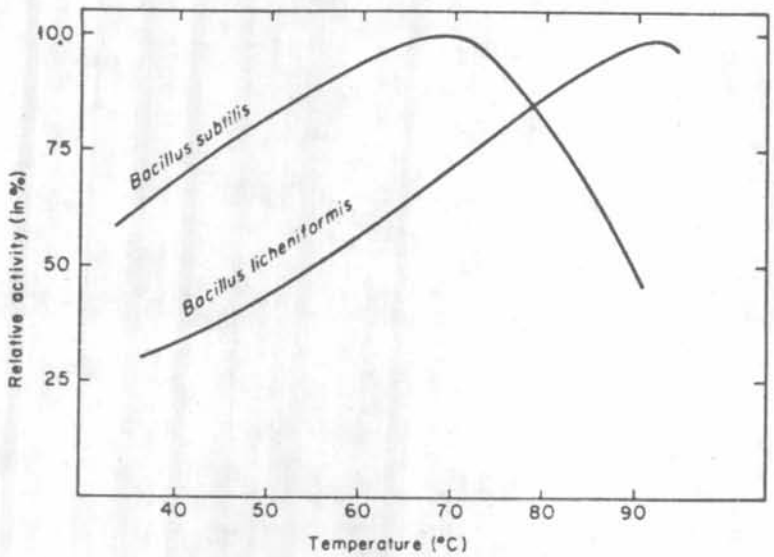


Figure 3 - 11 Influence of temperature on the activity of Bacillus licheniformis amylase and B. subtilis amylase (17)

stability (17, 21). Like the Bacillus subtilis enzyme the alpha-amylase from Bacillus licheniformis is calcium dependent but to a much lesser extent, as shown in Figure 3-12 (17).

The maximum temperature recommended for use with this enzyme is 115°C, since at 120°C it is rapidly inactivated. Since gelatinization of starch can be accomplished at 105°-110°C, use of this new enzyme at that temperature permits the simultaneous gelatinization and liquefaction with moderate levels of enzyme.

The requirement for calcium ions for full stabilization of the Bacillus licheniformis enzyme is on the order of 5 ppm at 70°C and pH 5.7 for a 0.1% solution of the commercial enzyme preparation, whereas the minimum calcium level for maximum activity for the Bacillus subtilis enzyme under the same conditions is about 150 ppm. The lowered calcium requirement for the Bacillus licheniformis enzyme is considered important because high calcium levels tend to be inhibitory to glucose isomerase if the sugar preparation is to be treated with that enzyme (17).

NOVO INDUSTRI has developed a new bacterial alpha-amylase and marketed under the trade name "Termamyl" (Termamyl 60 L) (22). It is a liquid enzyme preparation containing an outstandingly heat-stable bacterial alpha-amylase produced by a selected strain of Bacillus licheniformis. Termamyl is active and stable in starch solutions at temperatures up to 110°C and is recommended for high-temperature liquefaction (1).

Termamyl is standardized with an activity of 60 Kilo NOVO Units per gram (KNU/gm). One Kilo-NOVO alpha-amylase-unit (1 KNU) is the amount of enzyme which breaks down 5.26 gm. of standard starch solid per hour under standard conditions (23). The typical process conditions are shown in Appendix I(22).

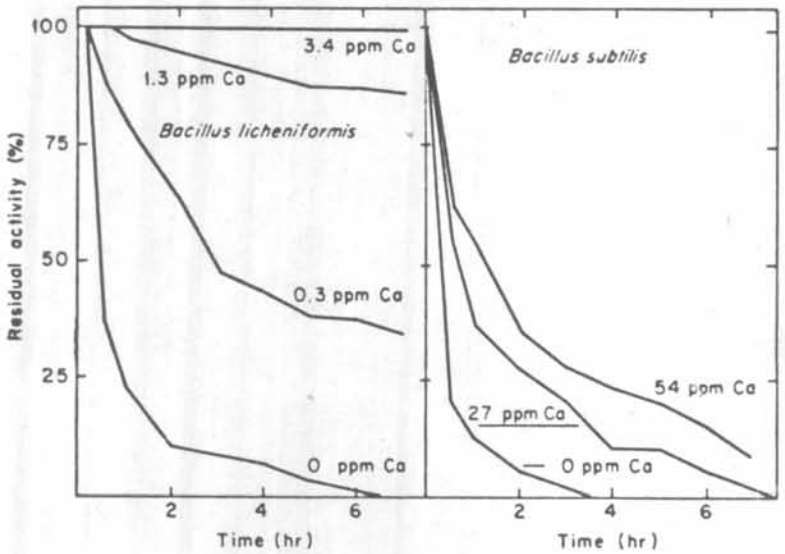


Figure 3 - 12 The effect of added calcium on amylase stability (70°C) (17)

3.3.4.2 Beta-amylases

Beta-amylase (alpha 1,4-glucan maltohydrolase, EC 3.2.1.2) is an exo-splitting enzyme which removes successive maltose units from the non-reducing ends of the polysaccharide chain (21). It hydrolyzes the alpha 1,4-glucosidic bonds in both amylose and amylopectin to yield maltose with an OH group in beta-configuration at C-1 as shown in Figure 3-13 (24). When this enzyme comes to an alpha, 1-6-linkages, as is found in amylopectin, the action on that particular chain stops (18, 21). The amylopectin branched are, however, trimmed down by the enzyme, producing, generally, 50-60% maltose. The remaining portion containing all the branch linkages is called "beta limit dixtrins" (17).

In developing a mechanism for the action of beta-amylase it is postulated that the enzyme has at least three specific groups, X, A and B, in the active site which are involved in binding and transformation of substrate as shown in Figure 3-14 (21). The X group recognizes the C-4 (OH) group at the non-reducing end of the polysaccharide chain. When there is proper interaction between X and OH of C-4 the second glucosidic linkage of the substrate will be in proper juxtaposition with respect to the catalytic group A and B as shown in the ES complex (reactive). The scheme also accounts for the formation of unreactive complexes where X has failed to perform its role and for inhibition by cycloamyloses.

The mechanism for inversion of configuration by beta-amylase may be postulated to occur as shown in Figure 3-15 (21). The proposed mechanism takes into account the four groups experimentally demonstrated to be in the active site. After formation of the enzyme-

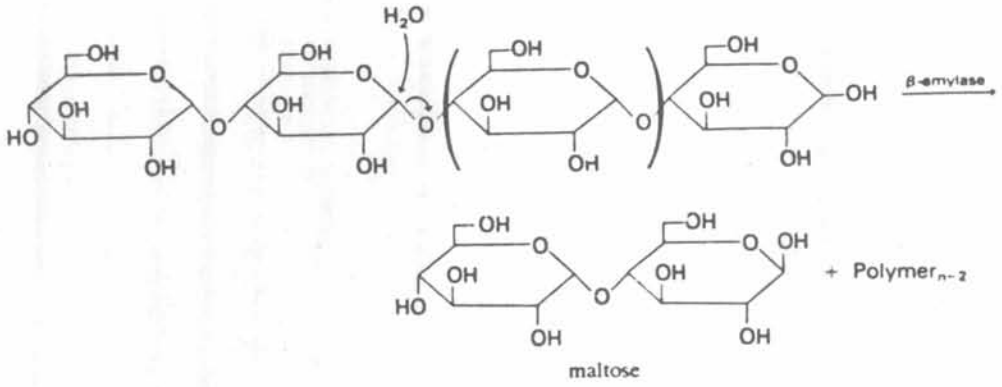


Figure 3 - 13 Maltose from beta - amylase - catalyzed hydrolysis of amylose (24)

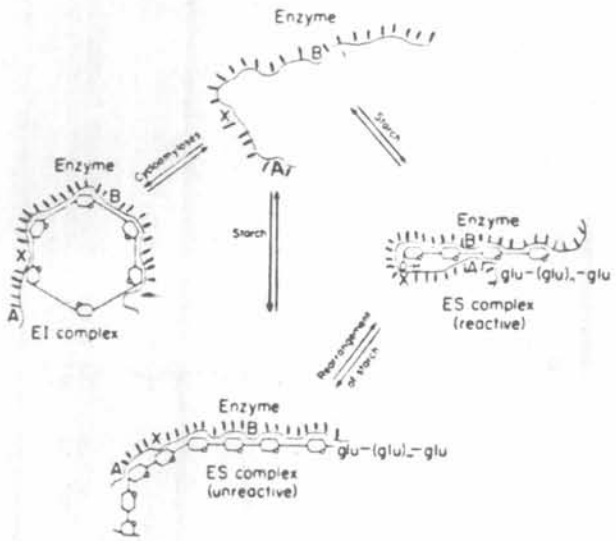


Figure 3 - 14 Schematic representation of beta - amylase mechanism involving a flexible active site (21)

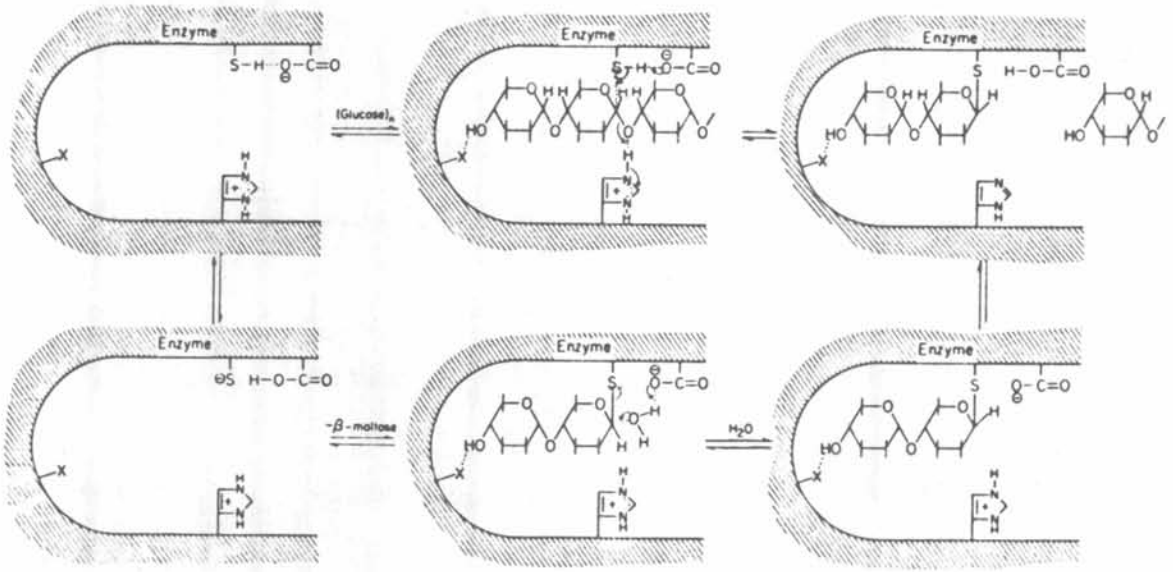


Figure 3 - 15 A proposed mechanism of beta - amylase - catalyzed hydrolysis of amylose (21)

substrate complex, a nucleophilic attack of the sulfhydryl group on C-1 is facilitated by the carboxylate group acting as a general base while the imidazolium group acts as a general acid to donate a hydrogen to the glucosidic oxygen. This leads to formation of a maltosyl-enzyme intermediate. In hydrolysis of the intermediate, the carboxylate group acts as a general base to facilitate a backside attack of water at C-1 to release beta-maltose and regenerate the enzyme. Group X serves to position the substrate properly at the active site.

