Enzymes

1.6. Enzymes

Introduction

First of all it should be noted that enzymes are proteins and that they are substrate specific. This means that a given enzyme only will work on a certain substrate and only do a very particular job. Secondly it should be remembered that, although they take part in a chemical (enzymatic) reaction, they do not change during that reaction. They are what we call biological catalysts that accelerate or facilitate chemical reactions.

Because they are proteins, they are heat sensitive and all enzymes have an optimum temperature and pH for activity. Within that range, activity increases with temperature until the denaturation point is reached. At that point the enzyme will lose its functionality. Apart from temperature and pH, enzymes are also dependent upon the availability of water, amount of enzyme used, the availability of the substrate and the time allowed for the reaction.

Enzymes are biological compounds, usually proteins, which expedite the conversion of one substance into another. Their presence accelerates the rate of a chemical reaction and they are often specific and act upon only one substrate, or catalyse only one kind of reaction, in different, but related, substrates.

Because they consist of long amino acid chains, enzymes are classified as proteins and share many protein-like qualities. An enzyme molecule, however, is folded into a specific three-dimensional globular structure. Within these contorted folds are cavities that match the external features of a substrate molecule - a fat, protein or starch, for example - much like a key fits into a lock.

When an enzyme's active site meets with a corresponding substrate molecule, they temporarily bind to form an enzyme-substrate complex. By forming the complex, the enzyme lowers the energy required for certain reactions to take place. These reactions may either break up the substrate molecule or join it with another molecule. In addition, the complex will limit the reaction to specific bonds on the substrate molecule.

Enzyme activity is highly specific. Depending on its three-dimensional structure, a particular enzyme may hydrolyse or synthesize only one type of molecule. Others are less specific to a given type of molecule, but promote a certain chemical reaction on entire classes of compounds sharing common

structural elements. Whatever the reaction, the enzyme itself will remain unchanged and this is why enzymes are considered catalysts.

Enzymes are named by adding the suffix "ase" to the end of the substrate. For simplicity, the substrate's name often is abbreviated. In baking applications, the general types of enzymes most commonly used are carbohydrases, proteases and lipoxygenases.

Basically enzymes can hydrolyse a polymeric substrate in two ways. Exo-enzymes remove a single polymer unit from the end of the polymer chain, whereas endo-enzymes can rupture the internal bonds in a random manner at any point along the chain.

The activity of enzymes is dependent upon temperature. Enzymes used in baking are usually stable at room temperature and the rate of enzyme activity doubles with each 10°C increase up to the temperature of denaturation, at which the enzyme is inactivated. Most enzymes are inactivated above 60°C. An exception would be bacterial α-amylase, which retains its activity up to 85°C.

Enzymatic activity is pH dependent; there will be a pH optimum where maximum activity is achieved. Enzymes are usually stable at pH values between 4 and 9. Most doughs have pH values between 5 and 6. There is seldom an issue of enzyme denaturation due to pH. However, the acidity does effect the ionization of groups at the active site of the enzyme, rendering the enzyme more or less effective, depending upon the pH of the dough. The usefulness of a given enzymatic activity depends in part on matching the pH optimum of the enzyme with the pH of the dough.

Enzymatic activity is dependent upon the concentration of the enzyme and the substrate. A higher concentration of enzyme will increase the reaction rate although not in direct proportion to substrate availability. The amount of time the enzyme and substrate are together directly affects the extent of reaction.

Many enzymes require the presence of a non-protein group, or coenzyme, to be active. There are also compounds that act as inhibitors of enzyme activity by binding reversibly or irreversibly to the enzyme and/or substrate or in some way inhibit enzyme action. Oxidizing agents, such as bromates and iodates, and some heavy metal ions, have this effect.
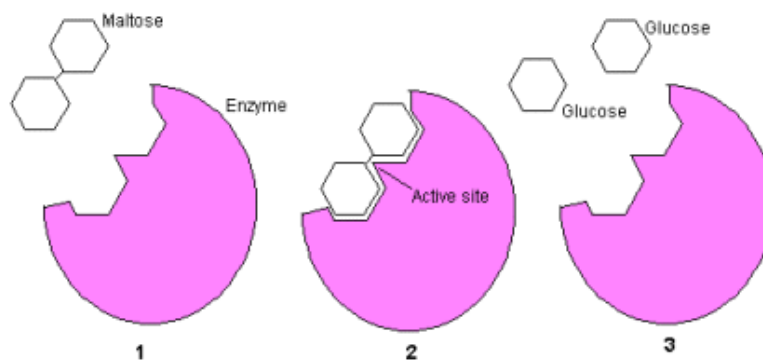
Production of enzymes

The starting point for enzyme production is a vial of a selected strain of microorganisms. They will be nurtured and fed until they multiply many thousand times. Then the desired end-product is recovered from the fermentation broth and sold as a standardised product.

A single bacteria or fungus is able to produce only a very small portion of the enzyme, but billions microorganisms, however, can produce large amounts of enzyme. The process of multiplying microorganisms by millions is called fermentation. Fermentation to produce industrial enzymes starts with a vial of dried or frozen microorganisms called a production strain.

