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ENZYMATIC MODIFICATION OF STARCH

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ABSTRACT

Enzymes are versatile catalysts largely used in industry. The starch industry – considered in terms of either starch extraction from corn (raw material) or starch modification (baking and syrups production) – uses huge amounts of enzymes (glucoamylase, bacterial α-amylase, fungal α-amylase, β-amylase, glucose isomerase, pullulanase, xylanase, fungal acid protease, among others). In this work, we discuss processes by which starch is partially (baking) or fully hydrolyzed (syrups production) and the extraction of pure starch from corn grains. Some health hazards related to the enzyme handling are also discussed.

KEYWORDS: Starch, enzymes, carbohydrases, proteases, syrups, baking.

INTRODUCTION

Starch is a source of life-sustaining chemical energy that is constantly replenished by plant photosynthesis. Thereby, the main sources of starch are plants in which it is found in granules. The main characteristics of starch granules are **composition** (15-30% of amylose and 70-85% of amylopectin), **birefringence**, **shape**, and **size**.[1]

Amylose and amylopectin – which are joined inside the granule through hydrogen bonds – are linear (glucose monomers are linked by α-1,4 glycosidic bonds) and branched (glucose monomers are linked to the main chain by α-1,4 glycosidic bonds and by an α-1,6 glycosidic bond at the branching points) polymers of glucose, respectively.[2] Aqueous solutions of amylose and amylopectin in presence of iodine acquire blue and reddish colors, respectively.

The starch industry involves either starch production (corn is the main source) or starch modification (for example, wheat flour in baking). Moreover, starches from potato, soya, cassava, barley, among others, are also available.

The aim of this work is to present a concise description of enzymes used in baking (low degree of starch hydrolysis), corn starch production and extensive starch hydrolysis (syrups). In addition, health hazards regarding the use of enzymes are discussed.

BAKING

The addition of enzymes is indispensable in baking because the natural constituents of wheat (mainly starch and proteins) must be modified in some extension for obtaining dough with extensibility and hardness suitable for handling at the high scale bakery automated machinery.[3]

The main enzymes used in bakery are amylases and proteases, although lipoxygenase, xylanase and pentosanase are also used to some extent.[4]

Although α-amylase, β-amylase and glucoamylase are highly available enzymes, α-amylase is by far the most used in bakery. The activity of β-amylase, present in wheat flour, must be considered because it can interfere with the amounts of fermentable sugars in flour, leading to uncontrolled dough growing.[5] The actions of α- and β- amylases and glucoamylase on amylose and amylopectin are presented in Figure 1.

α-amylase is found in plants, animals and microorganisms. Organisms of microbial origin are the most used in bakery. Amylases have maximum pH activities at the range 4.5-7.0. The heat stability varies according to the source of the enzyme (Table 1).

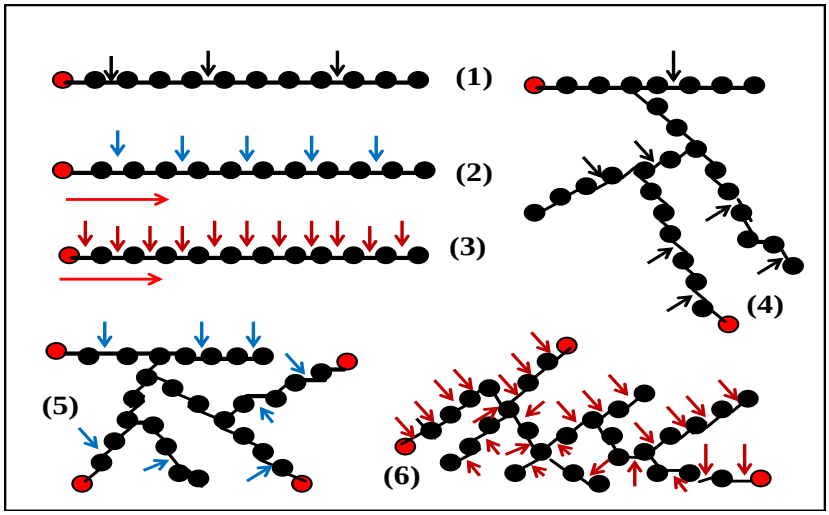

FIGURE 1: Hydrolysis of amylose and amylopectin, respectively, by α-amylase [(1) and (4)], β-amylase [(2) and (5)] and glucoamylase [(3) and (6)]. The red arrows indicate that β-amylase and glucoamylase disrupt the glycosidic bond from the non-reducing end of

the polymer chain (●). Only glucoamylase disrupts glycosidic bonds (α,1-6) at the ramification point. The actions of α- and β-amylases result in α- and β-dextrins, respectively.

Table 1: Effects of temperature on fungal and bacterial α-amylases.

Temperature (^{O}C)	Fungal α-amylase SKB	Bacterial α-amylase SKB
40	800	800
45	650	786
50	245	765
55	94	725
60	-	700
65	-	680

The fungal α-amylase – obtained from *Aspergillus oryzae*, *A. niger* and *A. awamori* – is less heat-stable than the bacterial α-amylase obtained from *Bacillus subtilis* and *B. stearothermophilus*. The use of fungal α-amylase in baking is favored over the bacterial one because its thermal sensitivity prevents the hydrolysis of gelatinized starch in the finished loaf, leading to soft or sticky crumbs. Bacterial α-amylase can also be used but at an adequate dose to avoid bread with a gummy taste.