3.3.4.3 Glucoamylases

Glucoamylase (alpha 1,4-glucan glucohydrolase, EC 3.2.1.3) is an exo-splitting enzyme which removes glucose units consecutively from the non-reducing ends of the starch chains (17, 21). Other names that have been used for this type of enzymic activity include amyloglucosidase, glucamylase and gamma-amylase (17, 18). The end product of its action is glucose which clearly differentiates this enzyme from the alpha and beta-amylases (17). It also hydrolyzes alpha 1,6 and alpha 1,3-linkages but more slowly than alpha 1,4 linkages (18, 19, 25). Although the action of glucoamylase is not stopped by the alpha 1,6-glucosidic linkages in branched molecule, a complete degradation is not accomplished. There appears to be a blockage in the substrate molecules, the type of which has not been defined as yet. It is possible that some of the alpha 1,6-linkages are present in some arrangements that enzyme finds it difficult to hydrolyze (17). However, in the presence of alpha-amylase a complete degradation takes place (17, 19).

Glucoamylase hydrolyzes alpha 1,4-glucosidic linkages but the product is beta-glucose, so there is an inversion of configuration as in the case of beta-amylase. The action of several exo-and endo-

splitting glucosidases have been examined and it was found that all exo-splitting enzymes caused an inversion of configuration about the anomeric C-1 position while all the endo-splitting enzymes caused a retention of configuration (21).

The detailed mechanism of the action of glucoamylase is largely unknown. There is a certain similarity between this enzyme and beta-amylase (17). The same rationale as presented in Figure 3-14 for beta-amylase may be used to explain the ability of glucoamylase to split off only glucose units. In the case of glucoamylase, the spatial distance between X and A and B would be such that when X is attached to the C-4 group, A and B would be juxtapositioned with respect to the first glucosidic bond (21).

The most recent extensive application of amylolytic enzymes is the use of glucoamylase for the production of glucose from starch. The presence of transglucosylases with glucoamylase is undesirable because of their production of glucose polymers having alpha 1,6-linkages which reduce glucose yields and interfere with glucose crystallization(18).

The final dextrose equivalent of glucose syrup using glucoamylase is dependent on enzyme and also substrate concentration. Figure 3-16 illustrates the effect of enzyme concentration on reaction rate and Figure 3-17 shows how the dextrose equivalent falls with increasing substrate concentration (19). These two effects are due to the resynthesis of higher sugars from glucose. The two most important sugars formed by this reversion or back polymerization are maltose and iso-maltose (18, 19).

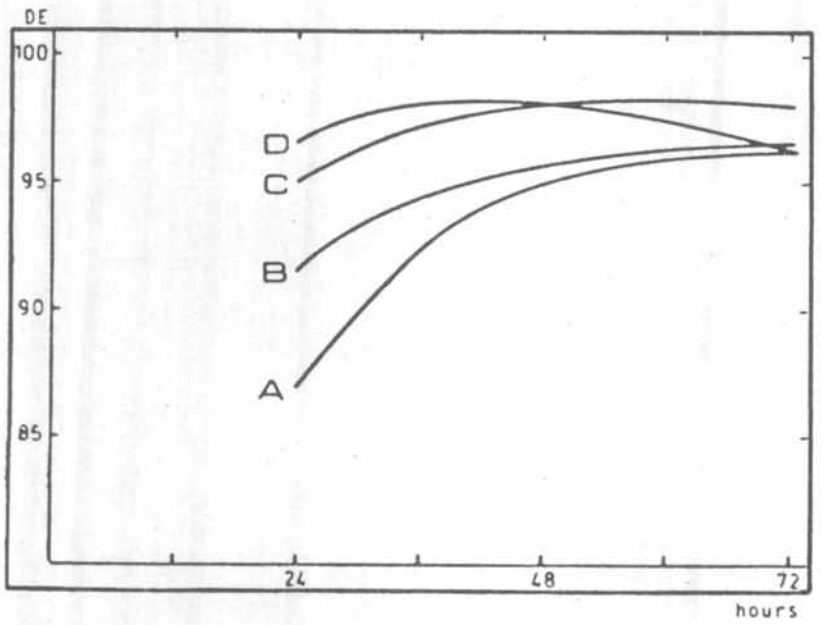


Figure 3 - 16 Effect of enzyme concentration on reaction rate. Substrate concentration: 33%. Temperature: 60°C. pH: 4.5. Enzyme levels: A = 1.5, B = 2.0; C = 3.0; D = 3.5 litres Amyloglucosidase NOVO 75 per ton of starch (19)

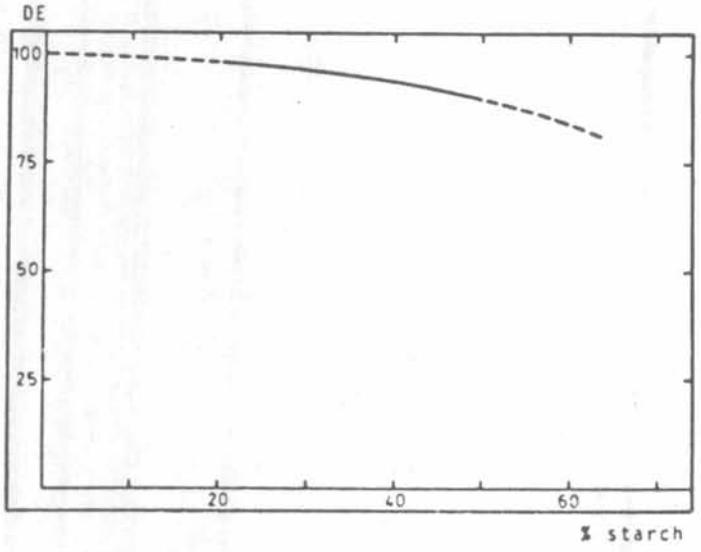


Figure 3 - 17 Dependence of final DE on substrate concentration (19)

NOVO INDUSTRI has developed glucoamylase and marketed under the trade name "Amyloglucosidase" (AMG 150 L) (22). It is produced from a strain of Aspergillus niger and is available in liquid form with a standardized activity of 150 Amyloglucosidase Units per ml (AGU/ml). One NOVO-amyloglucosidase-unit (1 AGU) is the amount of enzyme which splits 1 micromole maltose per minute under standard conditions (26). The typical process conditions are shown in Appendix II (22).

3.3.5 Glucose isomerase

Glucose isomerase (EC 5.3.1.5) catalyzes the isomerization of glucose, an aldose, to the corresponding ketose, fructose (and vice versa) (1). In many instances, the glucose isomerase occurs as an intra-cellular enzyme, although a number of organisms have been identified as producers of extra-cellular glucose isomerase (17).

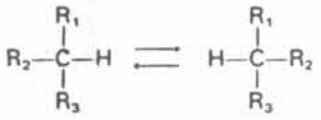
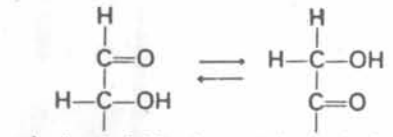
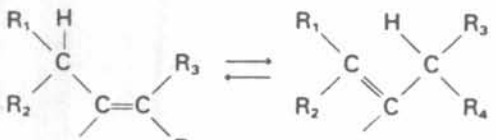
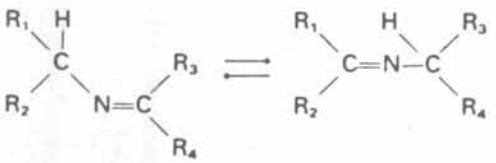
Already in 1957 it was discovered that an enzyme from Pseudomonas hydrophilia, known as a xylose-isomerase, could also isomerize glucose to fructose, although at a considerably lower speed (18). Since then, various teams have worked simultaneously on glucose-isomerizing enzymes from different organisms such as Lactobacillus species, Streptomyces species, Bacillus species (17, 27, 28). It was therefore not until around 1970 that commercial production of isomerized glucose syrups was taken up. In the beginning whole cells of Streptomyces species were predominantly used, but in recent years the development of viable commercial processes has been greatly enhanced by the introduction of immobilized preparations. These enzymes can be used and re-used several times in a batch operation or more important, they can be used in continuous column processes (1).