One very important aspect of fermentation is sterilisation. In order to cultivate a particular production strain, it is first necessary to eliminate all the native microorganisms present in the raw materials and equipment. If proper sterilisation is not done, other wild organisms will quickly outnumber the production strain and no production will occur.

The production strain is first cultivated in a small flask containing nutrients. The flask is placed in an incubator, which provides the optimal temperature for the microorganism cells to germinate. Once the flask is ready, the cells are transferred to a seed fermenter, which is a large tank containing previously sterilised raw materials and water known as the medium. Seed fermentation allows the cells to reproduce and adapt to the environment and nutrients that will be encountered later on.

After the seed fermentation, the cells are transferred to a larger tank, the main fermenter, where fermentation time, temperature, pH and air are controlled to optimise growth. When this fermentation is complete, the mixture of cells, nutrients and enzymes, called the broth, is ready for filtration and purification.

Filtration and purification termed as downstream processing is done after enzyme fermentation. The enzymes are extracted from the fermentation broth by various chemical treatments to ensure efficient extraction, followed by removal of the broth using either centrifugation or filtration. Followed by a series of other filtration processes, the enzymes are finally separated from the water using an evaporation process.

After this the enzymes are formulated and standardised in form of powder, liquid or granules.

Understanding the function of enzymes in baked goods

Many food product designers consider enzyme use new and innovative. While this is true for many categories, the baking industry actually has a long history of enzyme study and application. In fact, some references to the use of added enzymes in bakery foods are over 100 years old.

Even without this track record, enzymes are appealing functional ingredients for a variety of reasons. Enzymes are, for example, naturally occurring components of many bakery ingredients. If an enzyme is added, it often is destroyed by the heat of the baking process. In both cases, designers can obtain the functional benefits of the enzyme while maintaining a "clean label" image for the finished product. Enzymes also are specific to a particular function, eliminating concerns about undesired effects.

Nevertheless, getting the most out of enzymes in bakery products requires some planning on the part of the designer and a better understanding of what enzymes can do. The functioning of the some enzymes has been explained above, but there are other enzymes that can have a benificial effect on baked products.

Enzymes commonly used in baking

There are 3 main groups of enzymes which are commonly encountered in baking : enzymes that hydrolyse carbohydrates (amylases, cellulase, pentosanases), enzymes that hydrolyse proteins (proteases) and enzymes affecting fats and oils (lipase, lipoxygenases).

Enzymes that hydrolyse carbohydrates

Amylases are divided into α-amylase and β-amylase.
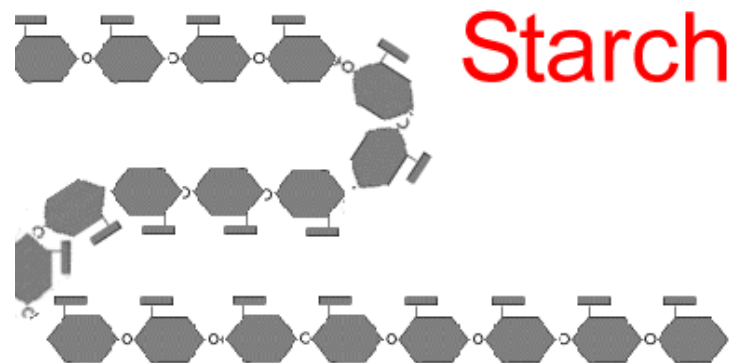
Starch

Two amylases are common to the baking industry, α-amylase and β-amylase also known as α-1,4-glucan glucanohydrolase and α-1,4-glucan maltohydrolase.

Amylases convert starch into sugar : the α-amylase will cleave the starch randomly (the so called 1-4 bonds in the starch) while the β-amylase can only chop off two sugar units at the time at the end of the starch chain. Normally there is enough β-amylase present in the flour but sometimes addition of α-amylase is needed. The α-amylase will cut the starch into smaller units called dextrins and the more α-amylase activity there is, the better for the β-amylase because there are more extremities available. So the substrate for the β-amylase is either starch or dextrins and the product is maltose.

α-amylase is an endo-enzyme that attacks linkages within the molecular structure. It randomly cleaves starch chains at interior α-1,4-glycosidic linkages producing short chains of glucose molecules or dextrins. β-amylase is an exo-enzyme and cleaves maltose units from the non-reducing end of the starch molecule. In order for these enzymes to function, the starch granule must be ruptured so that the individual starch molecules are available for enzymatic action.

Depending upon their origin, α- and β-amylases show differences in pH and temperature optima, thermostability, and other chemical stability. They do not require coenzymes for activity, although α-amylase activity is enhanced by the presence of calcium.

The pH optimum for α-amylase is 4.5 and it is inactivated at a pH of 3.3 to 4.0. This pH dependence decreases the efficacy of this enzyme in sourdoughs. β-Amylase is active across a much broader pH range, 4.5-9.2, with a pH optimum of 5.3. α-Amylase is relatively thermostable up to 70°C, whereas β-amylase loses about half of its activity at this temperature. Fungal amylase is the least temperature stable, followed by cereal amylase, while bacterial amylase is stable at higher temperatures. New intermediate stability enzymes have been developed that are active above the gelatinisation temperature of starch (60°C), but are totally inactivated at the later stages of baking (80-90°C). The objective is to maximize the anti-staling effect without creating a gummy, sticky product.