The α-amylase activity is expressed as SKB units. The test – originally proposed by Sandstedt, Kneen and Blish, therefore SKB unit – consists in the hydrolysis of a standard dextrin solution with α-amylase in presence of iodine up to a defined blue color end point. Thus, one SKB unit corresponds to the inverse of the reaction time required to reach the prescribed color end point.[6]

β-amylase is present in plants, absent in mammals, and present in some species of microorganisms. β-amylase hydrolyzes α-1,4-glycosidic linkages in starch and glycogen with an inversion of configuration on the C(1) position of the glucose from α to β. The cleavage of glycosidic bonds in α-1,4-glycans takes place in a stepwise (exo) fashion starting at a non-reducing end of the chain (Figure 1). Since the enzyme hydrolyzes alternate glycosidic bonds, the product of this reaction is mainly maltose. Thereby, bread obtained by using wheat flours rich in β-amylase can produce crumbs with high levels of maltose, which can interfere with the final quality of the product. β-amylase is a sulphydryl enzyme, which does not require any kind of cofactor and has a turnover number of about 250,000 bonds hydrolyzed/min (at

30°C and pH 4.8). The optimum pH for β-amylase activity ranges between pH 5.0 and 6.0, and the enzyme is stable between pH 4.0 and pH 9.0 at 20°C for at least 24 h. The heat stability of β-amylase depends on the source. For example, β-amylase from malted barley is inactivated at 70°C/10 min, whereas soybean β-amylase is inactivated at 70°C/30 min. Although β-amylase is seldom found in microorganisms, there are some patents describing its production from *Bacillus megaterium*, *B. circulans*, *Streptomyces tosaensis*, *S. hygroscopicus*, *S. viridochromogenes*, *S. albus*, *S. flavus* and *S. aureofaciens.* Another source of β-amylase is potato wastewater generated in starch factories. The potatoes are scraped and the separated starch is washed with water, resulting in an aqueous liquor rich in β-amylase activity. The enzyme precipitates from the wastewater by adjusting the pH of the solution to 3.7, followed by centrifugation and drying of the final product.[6][7][8]

Microbial **proteases** – obtained mainly from *B. subtilis*, *A. oryzae* and *A .niger* – are also used in baking aiming to hydrolyze partially the gluten of the flour in order to influence the extensibility of the dough, enabling an adequate manipulation through the bakery automated equipment.

Lipoxygenase is used when white crumb bread is the desired product. The bleaching effect presented by this enzyme results from the reaction between the atmospheric oxygen and the unsaturated fats in the flour. The lipid peroxides generated oxidize the colored components of the flour (e.g., carotenoids) into colorless compounds. Moreover, lipid peroxides improve the strength of the dough and the baking properties of wheat flour. The activity of lipoxygenase used in bakery is obtained by adding 0.5% defatted soy flour – based on the weight of the wheat flour – to the dough during the production of baked goods.[6]

Pentosanase hydrolyses non-starch polysaccharides generically called pentosans (or polymers of pentose sugars, mainly arabinogalactan and arabinoxylan). The presence of soluble pentosans creates gums and causes stickiness problems on the loaf produced. Some authors attribute the staling of bakery goods (such as biscuits) to the presence of pentosans in crumbs. However, there is some indication that hydrated gums combined with the protein matrix of the dough leads to an increase in the strength and stretching ability of gas cell structures (Figure 2). This improves the retention of gas and reduces the potential for gas cell breakdown and merging, producing unwanted large holes in the final bread. Thereby, the

action of pentosanase can contribute to reach a balance between gas retention and dough stickiness.

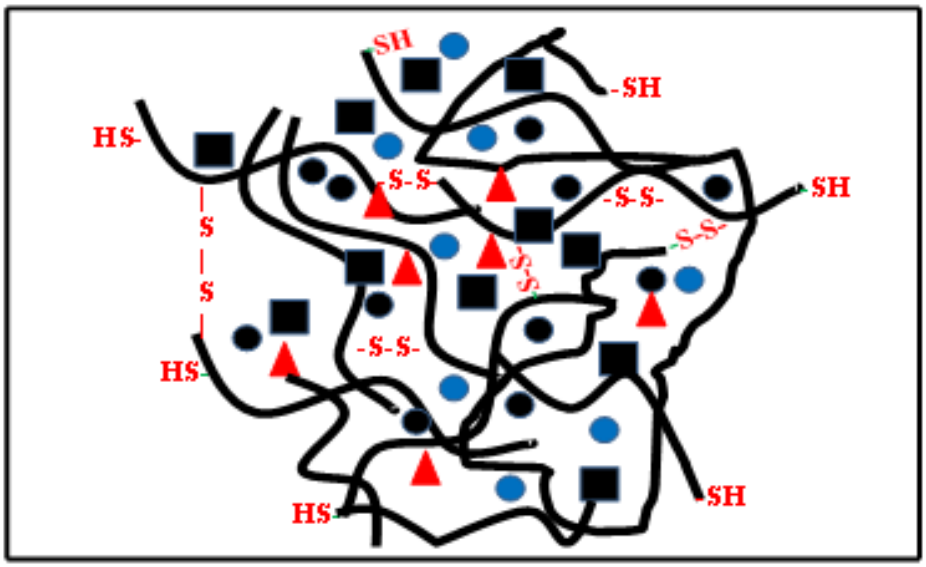

Figure 2: Pictorial entrapment of constituents inside the dough before baking. Symbols: starch granules (●), pentosans (■), lipids (▲) and bubbles of air and CO_2 (●).