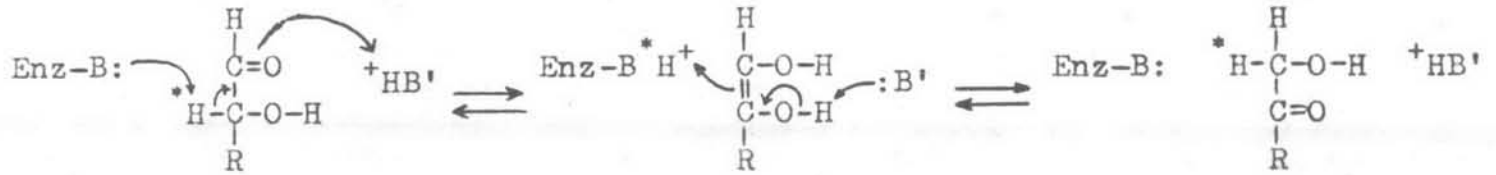
Rose classified the great bulk of enzyme-catalyzed isomerizations into reactions involving 1,1 - , 1,2 - , or 1,3 - hydrogen shifts as shown in Table 3-3 (24). The 1,-2-proton shifts involve formal hydrogen transfer between two adjacent carbon atoms, one undergoing oxidation, the other reduction; this describes the stoichiometry of aldose $\rightleftharpoons$ ketose isomerases. As shown in Figure 3-18 (24), Rose and Hanson have noted that one of the prochiral hydrogens at C-2 of the aldose substrate is removed by the enzyme and transferred, at least partially, to C-1 of the ketose formed. The partial transfer is in keeping with the suggested formation of an enediol or enediolate intermediate and a $BH^{\oplus}$ during isomerization. The intra-molecular hydrogen transfer implies that a single base removes hydrogen from C-2 and adds it back to C-1 of the enediol (or the opposite in the reverse direction).

Studies of the mechanism of glucose isomerase-catalyzed reactions by Takasaki suggest that metal ions participate in the binding of substrate at the active sites of the enzyme through a metal bridge complex. The enzyme bound metal ion is believed to coordinate with one or two of the oxygen atoms on C-1 of the substrate facilitating proton removal at C-2 by the basic group of the enzyme. This leads to a four-membered ring, the strain on which is relieved by elimination of a ring oxygen and formation of an enediol intermediate and subsequent transfer of the proton to C-1. The keto furanose which forms is then released from the enzyme (17).

Enzyme are commonly water-soluble, and for that reason, many enzyme are uneconomical to use in large-scale batch-type operations since the enzymes can generally be used only one time in the absence of rather

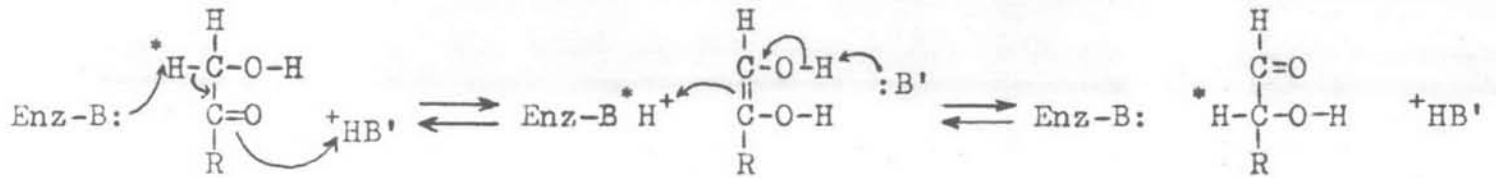
Table 3 - 3 Enzyme - catalyzed isomerizations classified as hydrogen shifts (24)

Type	Examples	Category
1,1-Shifts		Epimerases, racemases
1,2-Shifts	 α-hydroxyaldehyde β-keto alcohol	Aldose-ketose isomerases
1,3-Shifts		Allylic isomerizations
		Azaallylic isomerizations



Aldose

Ketose



Ketose

Aldose

Figure 3-18 A proposed mechanism of glucose isomerase (24)

costly enzyme recovery and purification steps. In recent years, however, techniques have been devised to fix active enzymes on mostly water-insoluble materials that can be readily removed from a reaction, thus permitting re-use of the insolubilized or immobilized enzyme (29).

Four principal methods have been used for immobilization. (1) Adsorption on inert carriers or synthetic ion exchange resins. This has inherent limitations because the adsorbed enzyme is weakly bound and is rather easily lost during use. (2) Entrapping enzyme in gel lattices, the pores of which are too small to allow the enzyme to diffuse but large enough to allow the passage of substrate and product. (3) Covalent binding to a wettable but water-insoluble carrier via functional groups non-essential for their biological activity. (4) Covalent cross-linking of the enzyme protein by an appropriate bifunctional reagent (18, 29, 30).

The immobilized enzymes have advantages over soluble ones: (1) they are chemically and thermally more stable (2) they are easier to recover and re-use; (3) they provide a choice of supporting materials that can be tailored to enhance the specific reaction and reactor design (18); and (4) they permit one to carry out continuous reactions (17). Factors adversely affecting use of immobilized enzymes include: (1) loss of enzyme from the support; (2) slower reaction rate; (3) blocking of enzyme sites and support structure by chemical reaction, by air, or by contaminating solid materials (18).

NOVO INDUSTRI has developed the immobilized glucose isomerase and marketed under the trade name "Sweetzyme" (1). It is produced from a strain of Bacillus coagulans as an intra-cellular enzyme, and consequently the yields are low compared to production of extra-cellular enzymes such as alpha-

amylases and amyloglucasidases (15). This enzyme is available in 2 types: Sweetzyme type A with an activity of about 500 GINU/gm for batch operation and Sweetzyme type Q with an activity of 150 IGIC/gm for continuous isomerization in a fixed bed reactor. One glucose isomerase-unit (1 GIU) is the amount of enzyme which converts glucose to fructose at an initial rate of 1 micromole per minute under standard conditions (31). One immobilized glucose isomerase for column-unit (1 IGIC) is the amount of immobilized enzyme which initially converts 1 micromole of glucose to fructose per minute under standard conditions (32). The typical process conditions for Sweetzyme type A and for Sweetzyme type Q are both shown in Appendix III (22).

3.4 Production of Glucose Syrup

The U.S. Food and Drug Administration (U.S. FDA) defines glucose syrup in the Standard of Identity, as "the purified, concentrated solution of nutritive saccharides obtained from edible starch". The dextrose equivalent is not less than 20. Later, the standards were expanded to the use of alternate and additional names for syrups derived from specific starch, i.e. "corn syrup", "wheat syrup", or "tapioca syrup". The Codex Alimentarius Commission recommends the following definition, "Glucose syrup is a purified concentrated aqueous solution of nutritive saccharides obtained from starch". The dextrose equivalent is not less than 20 (16).

Glucose is known commercially as dextrose (17). Dextrose is the term applied to the product obtained by the complete hydrolysis of starch to obtain D-glucose. Dextrose equivalent is a term widely used in the

syrup industry and is defined as the percentage of reducing sugars in the syrup, calculated as dextrose, on a dry weight or dry substance basis. It is abbreviated as DE (16).

The hydrolysis of starch for the production of glucose syrups is accomplished by three different methods which are acid conversion, acid-enzyme conversion, and enzyme-enzyme or multiple-enzyme conversion (16,18).

3.4.1 Acid conversion

When starch is hydrolyzed with acid as the catalyst, a cleavage of the C-O-C linkages occurs with the production of glucose and many of its polymers. The production is carried out in a pressure vessel termed a "converter". Starch is mixed with water to form a suspension, or slurry, containing 30-40% dry starch. The required amount of dilute acid, usually about 0.12% HCl, based on the weight of starch, is added and the temperature raised by live steam to the desired degree approximately 140°- 160°C. The heating continues for 15-20 minutes. The conversion is allowed to continue until the desired end point of conversion is reached. At that time heating is discontinued and the hydrolysis quickly ended by the introduction of equivalent amounts of a neutralizing agent, usually soda ash. The pH is adjusted to a range of 4 to 5.5 (16).

3.4.2 Acid-enzyme conversion

Glucose syrups are manufactured by means of a two-step hydrolysis process. The first of these steps is accomplished with acid as described above. The extent of the acid conversion is determined by the desired DE value and the carbohydrate composition of the finished syrup. The second step is carried out with starch-splitting enzymes such as alpha-amylase, beta-amylase and glucoamylase. The production of

different types of glucose syrup is dependent upon the selection of the correct enzymes or combinations thereof (16).

3.4.3 Enzyme-enzyme conversion

The initial step consists of gelatinizing the starch by heat, or other suitable procedure, and following this by the use of an alpha-amylase which can withstand the temperature required for gelation of the starch. After this initial step the alpha-amylase is inactivated and then followed by additional enzyme treatments. The selection of enzyme to be used is dependent on the type of syrup that is to be produced (16).

Enzymes have several distinct advantages for use in industrial processes: (1) They are of natural origin and are non-toxic. (2) They have great specificity of action. (3) They work best under mild conditions of moderate temperature and near neutral pH. (4) They act rapidly at relatively low concentrations, and the rate of reaction can be readily controlled by adjusting temperature, pH and amount of enzyme employed. (5) They are easily inactivated when reaction has gone as far as is desired (18).

The advantages of enzymatic methods over acid hydrolysis are due to the specific mode of action of enzymes, whereby much smaller amounts of undesirable by-products are formed than when acid is used. This means better yield of the desired products and less demand for purification, a difference which becomes more pronounced the higher the degree of conversion (1). The ability of enzymes to exert their effect at relative low temperatures further aids in avoiding undesirable side reactions (17).

Their removal from the reaction system after completion is often unnecessary since enzymes can usually be employed at very low concentrations (18). In addition, the enzymatic process also has the advantage of obviating the need for expensive corrosion-resistant equipment because the hydrolysis takes place under mild conditions as regards pH and temperature (1).

In recent years the acid thinning of starches has been replaced by bacterial alpha-amylase thinning of starches (17). The glucose syrup may be produced by either an acid-enzyme process or a dual enzyme process, but the highest final dextrose equivalent is obtained when using the dual process (1, 19, 28). However, enzymes have not yet totally replaced acid in liquefaction, partly because many plants are still equipped for the acid process, and partly because enzymatic liquefaction of certain starch types (corn, wheat) poses some filtration problems (1). The slow filtration rates for the glucose syrup are due to undigested residual solids. One method which obviates this difficulty is to employ a steam jet heater for the bacterial enzyme liquefaction step (18). Special heat treatment of the starch slurry by a steam jet heater or pressure cooker causes almost instantaneous gelatinization of the starch and coagulates the protein and fat impurities (1). The amount of residual insolubles is greatly decreased and filtration rates are markedly improved by this method (18).