Amylase supplementation can occur at the flour mill or at the bakery in doughs and sponges. Malted barley flour has been used as an ingredient in bread for more than a century. Today, malted barley flour, malted wheat flour, fungal and bacterial α-amylases are used. The use of amylases provides a source of sugar for yeast fermentation. The increased sugars also improve flavour and enhance crust colour. Through starch modification, amylases improve moisture retention, have a crumb-softening effect and decrease staling.

Flour tends to lack α-amylase and the miller will supplement the flour with α-amylase. The diastatic activity of the flour is expressed by the falling number or the Hagberg number. A good flour has a falling number between 200 and 250 seconds. The α-amylase the miller will add can come from three different sources : cereal source (malted barley), fungal source (Aspergillius oryzae) or bacterial source (Bacillus subtilis). Bacterial amylase do denature at relatively high temperatures and some will remain in the bread after baking. The enzyme will continue to chop up starch in the baked bread. As starch is one

of the main players in the staling mechanism, bacterial enzymes are used as crumb softeners because they will continue to work while the bread sits on the shelf of the supermarket. However there is a danger to it : the bread becomes softer and softer, it becomes more and more gummy and it is not uncommon that it will flatten, collapse while it sits on the shelf in case there is too much bacterial amylase left in the bread.

Fungal amylases work in exactly the same way bacterial and cereal amylases work. They however are denaturated at lower temperatures.

As said before, amylase converts starch to maltose and other simple sugars. Selected amylases in synergistic effect together with xylanases produce stable, fluffy dough and voluminous loaves of bread with a soft and elastic crumb. As all-round enzymes they can be utilized with most varied baking methods and flour grades worldwide.

Carbohydrases hydrolyse glycoside linkages in carbohydrates. This linkage specifically refers to the bond between one sugar molecule's reducing functional group and the -OH (hydroxyl) group of another molecule - usually a sugar molecule, as well. Amylases are the carbohydrases that offer the greatest number of potential functions in bakery foods. These hydrolyse amylose and amylopectin in starch, as well as starch derivatives such as dextrins and oligosaccharides.

The α-amylase enzyme hydrolyses starch into soluble dextrins. These dextrins may subsequently be hydrolysed by β-amylase to yield maltose, and/or amyloglucosidase to yield glucose. Because starch exists as a tightly packed granule, amylases must act upon starch granules that are damaged (as many are during flour milling) or on granules that have been gelatinized by moisture and heat (such as when a dough is mixed and baked).

The sugars resulting from amylase activity act as food for yeast in yeast-raised products. As a result, the presence of these enzymes in the proper proportions is critical to carbon dioxide generation. Most flour naturally contains both α- and β-amylase. The β-amylase is, however, the only one naturally present in sufficient quantities. Thus, controlling the gassing power of the dough requires added α-amylase.

Amylases also can affect the consistency of a dough. Damaged starch granules absorb more water than intact granules. This ability is reduced when the damaged granules are acted upon by amylases. With their ability to immobilize water reduced, the damaged granules release free water which softens the dough and makes it more mobile.

A third function of amylases is their ability to retard staling. Over time, the crumb of baked products firms due to a complex set of changes that includes recrystallisation (or retrogradation) of amylopectin in the starch. By hydrolysing the amylopectin into smaller units, bacterial α-amylase can maintain softness and extend shelf life.

One theory behind this suggests that amylopectin still crystallizes at the same rate with added enzymes, but that the shortened chain length maintains greater flexibility and softness when crystallized. Another theory is that the shortened amylopectin chains have a lesser tendency to retrograde. Either way, the enzyme must continue to hydrolyse starch after baking is completed. The fact that bacterial α-amylase is more thermally stable than other α-amylase sources is the reason it is used.

Because the enzyme is active in the finished baked product, it is possible for the enzyme activity to go too far. Rather than maintaining softness, the crumb can actually become gummy. The starting enzyme dosage is critical to preventing this. For even greater assurance against overdosing, amyloglucosidase or pullulanase may be added along with the α-amylase. These enzymes don't contribute to anti-staling when used alone, but help prevent gumminess when combined with the amylase.

A final use for amylases in bakery products is for replacing potassium bromate, an oxidizing agent that strengthens gluten strands. Strengthened gluten produces a dough with improved gas retention and, consequently, higher volume in the finished product. Based on various health studies, bromate use is on a sharp decline. Other oxidants -- such as ascorbic acid - can promote comparable volume, but they don't provide a direct match for bromate. To compensate, α-amylase can be added with ascorbic acid to improve the volume and increase the quality of the crumb. Bakeries may either add α-amylase and ascorbic acid separately or select a custom blend featuring an optimise mixture of the two components.