The effects of amylase and protease supplementation on bread quality are summarized in the following points (Figure 3): a) α-amylase addition: the use of this enzyme aims to increase the amount of fermentable sugars, which allow a uniform yeast fermentation and carbon dioxide production in the dough. The source of small mass sugars is the polysaccharides amylose and amylopectin, which are the substrates for α-amylase action. The extension of starch hydrolysis must be low in baking; the controlled hydrolysis is obtained by the presence of ruptured starch granules in the flour. When the wheat is milled, about 2-5% of the starch granules are ruptured, enabling them to swell in presence of water. The swelled granules are easily attacked by amylases, generating dextrins, which are subsequently hydrolyzed into glucose by β-amylase. Amylases also improve the gas retention properties of the dough through starch modification (Figure 2). The addition of fungal amylase reduces dough viscosity, leading to a bread with finer crumbs. The crust color is intensified by the cereal α-amylase but also by fungal α-amylase. The higher reducing sugar content increases Maillard browning and enhances the flavor. The amylases are supplemented during the milling of the flour (100 – 150 SKB/Kg of flour) and, if needed, during the first phase of baking, i.e., the preparation of the dough (fermentation stage). The amount added during baking depends on the composition of the dough, the type of bread desired, and the type of

machinery used; b) protease addition: the main aim is to influence the elasticity and texture of the gluten so that the handling properties of the dough improve. The proteases are used during the first phase of bread production (fermentation stage) to allow contact with the flour proteins (gluten) for an extended time. The proteases hydrolyze and shorten protein chains and allow them to realign into sheets of protein film. This action of the enzyme modifies the protein so that shorter mixing times (and a lower energy input) are required at the point of maximum extensibility. Mixing times are critically important in present day bakery operations. The use of automatic equipment and tight time schedules require mixing a given quantity of dough for a definite time. Exceeding this time schedule can cause expensive delays and variations in product quality. The proteases naturally present in the wheat flour are not adequate for the baking objectives because the low proteolytic activity and the optimum pH (4.0) differ from the dough pH (7.0). Moreover, in salty bread, where up to 3% of salt is added to the dough, proteases naturally present in the flour are inactivated completely.

Generically speaking, the use of enzymes in bakery increases due to constraints imposed by food legislation on the chemical modifications of flours concerning the elimination of natural pigments, for example. The use of proteases grows because marketed flours present a high protein content because of the genetic improving of cereal plants. The increase of pentosanase availability in the market is also of great importance since staling is an undesirable effect and must be avoided whenever possible as long as bread shelf-life increasing is a desired objective.

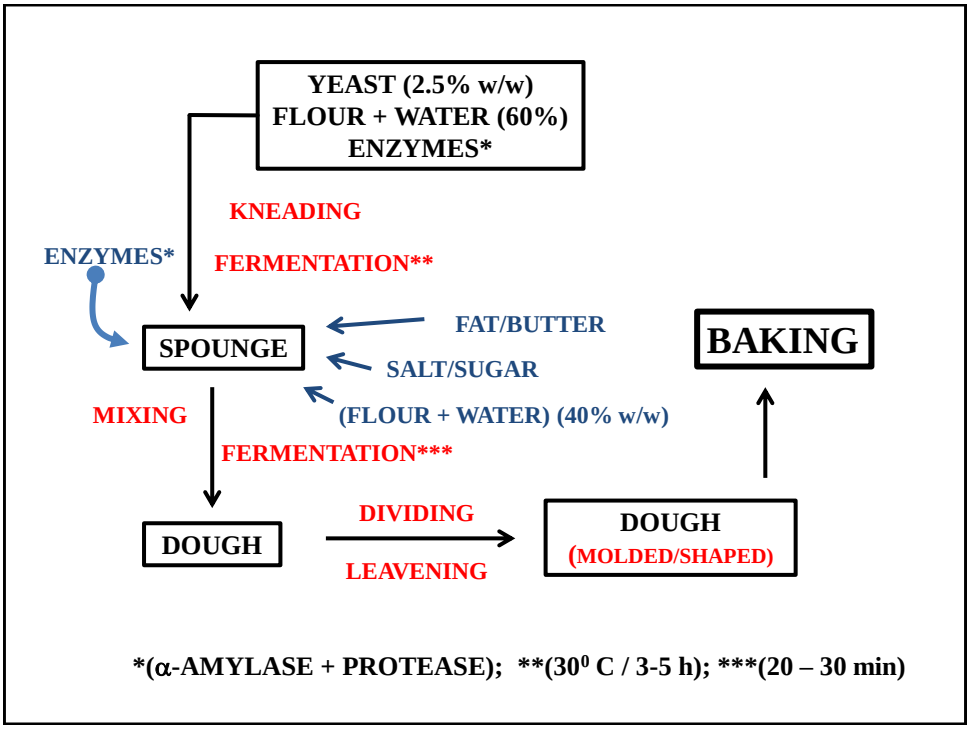

Figure 3: Dough process.