NOVO INDUSTRI recommends a dual enzyme process for the production of glucose syrup. Liquefaction of gelatinized starch is accomplished by use of bacterial alpha-amylase (Termamyl) and saccharification is allowed to proceed to completion by use of Amyloglucosidase (AMG).

Typical product is recommended to be composed of 95-96% D-glucose (22).

Glucose syrup finds extensive use throughout the food industry (18). Hoover has prepared a check list of functional uses of glucose syrup or corn syrup in specific food products as shown in Figure 3-19 (16). The selection of the type of syrup which is best suited to a given food formulation depends on the type of effect that is wanted.

3.5 Production of Fructose Syrup

A separate definition for fructose syrup has not as yet been officially adopted either by the corn refining industry or by the U.S. Food and Drug Administration. It is considered by FDA as a glucose syrup even though its chemical and physical properties are quite different. It is chemically more like an invert syrup because of the presence of glucose and fructose but the ratio of these sugars is different from invert syrup (16).

Fructose is known commercially as levulose (17). It has the highest sweetness value of any of the commercial sugars (16, 19, 27). If crystalline of sugars are compared, fructose is about 1.2-1.8 times as sweet as sucrose (16, 19) and more than twice as sweet as glucose (19). When in solution, however, certain factors affect the sweetness intensity. These include concentration of sugar, temperature, and pH (16). Fructose's primary industrial source is sucrose from sugar cane and sugar beets and its major commercial availability is in solution together with glucose in invert sugar (27).

Checklist of properties and functional uses of corn syrups in specific food products	Bodying agent	Browning reaction	Cohesiveness	Fermentability	Flavor enchancement	Flavor transfer medium	Foam stabilizer	Freezing point depression	Humectancy	Hygroscopicity	Nutritive solids	Osmotic pressure	Prevention of sugar crystals	Prevention of coarse ice crystals	Sheen producer
Baby foods	●										●				●
Bakery products		●		●	●						●				
Beverages, brewed	●			●											
Beverages, carbonated — non-alcoholic						●					●				
Breakfast foods									●		●		●		
Catsup, chili sauce, tomato sauce					●						●				●
Cereals, prepared									●		●				
Cheese spreads and foods	●		●								●				
Chewing gum	●		●			●					●		●		
Chocolate products											●				
Citrus juices, dried						●					●				
Condensed milk	●										●	●	●		
Confections	●				●	●	●		●	●	●	●	●		●
Cordials and liqueurs	●					●									
Licorice											●		●		
Malted products						●			●		●				
Marshmallows and related products	●						●		●	●			●		
Meat products	●	●	●								●				
Mince meat	●		●			●					●				
Mixes, prepared (cake, pie filling, puddings, etc.)		●				●	●		●		●		●		●
Peanut butter	●		●								●				
Pickles and pickle products						●					●				●
Pie fillings, cream and fruit						●					●		●		●
Pork and beans, canned		●				●					●				
Sauces		●				●					●				●
Sausages	●		●								●				
Soups, dehydrated									●		●				
Sweet potatoes, canned						●					●				●
Syrups, table, soda fountain, cordial						●					●				
Toppings						●					●	●	●		●
Wine	●			●											

Figure 3 - 19 Checklist of properties and functional uses of corn syrups in specific food products (16)

The conversion of glucose to fructose is one of a group of reactions collectively known as the Lobry de Bruyn - Alberda van Ekenstein transformation (6, 19, 27, 28). Such reactions are favoured by alkaline conditions and high temperatures and the equilibrium mixture will contain the other sugar as shown in Figure 3-20 (6, 28). The alkaline isomerization of glucose has been studied in some details as a possible means of producing fructose commercially (28). However, the lack of selectivity of the alkaline isomerization allows the production of non-metabolizable material such as psicose and objectionable, colored and off-flavoured materials which are costly to remove (17, 28, 33, 34). Consequently, chemical isomerization of glucose has not been employed commercially.

The discovery of enzyme systems by which glucose could be isomerized directly to fructose was a key factor in the development of commercial scale processes (17). The finding of glucose isomerase enzyme since 1957 and the development of this enzyme from different organisms make the commercial production of isomerized glucose syrups possible (27, 28). By the introduction of immobilized preparations, this enzyme can be used and re-used several times in a batch or continuous operation (1).

NOVO INDUSTRI recommends the process lay-out for the production of fructose syrup from glucose syrup with Sweetzyme (immobilized glucose isomerase) as shown in Figure 3-21 (35). The process steps are conveniently divided into three groups (36):

1. Pre-treatment: Filtration, carbon-treatment, ion-exchange, and evaporation.

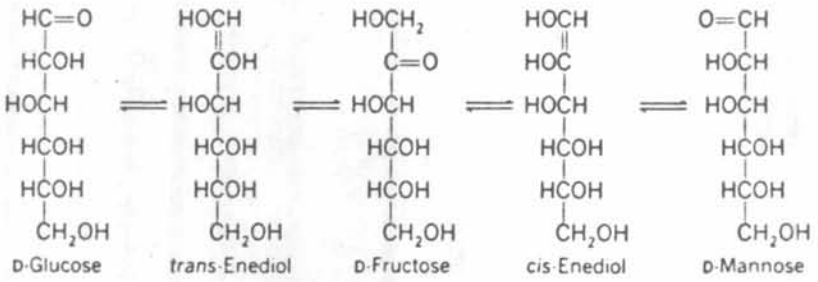


Figure 3 - 20 Isomerization of glucose in dilute alkali (6,28)

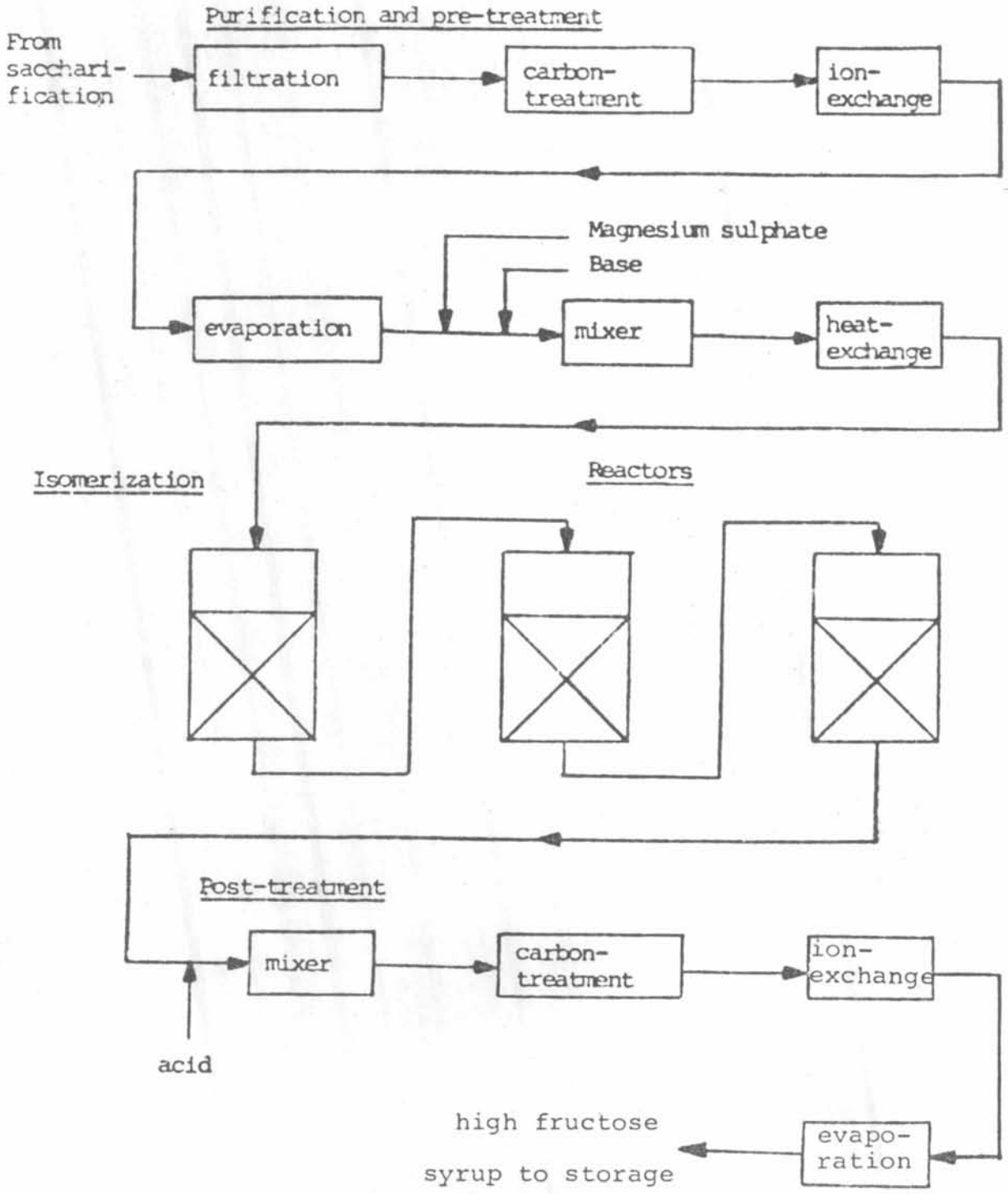


Figure 3 - 21 Process lay - out for the production of fructose syrup from glucose syrup (35)

2. Isomerization: Magnesium addition, alkaline pH-adjustment, temperature adjustment, isomerization and acidic pH-adjustment.