Amylases are not the only carbohydrases useful in bakery products. Pentosanases also can be added to improve quality. Both wheat and rye flour contain pentosans. These non-starch polysaccharides are highly hydrophilic and contribute significantly to the water absorption properties of a dough. In wheat flour-based products, pentosans also interfere with volume development.

Adding pentosanase to a wheat flour-based product can improve product volume by hydrolysing the pentosans present. At the same time, though, hydrolysed pentosan will release water, making the dough very slack. When using pentosanase, the water absorption of the dough must be adjusted to compensate. If the dough is too slack, not only will it be difficult to machine, but the volume-building benefits of the pentosanase will not occur.

In rye bread, the pentosans in the rye flour are critical to building structure since rye flour's gluten content isn't sufficient. If pentosan content is too high, though, it will compete for water with the starch and prevent it from swelling and gelatinising properly. Pentosanase will help control the pentosan content so there is enough to build structure, but not so much as to interfere with the starch functionality. Pentosanases that hydrolyse cellulose also are available. These may be added to high-fibre bakery products to help improve their eating qualities by breaking up the long cellulose chains that contribute to gritty mouth feel.

Flours contain naturally amylase in varying amounts, which may fluctuate from region to region and crop to crop. It is therefore common practice for the flour mills to add amylases to the flour in order to obtain a more standardised product.

There are several sub-classes of amylases and they are all starch degrading enzymes.

Alfa-amylase was the first commercial enzyme and it is commonly added to flour at the mill to standardise the performance of the flour. It provides yeast with a consistent source of sugars for fermentation and as a result they have a positive influence on bread volume, crumb grain, crust and crumb colour. If the alfa-amylase content is low there will to low dextrin production and poor gas production during proofing and the first stages of baking. This in turn leads to inferior quality i.e. bread with reduced volume and poor crust colour. The ability of alpha-amylase to delay staling is well known. They normally are of cereal or bacterial origin.

Pritchard proofed that another effect of alpha-amylase was the reduction in dough viscosity during starch gelatinisation. Gelatinisation of non-damaged starch starts at 55°C. Because alpha-amylase degrades the gelatinised starch, oven spring will be longer and as a result the volume of the bread will be higher.Fungal amylases have a limited effect on staling. These enzymes act predominantly on damaged starch, but at the temperature at which starch starts to gelatinise, fungal amylases are already deactivated and therefore cannot act on starch when it becomes accessible. Therefore enzyme manufactures have been looking for alpha-amylases with increases activity at lower temperatures. Maltogenic amylase is such an amylase. Maltogenic amylase can be used to improve the freshness of the final product. It can provide a better softness, moistness and resilience of the final product. They do have specific temperature stability profiles and by breaking down amylopectin they delay staling.

Pullulanase and isoamylase are also debranching enzymes. Both enzymes release side chains from the

branched amylopectin molecule. Beta-amylase and amyloglucosidase are exo-acting enzymes.

Also cellulase belongs to the group of enzymes that will produce, as amylases, glucose because cellulose is also a polymer of glucose. However the bonds between the glucose units are β, 1-4 bonds. The seemingly small difference in the bonds in cellulose make a big difference in the physical structure of the polymer and its susceptibility to enzymes. The alfa, 1-4 bond in starch imparts a coiled, helical structure to the amylose, whereas the β, 1-4 linkage results in an extended linear cellulose polymer. Amylases can hydrolyse starch but cannot hydrolyse cellulose at all. To break down cellulose, cellulases are needed and they do not break down starch. This illustrates the selectivity of enzymes in terms of their ability to bind to and hydrolyse only specific substrates with an unique spatial orientation.

For cellulose breakdown the combination of several enzymes is required. Cellulase is the most relevant one as it breaks down cellulose molecules into shorter units. There are 5 types of cellulases and the classification is based on the reaction they catalyse i.e. their influence on dough is a complex matter. Degradation of cellulose by cellulases aids the absorption of water by the flour, the development of the gluten network and improved proofing stability.. For this reason cellulases can improve oven spring and volume in products that are rich in fibres.

Unlike starch cellulose comes in several chemical forms and requires a significantly larger number of enzymes and processes to break it down to glucose. For example cellulose is converted from the crystalline form to the amorphous state, followed by conversion into cellobiose. Finally, cellobiose is converted to two glucose units. In this processes, endoglucanases hydrolyse the amorphous regions by random hydrolysis of β-glucosidic bonds.

Pentosans are polysaccharides comprised predominantly of the five-carbon sugars xylose and arabinose. While they are present in wheat flour in very small quantities, about 2 to 3 percent, they account for as much as one-quarter of the water absorption of dough made from wheat flour. This increases the viscosity of the dough and negatively affects loaf volume. Pentosanases cleave the polysaccharide chains thereby decreasing viscosity and improving loaf volume.

Enzymes that hydrolyse proteins

Enzymes that hydrolyse proteins are called proteases. The site of the attack is always at the peptide bond between the amino end of one amino acid and the carboxyl end of the adjacent amino acid. As is the case with other enzymes also here there are 2 types: exo-proteases and endo-proteases. The exo-proteases are further divided into two groups: carboxy-peptidases if they work on the carboxy end of the protein or amino-peptidases if they work on the amino end of the molecule.