The main events occurring during baking are shown in Table 2.

Table 2: Temperature effect on dough and baking.

T (^{o}C)	Dough changing Crumb formation	T (^{O}C)	Dough changing Crust formation
40-50	Yeast inactivation	**120-130**	Light browning*
60-70	Starch gelatinization	**140-160**	Caramel formation
80-85	Ethanol evaporation	**170-180**	Roasting products
100	Water evaporation	**200**	Charring

*Due to Maillard reaction (formation of flavor and aroma).

CORN STARCH AS RAW MATERIAL

Corn (Zea mayis) is a plant originated from wild species found in Mexico. During the centuries, this plant was submitted to hybridization and mutations, resulting in six main varieties, i.e., dent-corn, hard-corn, farinaceous, pop-corn, sweet-corn, and waxy-corn. The variety chosen for cultivation depends on the particular climate of a region. For example, the hard-corn and dent-corn are cultivated in tropical and warm climate countries, respectively. Corn varieties differ in size, shape, and internal composition of the grain. An average corn grain composition on dry-basis (DB) has starch (71.4%), protein (10.0%), fat (4.5%), ash (1.5%), pentose (6.4%), cellulose plus lignin (3.4%), and total sugars (2.8%), besides moisture (17.0%) and carotenoids (30.0 mg/Kg).[9]

The corn grain presents four main structures, i.e., fibrous extremity (0.8% (w/w) on DB), pericarp (husk; 5.2% (w/w) on DB)), endosperm (83% (w/w) on DB), and germ (11% (w/w) on DB). The fibrous extremity, from which the grain is fixed on the ear of corn, allows the penetration of water, other liquids and gases into the inflorescence matrix. The husk is the external membrane, which covers and protects the grain against external injuries. It is constituted by several types of cell layers and carbohydrates (hemicelluloses and cellulose), whose most external layer is covered by a waxy substance called cutine. The endosperm is mainly constituted by granules of starch embedded into a protein matrix (zein is the main protein). The hardness of this structure is a result from the strong starch-protein interaction. Colorants such as carotenoids are found into the endosperm. Roughly, the endosperm can be divided into three fractions, namely aleurone (the external part containing high amounts of protein and a rigid structure), vitreous endosperm (starch granules are fully covered by the protein rigid matrix; it is the part smoothed through the maceration process), and farinaceous

endosperm (starch granules are not covered by the protein matrix). The germ, in turn, contains all lipids and minerals of the grain, as well as high amounts of sugars and proteins. It is formed by three parts, namely plumule, radicle, and scutellum (a reservoir of nutrients quickly available for plant growing, in addition to oil droplets and high amounts of pentose; this structure is also a target for the maceration process). The radicle and plumule are responsible for the complete formation of the corn grain.[10]

Corn starch is constituted by amylose (26%) and amylopectin (74%). In the corn kernel, where starch constitutes about 70% of the kernel on DB, both polymers are tight joined, forming approximately spherical and birefringent granules. A pivotal characteristic of starch granules, which allows their extraction at a high purity through the maceration process (see below), is the capability to remain in aqueous suspension at a concentration of about 50% (w/v) on dry basis and the suspension will remain in a fluid-pumpable state.[11]

Corn is one of the most cultivated cereals worldwide. About 80% and 20%, respectively, are directed for producing livestock feed and commercial products derived from starch (such as corn meal, starch hydrolyzate, crystalline glucose, fructose-containing syrups, and bran). Moreover, a large variety of unmodified, modified and derivate starches is also available. Starch derivatization involves etherification and/or esterification of amylose and amylopectin followed by acid hydrolysis of polymers.

Because corn is a cheap, abundant raw material and stable during storage, it is readily available during the whole year. Such characteristics allow establishing large industrial corn-processing plants worldwide.

Corn can be processed through dry (corn is crashed against sieves by pressurized air) or wet (maceration in SO_2 saturated water) procedures. The choice between them depends on the variety and purity degree of the products desired. The wet procedure is chosen when purer products are desired; such products have high commercial values.[10][12]

The entire wet process can be summarized as shown in Figure 4. The first step consists of cleaning the grains in order to remove solid impurities (carried from the harvesting field) and broken grains. Thereafter, a sequence of unit operations, such as maceration, grinding, sieving and centrifugation, are carried out. For example, the full wet process, applied on dent-

corn could result in yields, on dry basis, for maceration water solids (3.53%), germ (6.78%), fiber (13.37%), protein (gluten) (10.91%), and starch (65.29%).[12]