3. Post-treatment: Carbon-treatment, ion-exchange and evaporation.

3.5.1 Pre-treatment

Glucose syrup resulting from enzyme liquefaction and saccharification of starch contains various impurities, which should be removed before isomerization. These may be of two kinds: Insoluble (particulate) impurities, e.g. fats and proteins, and soluble impurities, e.g. peptides, amino acids, and salts. The particulate impurities are removed by filtration or by centrifugation and filtration. The filtrate is then purified by treatment with powdered or granular active carbon and passed through an ion-exchange unit to remove soluble impurities and reduce the calcium content to less than 1 ppm. After this, the purified syrup is concentrated to 40% dry substance by evaporation under vacuum. The evaporation also serves to remove air dissolved in the syrup.

3.5.2 Isomerization

Various factors influencing activity and stability of Sweetzyme are shown in Figure 3-22 (36, 37). The following parameters will be dealt with:

3.5.2.1 Contact time

For a given conversion to be achieved with a given amount of enzyme, the lower the activity the longer the contact time. Long contact time gives rise to by-product and color formation. This will contribute to increased costs. By-products may block the active centre of the enzyme and thus decrease its stability. Furthermore, the consumption of carbon for decolorizing the syrup grows with increasing color formation.

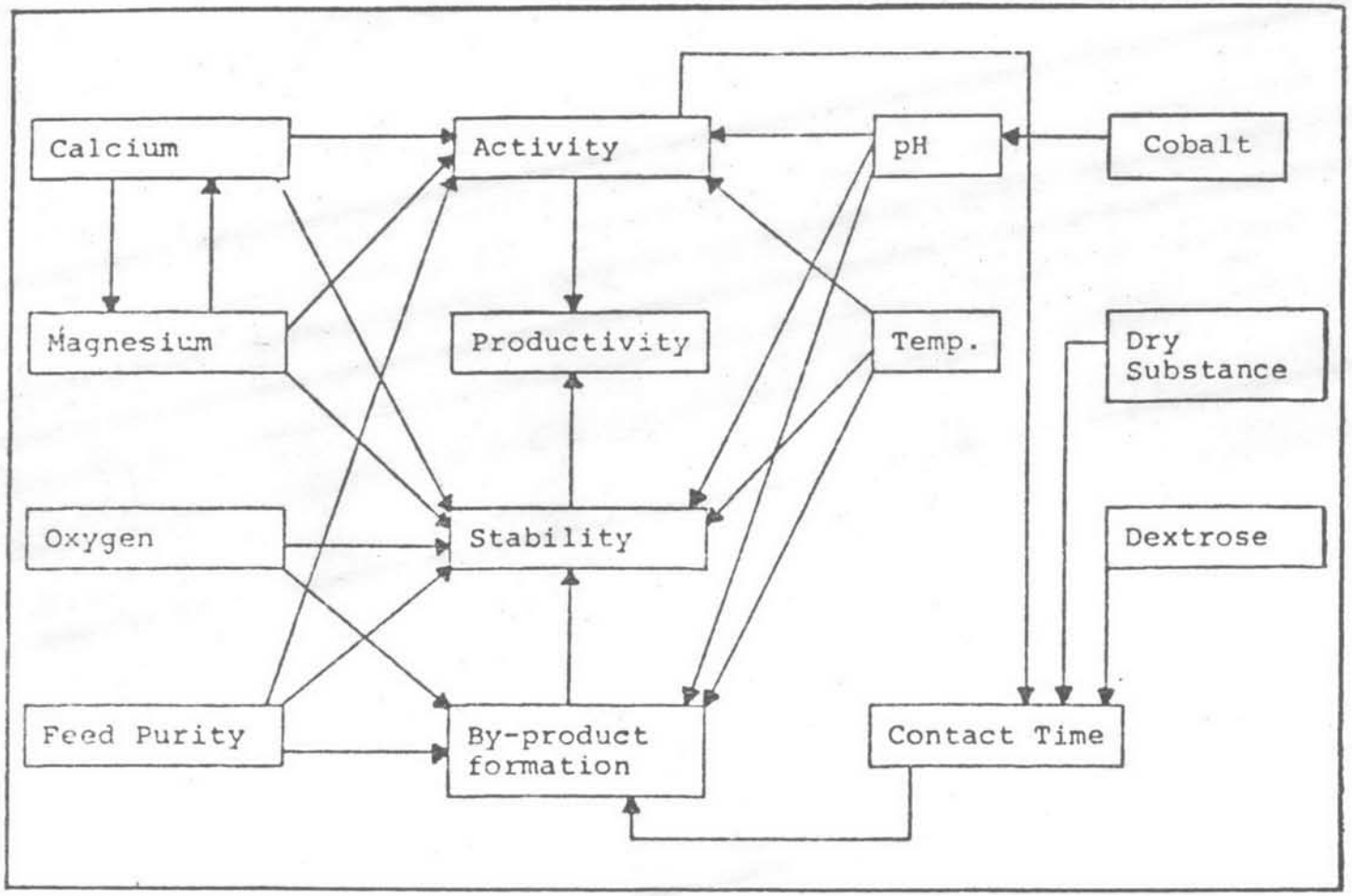


Figure 3 - 22 Influence of the individual process parameters on activity/stability and hence productivity of Sweetzyme (36,37)

3.5.2.2 Temperature

The temperature during the isomerization has an influence on activity, stability, and by-product formation. Figure 3-23 shows the relationship between temperature and activity and Figure 3-24 shows the relationship between temperature and time to 50% residual activity (37).

3.5.2.3 pH

The pH of the feed syrup has an influence on activity, stability and by-product formation. Figure 3-25 and 3-26 show the relationship between pH and activity and between pH and stability respectively (35).

3.5.2.4 Cobalt

It is a well-known activator of glucose isomerase enzymes. Cobalt ions at 10^{-3} M and higher activate the enzyme in the presence of magnesium ions. They also protect the enzyme against heat denaturation (28). Cobalt must be added to the glucose syrup feedstuff to stabilize and preserve the activity of glucose isomerase. However, NOVO INDUSTRI reveals that cobalt addition to the syrup can be omitted at a pH value above 8. Table 3-4 shows productivity and residual activity for continuous isomerizations at different pH values with and without cobalt addition. It can be seen that at low pH values, cobalt addition is essential for the total productivity, whereas no improvement is seen at high pH (37).

3.5.2.5 Magnesium and calcium

Magnesium ions in the feed syrups activate and stabilize the enzyme while calcium ions acts as inhibitors (35). Experimental work with Sweetzyme, however, shows that magnesium and calcium are competitors

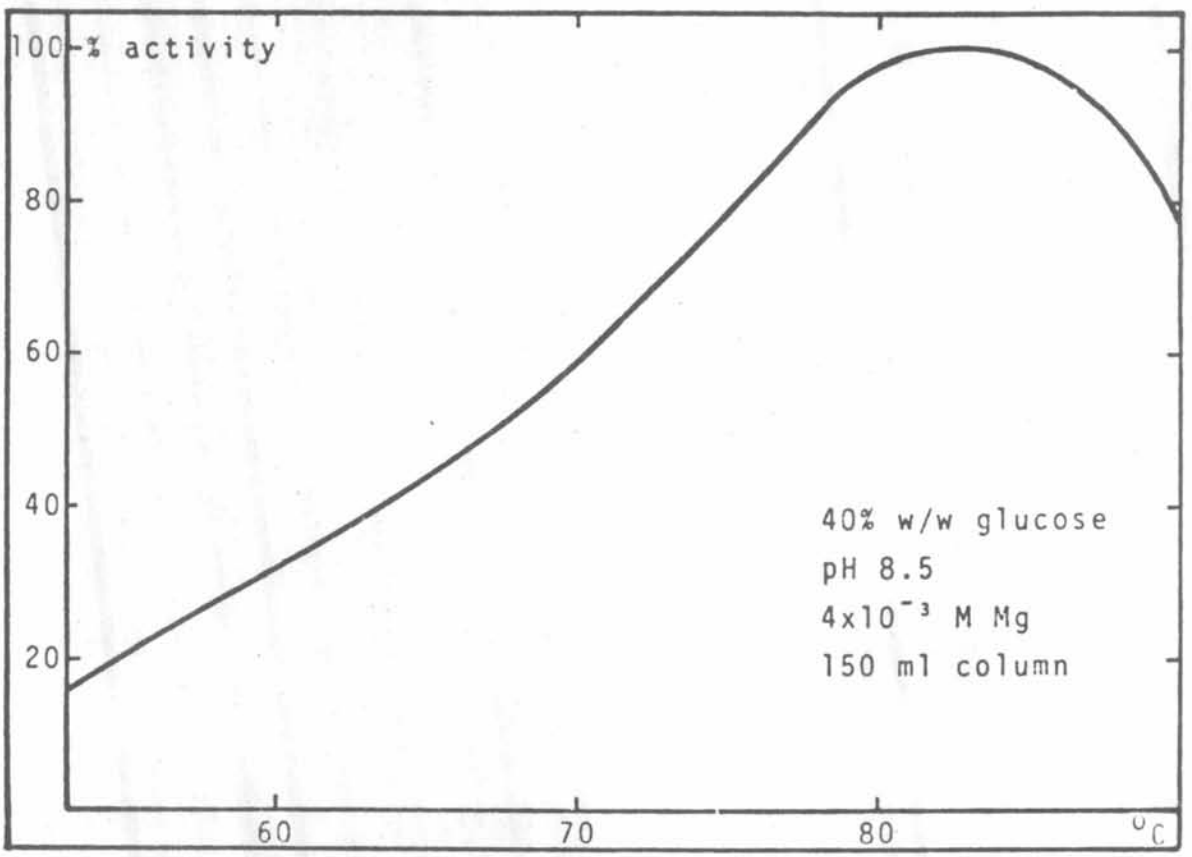


Figure 3 - 23 Temperature/activity profile of Sweetzyme (37)