Protease will react with proteins and weaken them. As a result mixing time will be reduced, machinability will be improved as well as pan flow (the dough will fill more easily the shape of the pan). These effects are accomplished by breaking the long protein chains, cutting peptide bonds, into smaller units.

Proteins are made up of long chains of amino acids. Proteolytic enzymes, or proteases, include proteinases and peptidases. Proteinases split proteins at the CO-NH linkages, creating polypeptides, peptides and peptones. These are then further hydrolysed into amino acids by peptidases.

Proteases are "more" specific than amylases. The specificity of starch degrading enzymes depends on which kind of linkages between the individual glucose units they can hydrolyse. In proteins however, all the bonds are peptide bonds but a protein is composed of about 20 different amino acids and not a single chemical unit like the glucose in starch. Hence the characteristics of the various amino acids add an extra level of complexity to the proteins. The result is a "higher" level of specificity of protease enzymes.

In addition to supplying amylases, malted barley flour was also once relied upon for proteolytic enzymes, but these have been replaced by proteases from plant and fungal sources. Addition of <u>proteases</u> enables high speed bread production by decreasing the mixing time needed to achieve pliable dough. A protease acts to decrease the size and binding ability of the gluten molecules. Their action has much the same effect as reducing agents, except their effect is permanent; it cannot be reversed by the addition of an oxidizing agent.

<u>Enzymes affecting fats and oils</u>

The number of carbon atoms in the fatty acid chain can range from 4 to 24. Saturated fatty acids contain all single bonds between the carbon atoms. Unsaturated fatty acids have one or more double bonds between the carbon atoms. Monounsaturated fatty acids contain only one double bond and polyunsaturated fatty acids have more than one. The double bond can be in cis or trans configuration. The cis form is commonly found in nature while the trans form, the less chemically reactive of the two, is normally the result of a hydrogenation process.

Fatty Acids
Saturated Fatty Acids
Unsaturated Fatty Acids

Structure of a Phospholipid
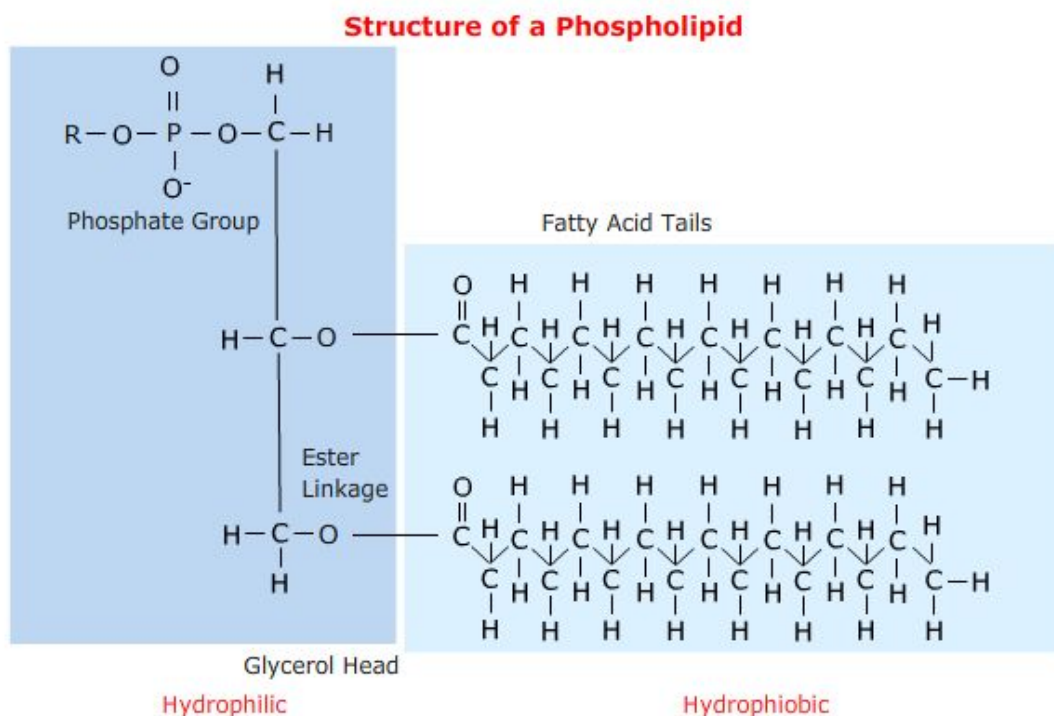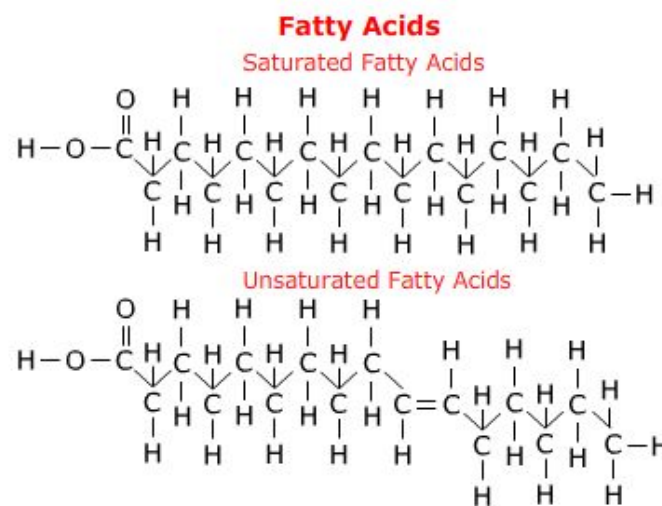
Normal wheat flour contains 1 - 1,5 % lipids, both polar and non-polar. Some of these lipids, especially