Maceration, which is the second step of the whole corn wet processing (Figure 4), can be carried out under the best values of temperature (40-50^{o}C), lactic acid (10-30 g/L), and sulfur dioxide (150-1440 ppm) concentrations to soften the grains adequately. The high yields of intermediates and final products obtained depend on the maceration efficiency. The maceration, whose average duration varies between 30 h and 50 h, begins by mixing one ton of corn with water (1.2-1.48 ton).[9] The whole process, which is constantly supplied with SO_2, can be divided into three steps: **1**) **complete corn hydration**, leading to an overall grain weight increase (about 40-45%). Moreover, the microorganisms naturally present into the grain – mainly *Lactobacillus sp.* – metabolize the carbohydrates and proteins released to the aqueous medium by the grain during hydration. Both substrates correspond to about 6.5% (dry basis) of the total amount of corn processed. At the end of fermentation, about 20 g/L of lactic acid are formed, which play a role (together SO_2) in avoiding the growth of undesirable microorganisms and softening the corn grains. Moreover, temperature and lactic acid act synergistically to destroy the vegetative cells of the grain. Its membrane becomes more porous to SO_2 absorption. This step, which lasts about 13 h, finishes when the SO_2 concentration into the medium is over 300 ppm, which is a deleterious condition for Lactobacillus sp.; 2) **free SO_2 diffusion throughout the corn grain**. As the SO_2 penetrates into the grain, proteins are disarranged – mainly due to the disruption of disulfide bonds among cystein molecules. Therefore, starch and other corn components (oil, for example) are released; 3) **saturation of the grain by SO_2 at a concentration of 2,500 ppm**.

The wet maceration process could be carried out by using hydrolytic enzymes (proteases and carbohydrases) in association with sulfide dioxide.[13][14]

Considering that the pericarp structure is constituted by intricately disposed lignin and polymeric carbohydrates (hemicelluloses, cellulose, and xylans), and the endosperm in turn is composed mainly by starch and proteins, the use of hydrolytic enzymes could be a valuable tool.[15] Thereby, the xylanase and the fungal acid protease must be added separately during maceration (Figure 4). The disruption of the outer layers of the grain by xylanase facilitates the diffusion of SO_2 and protease. Consequently, the amount of SO_2 needed to saturate the grain at the third maceration stage can decrease by about 25% and the starch extraction can be improved by the second grinding.[9] On the one hand a drastic decrease in SO_2 is not

advisable because it is also important for the microbial control of the fermentation; on the other hand, its use in minor amounts is desirable for economical reasons, environment protection and decrease of allergenic potential to corn-processing workers. A clue for achieving such results was demonstrated by adding xylanase and corn grain previously hydrated and little grinded at the first maceration stage. This approach led to the reduction of about 4 h of the overall duration of the maceration process.[14][16]

Caution must be taken when enzymes are used because of the eventual residual activity in the final product. The presence of xylanase in the product is irrelevant because its substrate was already hydrolyzed at the first maceration stage. However, protease, which is added later to the maceration process, could remain active on hydrolyzing proteins in the final product, leading to undesirable organoleptic and physical alterations. Therefore, the xylanase/protease ratio must be balanced.

The criterion for setting enzyme concentrations is based on both the enzyme-producer technical information and previous tests, in which the minimal amount of enzymes which leads to a significant modification of the corn texture is determined (from 92N to 53N, for instance). Moreover, attention must be paid on the fact that the xylanase substrate is found in insoluble form in the granule, which implies the occurrence of heterogeneous catalysis during maceration. Such situation leads to an increase in hydrolysis time, which can be offset by using an amount of enzyme higher than that predicted by the hydrolysis of the soluble substrate (normally used for determining the xylanase activity).[17]

Finally, the waste water of wet corn processing – commonly called corn steeping water and constituted, on a dry basis, by total solids (53%), total nitrogen (5%), lactic acid (30%), total sugars (8%), and ashes (4%) – is a valuable byproduct as a nitrogen source for penicillin production by fermentation.[18]

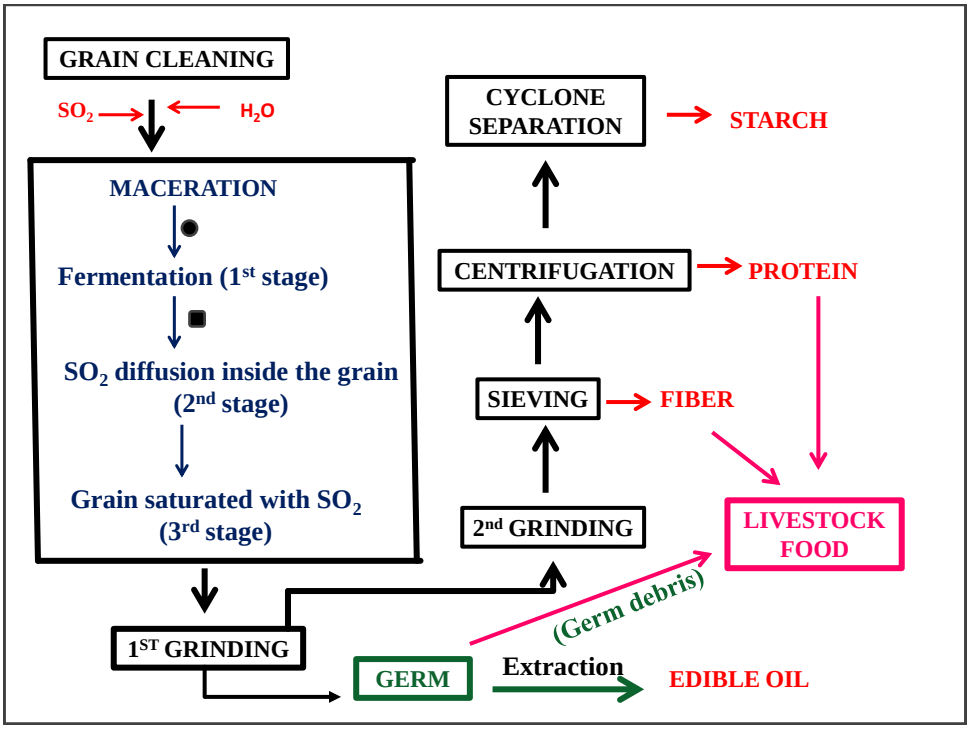

Figure 4 – Wet corn processing diagram. Enzymes: xylanase [(0.08-0.2% w/w); (●)] and fungal acid protease [(0.25-1% w/w); (■)]. Operational conditions: grain/water ratio equal to 1:1.5; SO_2 added (150-1,500 ppm); total time of maceration (45-50 h).