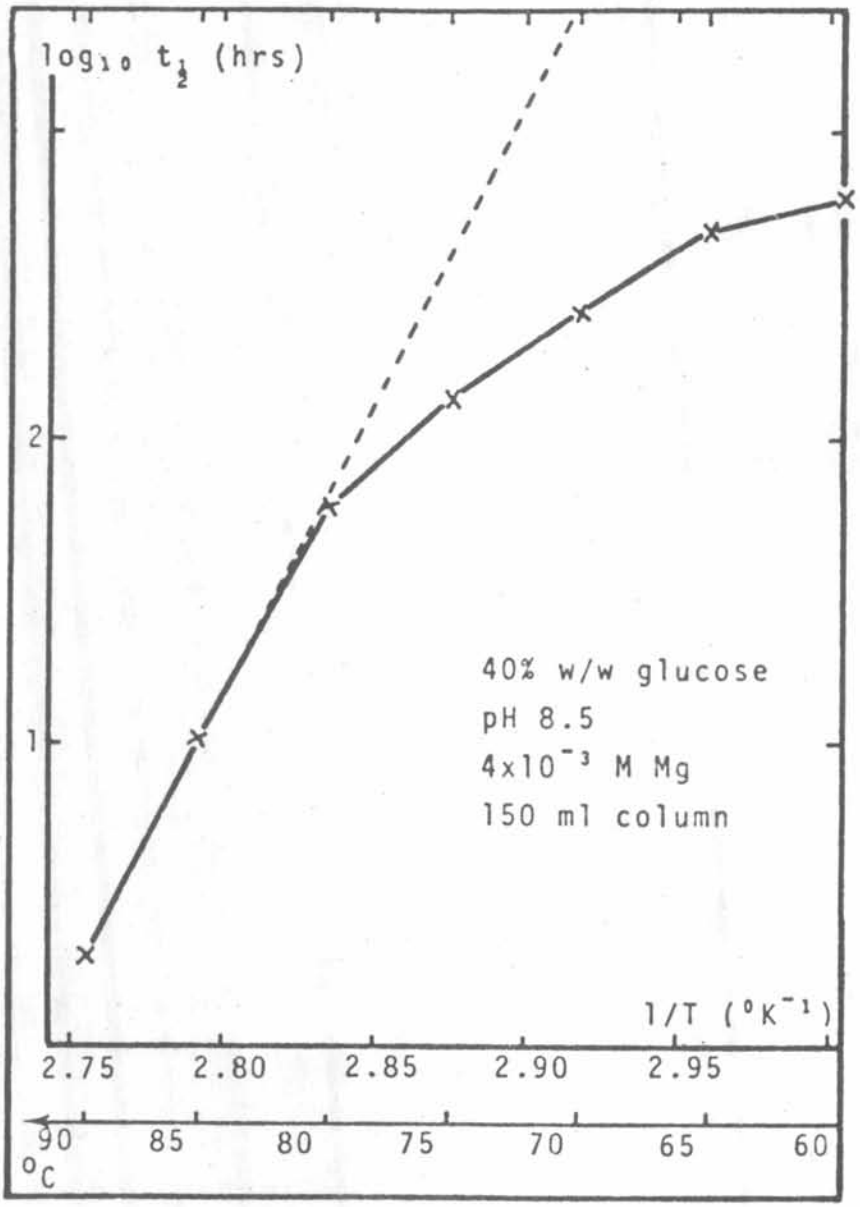


Figure 3 - 24 Temperature/stability profile of Sweetzyme (37)

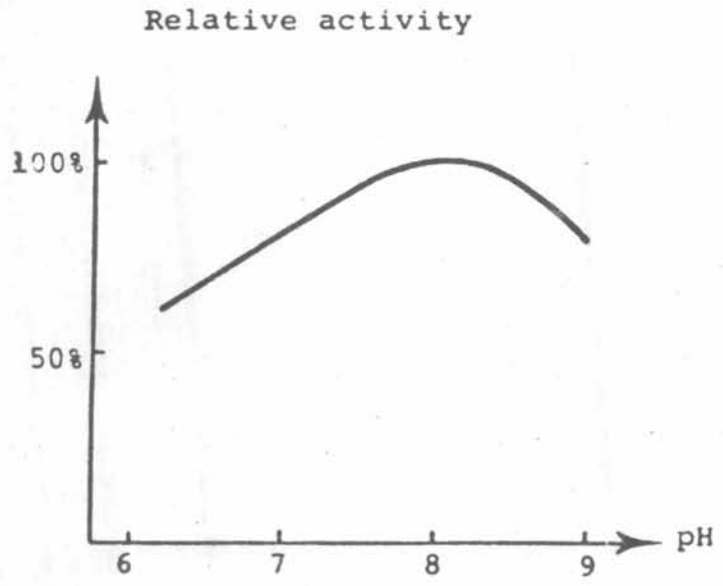


Figure 3 - 25 Initial activity of Sweetzyme versus pH (35)

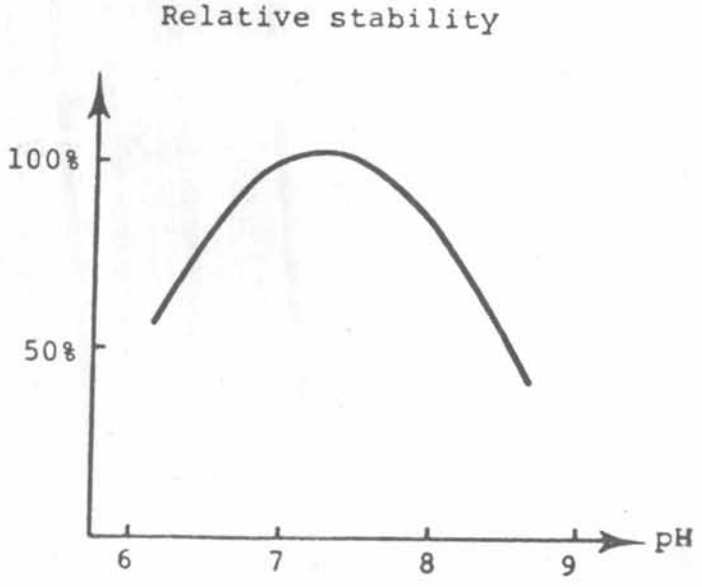


Figure 3 - 26 Stability of Sweetzyme versus pH (35)

Table 3 - 4 Influence of cobalt addition to the syrup on the stability of Sweetzyme (37)

	pH 7.6		pH 8.5	
	3.5×10^{-4} M Co	No Co	3.5×10^{-4} M Co	No Co
Productivity kg/kg enzyme	615	560	590	630
Residual activity	41%	28%	42%	52%
Process parameters:				
Syrup:	40% w/w glucose			
Temperature:	65°C			
Mg:	8×10^{-3} M Mg			
Activity:	150 IGIC			
Isomerization time:	450 hours			

and that the inhibitory effect of calcium can be overcome by extra addition of magnesium (37). Table 3-5 shows the influence of the addition of magnesium on the stability of immobilized glucose isomerase (IGI). These results show that the addition of magnesium does not affect the productivity or the residual activity of IGI if no calcium is present in the syrup (32). Figure 3-27 shows the influence of magnesium and calcium on the activity of IGI. On the basis of experiments of this type, it is concluded that calcium has no effect on productivity provided that the molar ratio of magnesium to calcium exceeds 10 (32).

3.5.2.6 Oxygen

Presence of oxygen in the feed syrup will increase the by-product formation and this may impair the activity of enzyme. Figure 3-28 shows the effect of short repeated exposure to oxygen on the stability of the enzyme. The figure illustrates the stability in batch operation with and without contact with air between re-uses (37).

3.5.2.7 Feed purity

The purity of the syrup is important to enzyme stability as shown in Figure 3-29. The high DE syrup resulting from liquefaction and saccharification of starch should be purified by filtration, carbon-treatment, cation and anion-exchange before it is isomerized. It can be seen from the figure that filtration alone leads to a drastic decrease in activity, whereas the full purification increases the stability considerably (37).

3.5.2.8 Dry substance content

The concentration of the syrup during isomerization influences the contact time between enzyme and syrup (37).

Table 3 - 5 Influence of the addition of Mg^{++} on the stability of immobilized glucose isomerase (32)

	Addition of Mg M				
	0	4×10^{-4}	8×10^{-4}	4×10^{-3}	8×10^{-3}
Productivity kg/kg enzyme	590	630	635	620	630
Residual activity, %	50	50	50	48	52

Process parameters

syrup ..... 40% w/w glucose
temperature ..... 65.0°C
pH 8.5
activity ..... 150 IGIC/g
isomerization time ..... 450 hours

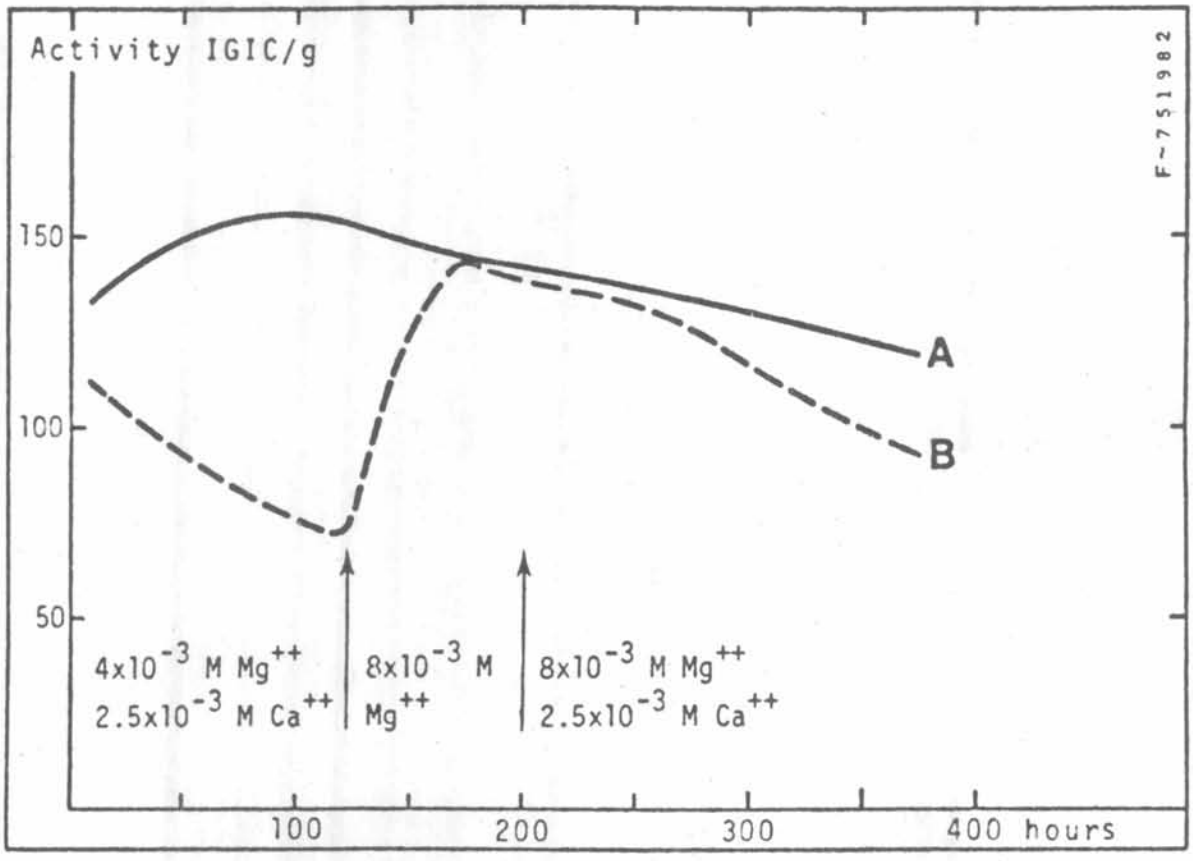


Figure 3 - 27 Influence of magnesium and calcium on the activity of immobilized glucose isomerase