the polar lipids such as phosphorlipids and galactolipids are able to stabilise the air bubbles in the gluten matrix. The addition of functional lipases modifies the natural flour lipids so they become better at stabilizing the dough. This ensures a more stable dough in case of over-fermentation, a larger loaf volume, and significantly improved crumb structure. Because of the more uniform and smaller crumb cells, the crumb texture is silkier and the crumb colour appears to be whiter. It also reduces the need for addition of emulsifiers like DATEM and SSL that otherwise are commonly added to dough in order to stabilise it. This in turn means that emulsifiers can be removed from the label.

Lipase removes the a fatty acids from the glycerol backbone. Its pH optimum is generally 7 - 8. Most lipase enzymes remover the two outside fatty acids, leaving the middle one attached to the glycerol. As a result two free fatty acids and a monoglyceride are formed. These free fatty acids tend to be chemically reactive especially when they are unsaturated. Free unsaturated fatty acids can react with the oxygen in the air and cause rancidity.

Proteases hydrolyse the peptide bond between the amino group of one amino acid and the carboxyl group of the next amino acid in a protein. In dough, this serves to weaken the gluten chains. This can affect the dough in two ways, depending on when the protease is added. If the protease is allowed to hydrolyse a portion of a dough early in the process - added to the sponge of white pan bread, for example - it will reduce the mixing time necessary to develop the dough. Early addition of a protease to a complete dough, however, will cause the gluten to become too weak to build structure properly, resulting in a course, uneven crumb.

Nevertheless, protease could be added to an entire dough later, at the mixing stage. This won't reduce the mixing time because the enzyme will not have had enough time to hydrolyse much gluten. Still, as hydrolysis occurs through shaping, floor time and proofing, the protease will help improve the flow of the dough. This procedure might be used to eliminate short pan fills in a straight (non-sponge) dough system or to help the pan flow of buns and English muffins.

Another application for proteases is in replacing sodium sulphides in cracker doughs. Cracker doughs contain low levels of fat and water, making them rather stiff. This stiffness makes it difficult to laminate the dough into layers and to sheet it to cracker thinness. Sodium sulphates hydrolyse the disulfide bridges on the gluten molecule, reducing its resistance to extension and making the resulting dough more plastic.

Sulphates have undesirable side effects, however. They break down vitamin B2, inhibit browning reactions that are desirable in baked products, and are a marketing no-no because some consumers exhibit allergic reactions to the substance. In fact, many countries have banned or are considering banning sulphate's use in bakery products. Adding a protease to the formula and allowing sufficient time for the enzyme to act (sulphates, by comparison, react more rapidly) can achieve the desired workability in the dough without the negative side effects.

While proteases help make dough more slack, lipoxygenases can help do the opposite. Lipoxygenases catalyse the addition of an oxygen molecule to polyunsaturated fatty acids to form peroxides such as indeterminateness acid. These then will interact with a gluten side chain, making the gluten more hydrophobic and, subsequently, stronger. With stronger gluten, the dough will have better gas-retention properties and increased tolerance to mixing.

In a way, lipoxygenases offer results similar to those obtained with dough strengtheners such as sodium stearoyl-2-lactylate, but they also offer additional benefits. Although the exact mechanism behind it is not fully understood, lipoxygenase can bleach fat-soluble flour pigments to produce a whiter crumb in finished bread and rolls.

Lipoxygenase is used in the bakery and it is found in soy flour. This enzyme will bleach the flour. Flour

contains a yellowish pigment that will be broken down by lipoxygenase. As a result one will obtain a whiter crumb.

Asparaginase

Acrylamide is classified as "probably carcinogenic to humans". The WHO (World Health Organisation) recommended producers of carbohydrate rich food prepared by baking or frying, to reduce the concentration of acrylamide in their products.

During recent years it has been shown that the amount of the potentially carcinogenic substance acrylamide is relatively high in a number of cereal and potato based products like biscuits, crackers, crisp bread, French fries and potato crisps. This is a substance that is formed at high temperatures when the amino acid asparagine reacts with a reducing sugar like glucose. To meet this issue the enzyme asparaginase have been developed in order to reduce the formation of acrylamide. Asparaginase converts asparagine to aspartic acid which does not take part in the formation of acrylamide. The use of an asparginase is able to reduce the formation of acrylamide with up to 90%.