STARCH CONVERSION

The production of syrups requires an extensive hydrolysis of the starch, contrarily to baking, in which the extension of starch hydrolysis is small. Table 3 shows the main products derived from starch hydrolysis.

Industrial processes for starch hydrolysis into glucose rely on inorganic acids or enzyme catalysis.[19] The use of enzymes is preferred because they lead to increases in yield and reduction in the cost of the process. Moreover, enzymes allow controlling starch depolymerization due to their specificity hydrolysis pattern and prompt inactivation (by changing the pH and/or temperature) when a desired depolymerization degree is reached. Milder reaction conditions involve lower temperatures and near-neutral pH, which minimizes unwanted side reactions. Thereby, the formation of side compounds does not occur, such as 5-hydroxy-2-methylfurfuraldehyde – responsible for off-flavors and off-colors in the final product. Moreover, enzymatic methods are favored because they require less energy and do not need the neutralization step (Figure 5).

The beginning of syrup production can be situated in the thirties of the 20th century, when the acid hydrolysis of maize was performed at an industrial scale (Figure 5). Since then, syrups have been used as sweeteners and ingredients in formulations of culture media for fermentative processes.[20]

However, the syrup industry began to grow effectively in the 1970s, when the enzymes glucoamylase – which catalyzes the hydrolysis of oligosaccharides into glucose (Figure 1) – and glucose isomerase, which isomerizes glucose into fructose, became available in huge quantities. Figure 6 shows the main steps of the complete starch hydrolysis.

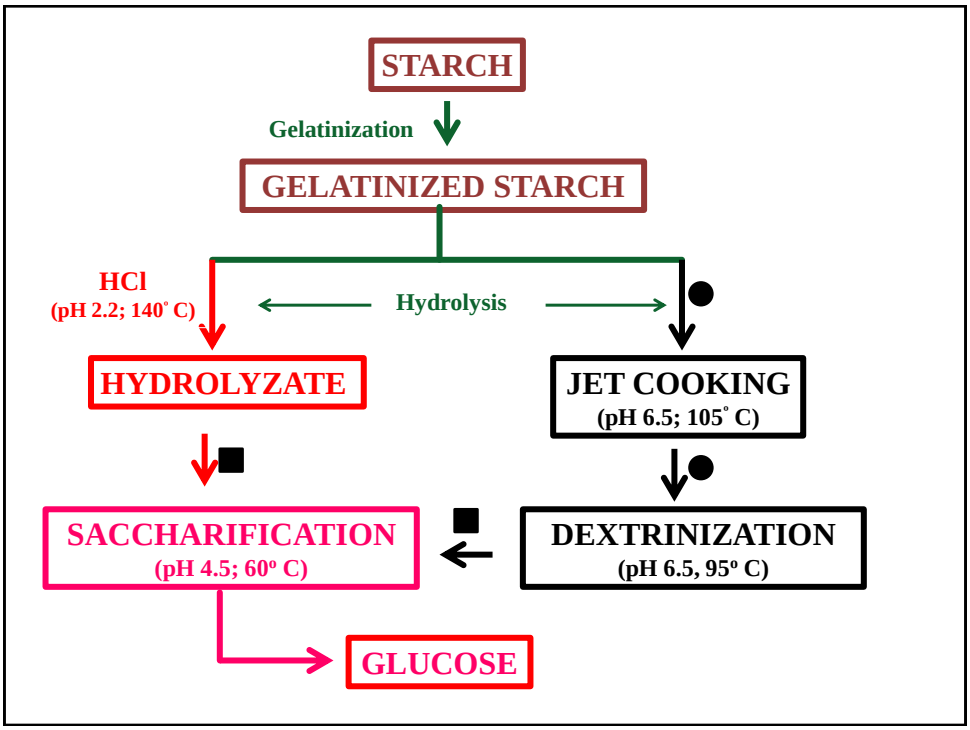

Figure 5: Starch conversion to glucose by acid and enzymatic hydrolysis. Enzymes: bacterial α-amylase (●) and glucoamylase (■).

Dextrinization, saccharification and isomerization are unit operations carried out by bacterial α-amylase (or hydrochloric acid), glucoamylase (or fungal α-amylase) and glucose isomerase, respectively.

Gelatinization is carried out by heating the aqueous starch paste up to 80°C (the temperature varies according to the type of starch; for example, corn gelatinizes at 70°C, oat at 58°C, wheat at 60°C, rice at 76°C, and potato at 65°C). During gelatinization, the starch hydrolysis

is low but the viscosity of the solution increases markedly. During gelation, the granules swell and disrupt partially, so that they become a substrate for amylases. Sometimes, white granules formed by amylose and/or amylopectin appear in the bulk of gelatinized starch. They re-precipitate from the viscous aqueous starch solution. This phenomenon is called starch retrogradation.