A: Control column - B: Calcium - treated column (32)

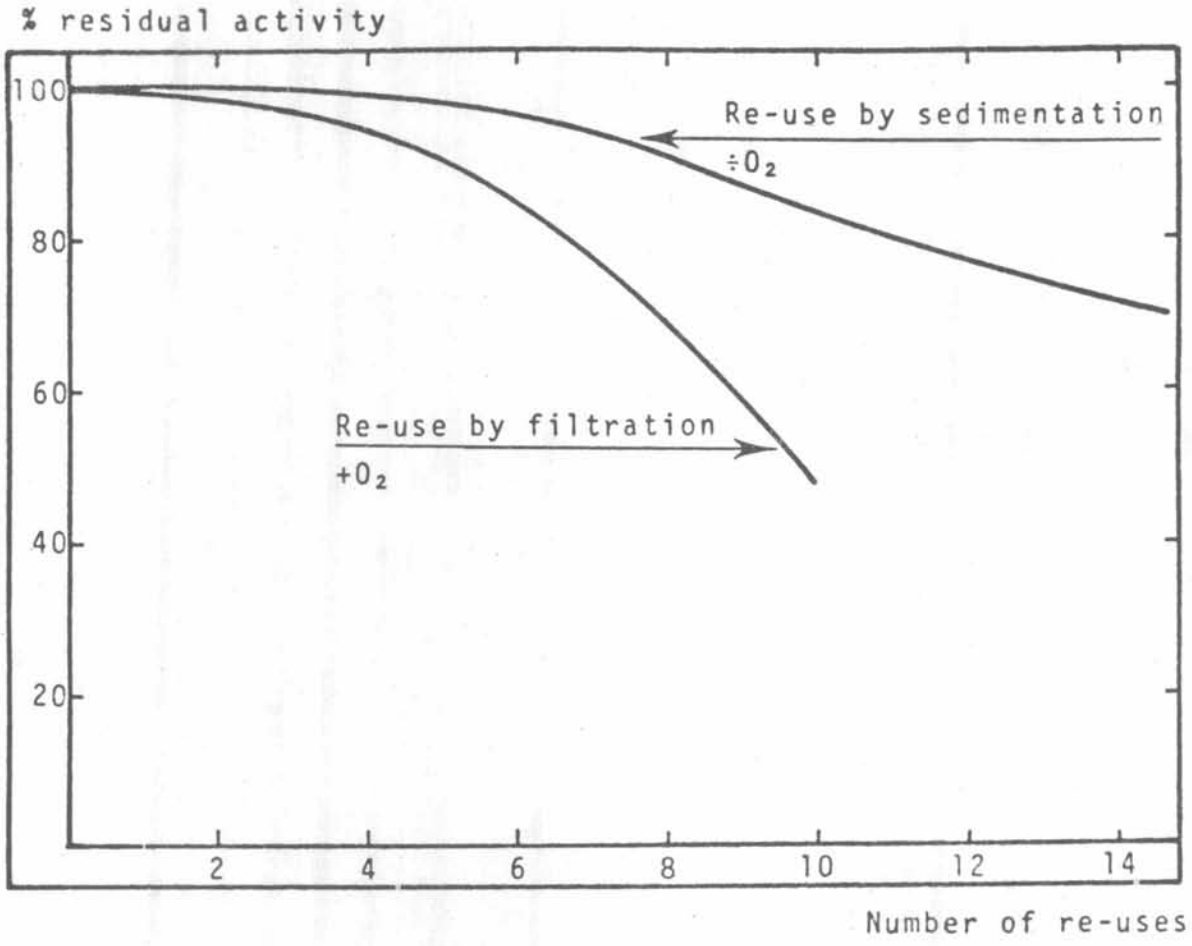


Figure 3 - 28 Influence of oxygen on the stability of Sweetzyme. Batch operation, 20 hour isomerizations (37)

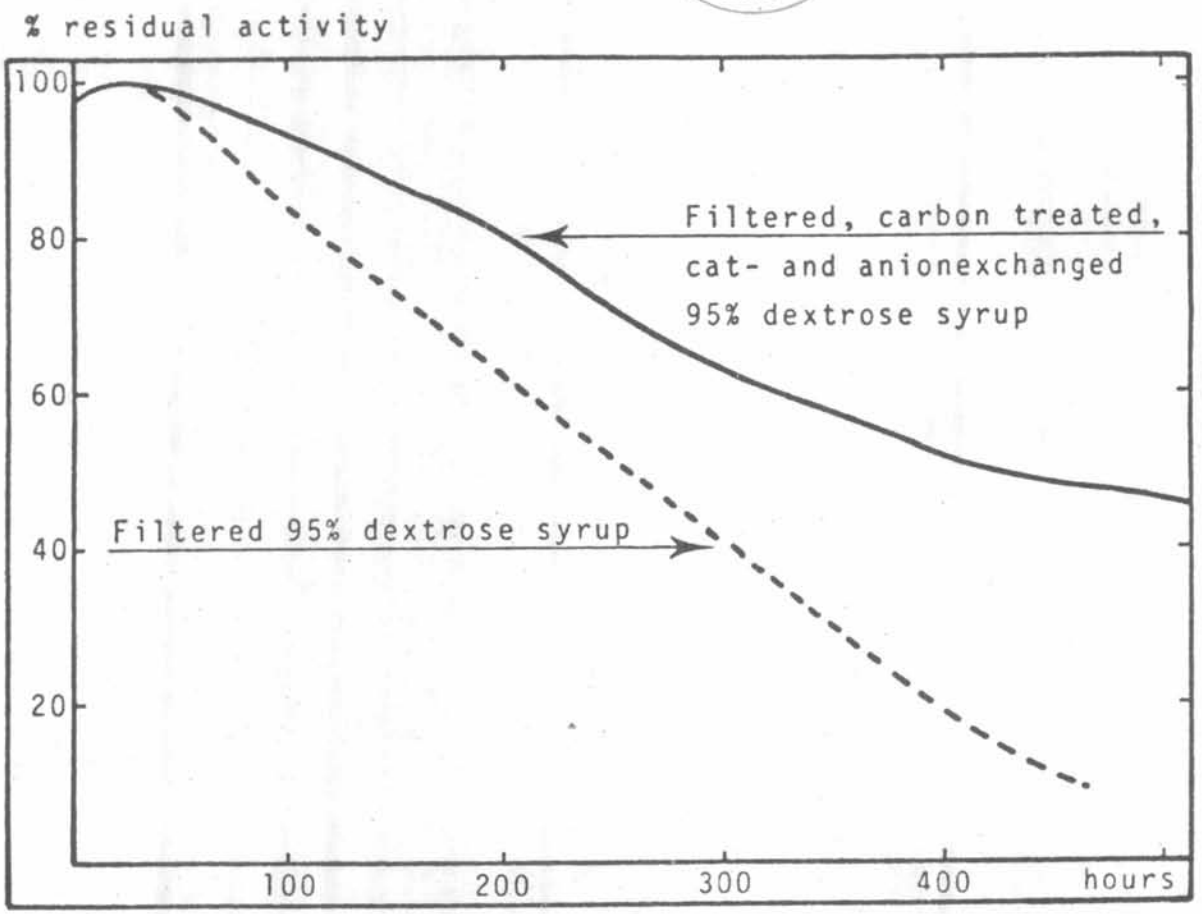


Figure 3 - 29 Influence of syrup purity on the stability of Sweetzyme (37)

3.5.2.9 Dextrose content in the feed syrup

Glucose isomerase is completely specific for monomeric D-glucose. The maltose, maltotriose and higher maltooligosaccharides present in glucose syrup remain untouched by the enzyme. Consequently the final amount of fructose which may be produced in syrup is dependent on the amount of glucose present in the substrate (28). Any reduction of the glucose content in the inlet syrup will require an increase in the degree of conversion. In other words, the glucose content of the inlet syrup should be as high as possible and preferably not less than 93% (1).

The advantage of the immobilized enzymes is that they permit one to carry out batch or continuous reactions. The batch operation is carried out in a heated tank equipped with pH control under gentle stirring while the continuous operation is carried out in a column (37). The enzyme concentration in column is extremely high compared to much lower concentration in tank (17). Preferred process parameters are shown in Table 3-6 for both batch reaction and continuous isomerization (27).

Sweetzyme type A can be re-used to isomerize the next batch of syrup. Figure 3-30 shows the activity decrease with re-uses. The enzyme does not show any sign of physical degradation after more than 20 re-uses, but the activity decreases slowly after about 5-6 re-uses. To maintain a constant fructose content in the fructose syrup, the reaction time has to be prolonged or extra enzyme must be added (37).