Asparaginase catalyses the hydrolysis of the amide group of the side chain of asparagine, which results in the reduction of acrylamide. Reducing acrylamide is getting crucial in bakery products: asparaginase reduces acrylamide from 50 – 90 % and does not interfere with the organoleptic characteristics of the final product. Asparaginase can successfully be applied for acrylamide reduction without changing the appearance of the final product.

The rate of enzymatic hydrolysis of asparagine is dependant upon physical process parameters such as temperature, pH, water activity and time, as well as interactions among these parameters. In addition the presence of precursors such as reducing sugars will influence acrylamide formation which means that the reaction limiting factor differs from product to product.

Hemicellulase and xylanase

Such enzymes work on different parts of the insoluble hemicellulose fraction of the flour. During this process they mainly form xylose and arabinose. This whole group of related materials is often described as wheat pentosans. Pentosans roughly bind seven times their own weight in water. This leads to greater extensibility and stability of the dough (better machinability) and in turn leads to a better oven spring, volume and finer cell structure of the baked product. This in turn leads to improved softness and resilience.

However the whole picture is more complicated then described here. There are various kinds of xylanases (high molecular weight, low molecular weight) and there is no complete understanding of the mechanisms and effects of the different xylanases. There is not one single xylanase which performs equally well under all circumstances. Bakeries and bread improver companies need to establish optimal dose rates and optimal xylanase blends for each application and they can do this only by trial and error. There is no way to predict the performance of a xylanase in a given bread process.

Flour contains 2,5 - 3,5 % non-starch polysaccharides, which are large polymers (mainly pentosans) that play an important role in bread quality due to their water absorption capability and interactions with gluten. Although the true mechanism of hemicellulase, pentosanase or xylanase in bread-making has not been clearly demonstrated, it is well known that the addition of certain types of pentosanases or xylanases at the correct dosage can improve dough machinability yielding a more flexible, easier-to-handle dough. Consequently, the dough is more stable and gives better ovenspring during baking, resulting in a larger volume and improved crumb texture.

Transglutaminase

When using so-called "weak" flours the use of transglutaminase is beneficial. Weak flour will also lead to a weak gluten network. By adding transglutaminase the dough will be able to retain better the CO2 formed during fermentation. Transglutaminase has a cross-linking effect on proteins independent of the redox system of the dough.

The effect on the rheological properties of dough is similar to oxidation and the effect is due to an increased number of disulphide bonds. It is often added by the mill to the flour. This has the advantage that the dosage can be based on the properties of the flour.

Studies also have been done of the effects of transglutaminase in frozen dough (W. N. Huang et al). After five weeks of frozen storage at –18°C, the gluten structure in the control dough appeared less continuous, more disrupted, and separated from the starch granules, while the dough containing 0.5% transglutaminase showed less fractured gluten network. Addition of transglutaminase increased specific volume of bread significantly with softer bread texture. Even after the five weeks of frozen storage, bread volume from dough with 1.5% transglutaminase was similar to that of the fresh control bread. The improving effects of TGase on frozen dough were likely the result of the ability of transglutaminase to polymerise proteins to stabilise the gluten structure embedded by starch granules in frozen doughs.

Also in gluten free products transglutaminase can play an interesting role. One of the main problems of gluten free bread is the creation of a good crumb structure. Transglutaminase is a tool to improve the structure of gluten free breads. The quality of these products is significantly better with the formation of a stable protein network. However the efficiency to create a protein network depends on the source of the protein (skimmed milk powder or egg proteins for instance) and the quantity of transglutaminase added to the recipe. But the use of transglutaminase will improve the volume, the crumb structure and the overall quality of gluten free bread.

Understanding and selecting enzymes

Understanding what different types of enzymes do to bakery products is the first step in enzyme selection. Considering how specific enzyme action is, once the desired results are determined, the enzyme to use will be a straightforward decision. Other factors in enzyme selection and use aren't so easy. These include the enzyme source and form, the strength of the enzyme activity and how much to use, and the conditions under which the enzyme will be used and handled. Amylases used in bakery foods come from three primary sources.

1. Malt ingredients. As previously mentioned, flour contains naturally occurring amylases. The same is true for cereals other than wheat. When a cereal kernel becomes moist and germinates, it experiences a dramatic increase in α-amylase. Consequently, malting grains such as barley and wheat can serve as the basis for many α-amylase-containing ingredients. Malt flour is most frequently used by millers to standardize the α-amylase content of wheat flour, although it is also often found as an ingredient in crackers and certain breads. It is made from wheat or barley that has been germinated, dried and ground to flour fineness. Malt extracts and syrups start with germinated barley. Rather than grinding the kernels after drying, these ingredients are made through a series of liquid extraction and concentration steps that preserve the grain's α-amylase activity. Diastatic malt syrups are made the same way, but start with a blend of corn and barley. This causes diastatic syrups to have less of the malt flavour contributed by regular syrups and extracts, yet provide the same level of enzyme activity. The non-diastatic malt syrup process is similar, but produces an ingredient without the amylase activity. This is then used for non-enzyme related benefits such as flavour and improved crust colour.
2. Fungal amylase. During growth, certain fungi synthesize α-amylase. Cultures of Aspergillus oryzae are extracted, concentrated and dried to yield fungal amylases. These are available both in ready-to-use tablet form and blended to a predetermined activity with flour or starch to yield a

powdered form. Fungal amylases can be used to standardize wheat flour, but are most often added at the production facility to aid with dough conditioning.