Depending on the source of starch granules, amylopectin can be a highly branched polymer that is not completely decomposed by the combined use of α-amylase and glucoamylase. In this case, a bacterial pullulanase might be used.

Isomerization can be considered the last step of starch hydrolysis (Figure 6). The resulting glucose is isomerized into fructose through the catalytic activity of glucose isomerase. The enzyme is used in the immobilized form, and the full process is carried out continuously in a fixed-bed reactor.[20] Two points must be stressed regarding the use of glucose isomerase. First, enzyme catalysis leads to an equilibrium between glucose and fructose, which has to be overcome; second, the formed fructose acts as a competitive inhibitor of glucose for the catalytic site of the enzyme. Both problems are eliminated by using a chromatographic separation column.

The fructose syrup production began in 1970 as soon as the immobilized glucose isomerase appeared in the market, allowing countries deprived of sugar-cane or beet to obtain a sweetener comparable to sucrose.

The perspectives of the use of enzymes in syrup production are based on the improvement of catalytic characteristics of the immobilized glucose isomerase, availability of thermostable α-amylase (stable at temperatures over 105^{o}C), immobilized glucoamylase, and heat stable debranching enzymes (pullulanases).

Table 3: Products resulting from starch hydrolysis.

PRODUCT	USES
Maltodextrins	Stabilizers, gums, pastes, thickeners
Mixed syrups (42 < DE*<63)	Confectionary, soft drinks, ice creams, baby foods, canned food
Maltose syrup	Confectionary
Glucose syrup	Soft drinks, culture medium, candies
Fructose syrup	Soft drinks, canned food, yoghurt

*DE (dextrose equivalent) is the reducing power of liquefied starch solution regarding a standard glucose solution considered 100% of reducing power. Total reducing sugars are measured by special reagents such as Fehling, Benedict, dinitrosalicylic acid (DNS), or Somogyi-Nelson.

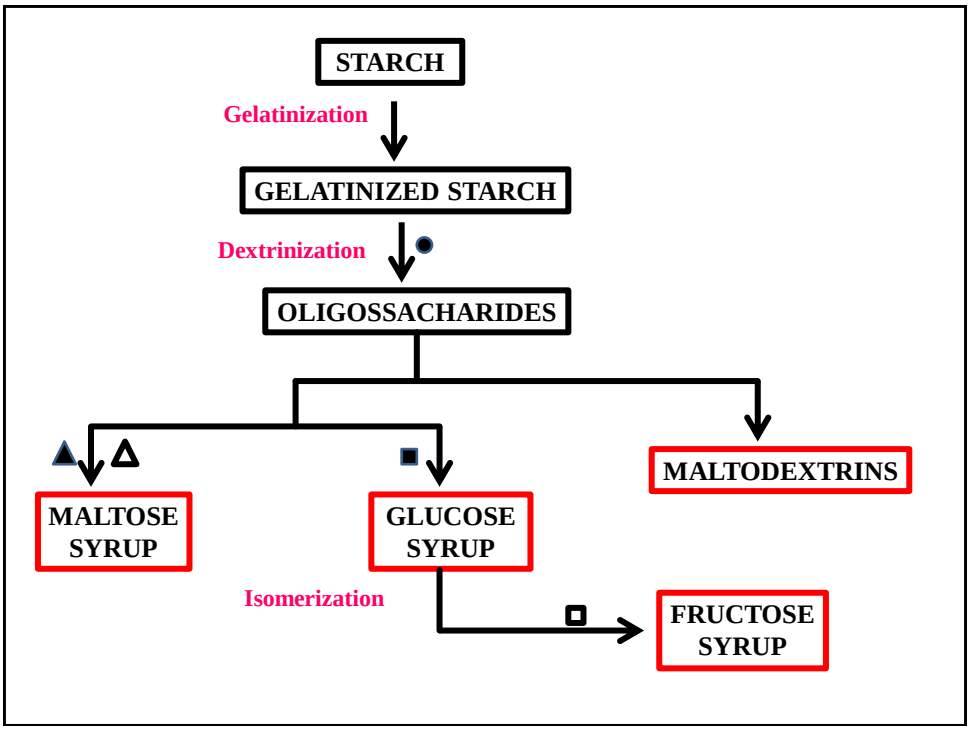

Figure 6 – Conversion of starch into syrups. Enzymes: bacterial α-amylase (●), glucoamylase (■), β-amylase (▲), fungal α-amylase (Δ), and glucose isomerase (□).

ASPECTS ON SAFETY IN HANDLING ENZYMES

Due to the huge amounts of enzymes handled in industry, there is a great concern about risks to human health. Health hazards can be divided into two main groups: allergic reactions and direct contact with the body.

Allergic reactions result from the protein nature of the enzymes, which stimulates the body immune system, leading to the well-known antigen-antibody reaction. The main way to develop an allergenic response, being asthma the most conspicuous, is by inhalation of enzyme dust. The intensity of response depends on the physical condition of each individual. Some individuals who have allergic reactions – such as hay fever, rhinitis, migraine and urticaria, among others – are known to have a built-in genetic susceptibility or weakness. These people may become extremely sensitive to foreign proteins; the large majority of the population is unaffected by it.