Table 3 - 6 Immobilized glucose isomerase: optimal values for practical application in a plug flow process compared to proposed values for batch operation with soluble glucose isomerase (27)

	Immobilized glucose isomerase plug flow process	Soluble glucose isomerase batch operation
Temperature	65°C	65°C
pH	8,5	7,0
Co^{2+}	0	$3,5 \times 10^{-4}$ M
Mg^{2+}	4×10^{-4} M	8×10^{-3} M
Ca^{2+}	$Mg^{2+}/Ca^{2+} > 10$	not specified
Substrate	2,6-3,0 M glucose	2,6-3,0 M glucose

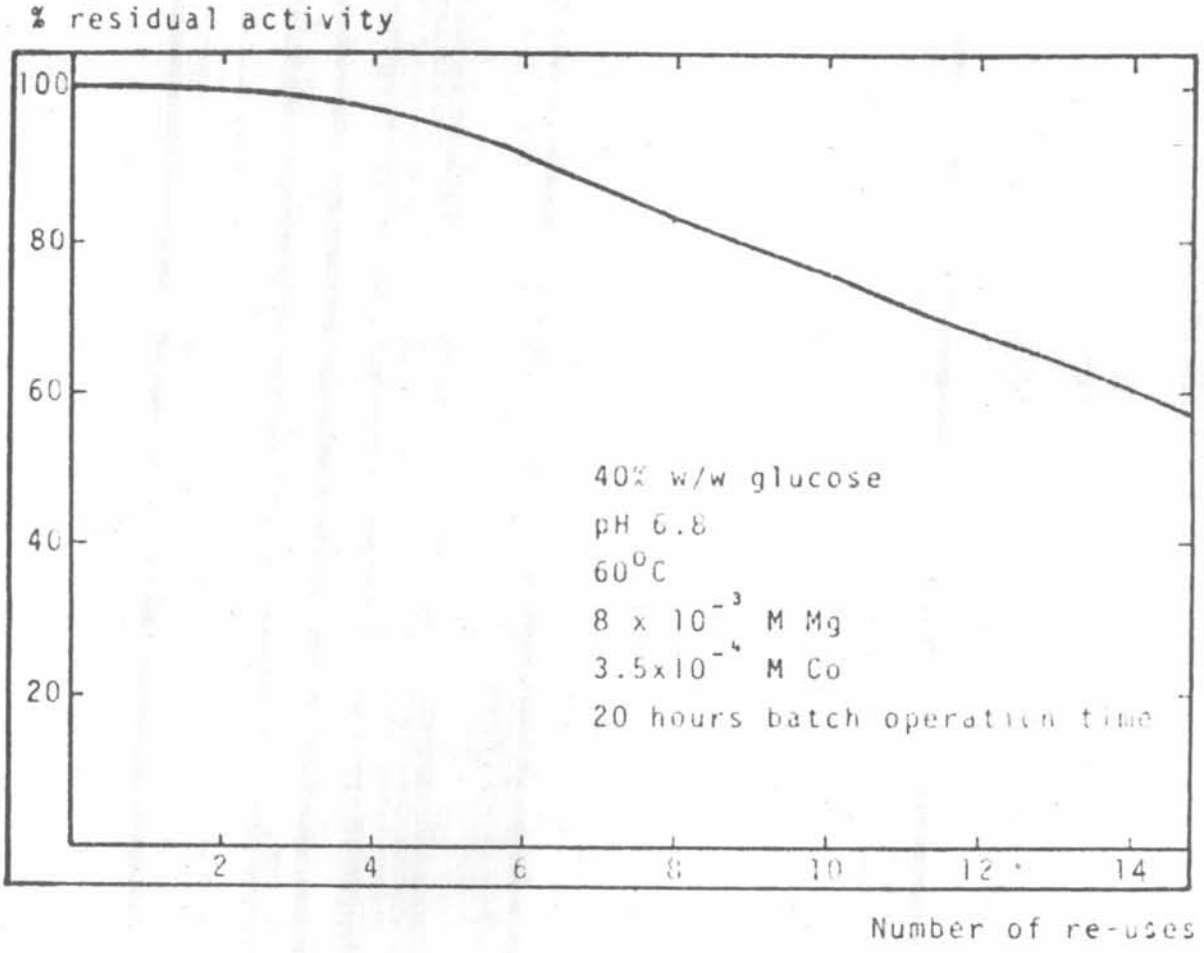


Figure 3 - 30 Stability of Sweetzyme - batch re-use (37)

Sweetzyme type Q is used in a continuous operation in which the enzyme is loaded into a column and a high glucose syrup passed through the enzyme bed is characterized by a short contact time between enzyme and syrup. The main advantage of continuous process is that the addition of cobalt can be omitted and the addition of magnesium can be considerably reduced. This will greatly influence the overall isomerization cost. The cost for additives will be greatly reduced, but more important, the cost of post-isomerization purification will be considerably lower (37).

After isomerization the syrup from the reactor is immediately combined with acid (to pH 4-5) in order to keep the residence time at high pH at a minimum (36).

3.5.3 Post-treatment

The syrup is purified by carbon-treatment to remove color and by ion-exchange to remove cobalt and magnesium. In the cases of syrup from continuous isomerization, the syrup should be cation-exchanged if a specially low ash content of syrup is desired (37). After this, the syrup is concentrated to 71% dry substance by evaporation under vacuum and stored. A typical fructose syrup may have 42% fructose, 53% glucose and 5% oligosaccharides of dry substance (35).

Products in which the fructose syrup can be used and the advantages provided by the syrup are listed in Table 3-7. The certain of the advantages noted may be observed in other related products (33).

Table 3-7 Advantages of fructose syrup in food products (33)

Food Products	Advantages
Spaghetti Sauce	1. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.
	2. Osmotic pressure may be higher thus decreasing the possibility of bacterial growth.
	3. A higher degree of sweetness is provided than can be provided by the use of corn syrup.
Peanut Butter	1. A higher degree of sweetness is provided than can be provided by the use of corn syrup.
	2. Osmotic pressure may be higher thus decreasing the possibility of bacterial growth.
Maraschino Cherries	1. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.

Table 3-7 (cont.) Advantages of fructose syrup in food products

Food Products	Advantages
	2. Cherries are crisper than when corn syrup and/or sucrose is used.
	3. A higher degree of sweetness is provided than can be provided by the use of corn syrup.
	4. Osmotic pressure may be higher thus decreasing the possibility of bacterial growth.
Whipped Toppings	1. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.
	2. Osmotic pressure may be higher thus decreasing the possibility of bacterial growth.
	3. Viscosity of syrup may be lower thus improving the quality of the product.
Canned Sweet Potatoes	1. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.

Table 3-7 (cont.) Advantages of fructose syrup in food products

Food Products	Advantages
	2. A higher degree of sweetness is provided than can be provided by the use of corn syrup.
Ice Cream and the like	1. A higher degree of sweetness is provided than can be provided by the use of corn syrup.
	2. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.
Jams, Jellies and Preserves	1. Flavor is enhanced; Seems to be a synergistic effect between the fructose-containing syrup and the natural flavor of jams, jellies and preserves which brings about an enhancement of flavor beyond that obtained when either a corn syrup or sucrose is used.
Confectioneries - nougat, caramel and jellies (starch and pectin)	1. The grain size and crystal growth may be effectively controlled.

Table 3-7 (cont.) Advantages of fructose syrup in food products

Food Products	Advantages
	2. The tenderness of the confectioneries can be enhanced to a greater degree than can be achieved by the use of corn syrup and/or sucrose.
	3. Moisture control is easily achieved.
	4. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.
Catsup	1. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.
	2. A higher degree of sweetness is provided than can be provided by the use of corn syrup.
Toppings	1. Osmotic pressure may be higher thus decreasing the possibility of bacterial growth.
	2. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.

Table 3-7 (cont.) Advantages of fructose syrup in food products

Food Products	Advantages
	3. Viscosity may be less than that which can be achieved by the use of corn syrup and/or sucrose.
Marshmallows	1. Tenderness can be enhanced to a greater degree than can be achieved by the use of corn syrup and/or sucrose.
	2. Air cell size may be decreased to a greater extent.
	3. Shelf life increased due to humectant action of the syrup.
	4. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.
Soft Drink and Fountain Syrups	1. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.
Salad Dressings	1. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.

Table 3-7 (cont.) Advantages of fructose syrup in food products

Food Products	Advantages
	2. Osmotic pressure may be higher thus decreasing the possibility of bacterial growth.
	3. Viscosity may be less than that which can be achieved by the use of corn syrup and/or sucrose.
Table Syrups	1. Possibility of sucrose inversion is eliminated thus decreasing the chance for dextrose crystallization.
	2. Osmotic pressure may be higher thus decreasing the possibility of bacterial growth.
	3. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.
Pork and Beans and Baked Beans	1. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.
	2. Improved color control can be achieved.

Table 3-7 (cont.) Advantages of fructose syrup in food products

Food Products	Advantages
Frozen Fruits	1. Improved sheen or gloss and color are achieved. 2. A more thorough coating of the fruit is achieved in the case of corn syrup. 3. Greater penetration of the fruit is achieved by the fructose-containing syrup than is achieved in the case of sucrose.
Glazed Fruits	1. Moisture control is easily achieved thus increasing shelf life. 2. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage. 3. Color will be enhanced. 4. Flavor is enhanced; seems to be a synergistic effect between the fructose-containing syrup and the natural flavor of the product which brings about an enhancement of flavor beyond that obtained by the use of corn syrup or sucrose.

Table 3-7 (cont.) Advantages of fructose syrup in food products

Food Products	Advantages
Sweet Pickles	1. Osmotic pressure may be higher thus decreasing the possibility of bacterial growth. 2. Crispness is enhanced due to the ease with which the fructose-containing syrup penetrates the pickle. 3. Aging is eliminated since sucrose inversion is not required. 4. Tissue damage is reduced due to the ease with which the fructose-containing syrup penetrates the pickle.
Cookies	1. Desirable color may be more easily obtained. 2. Possibility of sucrose inversion is eliminated thus providing a constant sweetness upon storage.