Measures for avoiding allergy in the enzyme-handler must be taken since the first moment the individual enter in contact with the substance. The local where the manipulation takes place must be constantly monitored, for example by determining the amount of powder suspended in the room air (mg/m^3). To do this, a volume of air is suctioned during a fixed period of time (in general 8 h) through a micro-filtration membrane (whose superficial area is well defined), which is then weighted before and after exposure so that the concentration of particles in suspension is evaluated (values up to $1x10^{-5}$ mg/m^3 are acceptable). The exigency for such a small concentration in the air is due to the high possibility that a sensitized person suffer an anaphylactic shock soon after the person inhales the protein powder. It is recommended that the operator be submitted to a complete and rigorous medical check-up and clinical tests (evaluation of pulmonary capability and serum level of α-1-anti-trypsin, for example) before admission. Periodical check-up is obligatory for enzymes-handlers.

The direct effects on the body of the operator depend on the nature of the enzyme handled. It is common to observe that even enzymes with similar catalytic mechanisms may cause different types of hazards.

The direct contact of the enzyme with the body of the operator deserves attention because more or less serious injuries to body tissues can occur depending on the part of the body affected and the time of contact. Mucous membranes of cells, which cover external organs such as eyes, mouth, nose, ears, teeth, genitalia and skin, as well as sensitive tissues such as those of respiratory and digestive tracts, must be fully protected.

The safety measures employed at the industrial level must guarantee that enzyme dust or droplets are absent in the air of the handling room. Moreover, the production place must be well sealed in order to impede the scattering of the suspended material throughout the factory and around the facility. To reach this aim, the local exhaustion and ventilation must be dimensioned. The air must be filtered and monitored.

The used filters and the operator protective equipment (goggles, gloves, boots, mask etc.) must be disposed of inside a hermetic sealed recipient as soon as possible. Another important measure is warning the enzyme-handler to watch for and report immediately skin reactions (reddening and itching) and any respiratory problems (wheezing and shortness of breath). Moreover, the company should instruct the person on the whereabouts of the enzyme manipulation through suitable means (notices, literature, and training).

Dissolving and stabilizing enzymes in solution is a valuable procedure for handling enzymes. The enzyme manufacturer often offer a liquid enzyme preparation in more concentrated forms aiming user safety (easier handling, less splashing, lower aerosol formation), and reduction costs of transport and storage. In addition, powdered enzymes must be constituted by dense particles that settle quickly.

Some attention must also be paid on the manipulation of enzymes at a laboratory scale, where in general concentrated enzyme preparations are handled at small quantities. Such condition often induces the personnel to assume a negligent posture, as they do not protect themselves appropriately. Thereby, they become strong candidates to present either allergic symptoms at best (reddening, itching) or more hazardous illnesses at worst (asthma, lungs emphysema) in the long-term.

In short, a summary of safe enzyme operation principles are a) containment: enclosed plant, total enclosure of process equipment, controlled general air and separate exhaust air, strictly controlled access, protective clothing, washing and decontamination facilities, and separate eating area; b) environmental control and monitoring: regular plant cleaning, planning of processes to minimize risks, initial and periodic background air monitoring, initial and periodic air-flow measurements, and monitoring of equipment during operation; c) personnel screening: initial and periodic health examination, continual recording of absences and illnesses, established procedure for accident, and illness reporting and liaison with hospital experts.

The regulations related to enzyme safety consider as a starting point the recommendation to use enzymes attained from well-known and established sources, mainly catalysts used in food technology. Thereby, the sources chosen are animals (catalase, lipase, pepsin, rennet, trypsin, chymotrypsin) and plants (bromelain, papain, ficin) eaten by humans since ancient times. Regarding microbial sources, they have been accepted only after a prolonged field scrutiny over time. The enzyme-producing microbial strains fully accepted are *Bacillus subtilis*, *Aspergillus oryzae* and *Aspergillus niger*. The maximum content of residual enzymes in food products vary from 10 ppm (papain in beer) to 750 ppm (glucose oxidase and catalase in solid egg products).[21]

CONCLUSION

Starch can be either a product *per se* or a raw material for producing derivatives of commercial interest (syrups, glucose, fructose, maltodextrins etc.). In baking, the partial starch hydrolysis catalyzed by fungal α-amylase is pivotal to obtain bread with adequate flavor, aroma, crumbs, and crust. In starch extraction from corn, xylanase and fungal acid protease can promote the shortening of maceration time and a SO_2 reduction of about 20%. In the syrup production, bacterial α-amylase, fungal α-amylase, β-amylase, glucoamylase and glucose isomerase allow obtaining a variety of syrups (maltose, fructose, and glucose). Due to the huge amount of enzymes used in industry, caution must be taken regarding the health hazards to which enzyme-handling workers can be exposed. An adequate control on working conditions in the plant (calibrated exhaustion and ventilation systems), an adequate disposal of equipment used (goggles, mask, boots, suits etc.) and residues generated in the plant (filter membranes embedded with enzyme particles) are effective measures to avoid allergic injuries or direct enzyme contact with the skin and other mucosal tissues of the worker.

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