3. Bacterial amylase. Certain bacteria, such as Bacillus subtillis, also synthesize α-amylase. This can be extracted and dried much like fungal amylases. Bacterial amylases, however, tend to be more thermally stable and are, therefore, useful for maintaining softness in finished baked products.

Like amylases, proteases for bakery applications can be extracted from both fungi and bacteria - most often with the same species used for α-amylase production. Different types of protease have different catalytic mechanisms. The different mechanisms primarily control how the enzyme responds to different pH conditions.

1. Acid proteases can be found in flour and have a low pH optimum. They are thought to mellow gluten during long-term, low-pH fermentation of saltine cracker sponges.
2. Sulphydryl proteases are found in many grain-based ingredients such as flour and malt. They also are extracted from pineapple stems (bromelain) and papayas (papain). Sulphydryl proteases have a pH optimum range from around 3.5 to nearly 8.5.
3. Serine proteases often are called alkaline proteases because their activity is optimum above pH 7.5.
4. Neutral proteases make up most of the commercially available proteases. Here, the pH is optimum in a narrow range around 7.

Lipoxygenases aren't available in concentrated forms like proteases and amylases. They are added as a natural constituent of full-fat and defatted soy flour. These flours often are offered with other functional ingredients such as calcium peroxide for additional oxidation, dicalcium phosphate for dough conditioning, and corn flour to improve absorption and mix tolerance.

Activity problems

Because they are a naturally occurring component of soy flour, lipoxygenase activity is not as standardized as it is with the tablets, powders, etc. available with amylases and proteases. Though these forms sport standardized activity levels, product designers still may be confused by the different methods of activity measurement. The amylose activity of malt extracts and syrups, for example, is typically expressed as degrees Lintner, while concentrated amylase sources are often expressed in Sandstedt-Kneen-Blish (SKB) units.

On top of the tremendous number of standardized tests, individual enzyme suppliers often have a custom method of determining enzyme activity. This presents a challenge to product designers trying to compare activities in order to predict usage levels and cost impact.

The goal of most methods of measuring enzyme activity is to determine how quickly the enzymes convert substrate molecules to product molecules. Because of this, the activity measurements often have little to do with the enzyme's activity in actual use, particularly in baked products. Designers will probably wish to create their own assay by testing enzymes at different levels in actual doughs. The observed effects can then be related to the amount of enzyme added.

By using the level of activity per gram of enzyme as the measuring unit, product designers will have a common basis for comparing enzymes. In addition, the activity measurement will include a weight that can be directly related to the price of the ingredient in order to determine the cost of a given degree of effectiveness.

Synergistic effects

Each of the enzymes mentioned above has its own specific substrate in wheat flour dough. For example,

lipases work on the lipids, xylanase works on the pentosans, and amylases work on the starch. Because the interaction of these substrates in dough and bread is rather complex, the use of enzyme combinations can have synergistic effects that are not seen if only one enzyme is used, not even at high dosages. Quite often an overdose of enzymes will have a detrimental effect on either the dough or the bread. For instance, an overdose of fungal alpha-amylase or hemicellulase / xylanase may result in a dough that is too sticky to be handled by the baker or baking equipment. It is therefore beneficial for some types of bread formulation to use a combination of lower dosages of alpha-amylase and xylanase with low dosages of lipase or glucose oxidase to achieve optimum dough consistency stability and bread quality. Another example is to use maltogenic alpha-amylase in combination with fungal alpha-amylases and xylanase or lipase to secure optimum crumb softness as well as optimum bread quality in terms of crumb structure, bread volume, etc.

<u>Factors influencing the performance of enzymes</u>

When creating a test for comparing enzyme activity and when preparing to put enzymes to work in the formula, remember that conditions the dough encounters through the process will greatly affect enzyme activity.

Time is critical for successful application of many enzymes. Put simply, the chemical reaction must have enough time to proceed. An enzyme's catalytic reaction can, of course, be sped up by increasing the enzyme level to increase the amount of available catalyst. However, this can be expensive and, in the case of bacterial amylases for shelf life extension, be impossible due to detrimental effects in the finished product.

Keep in mind also that amylases can only act on damaged or gelatinized starch granules. A certain amount of mixing and/or dough development will be required before these enzymes begin to work. A protease will start to act as soon as a dough is wetted. On top of that their performance depends on the fat and sugar content of the product. An amylase that performs well in bread will not give as good results in cake for instance.

Temperature influences enzyme activity in both a positive and negative way. Every 18 degree F increase in dough temperature increases the enzyme activity up to two-fold. On the down side, the same temperature increase also will accelerate the rate of enzyme denaturation by a factor anywhere from 10- to 30-fold. At a high enough temperature, the rate of denaturation catches up with the reaction rate, slows it and eventually stops it. Just as the time and enzyme amount must be optimally balanced, so must the time and temperature. A longer reaction time can actually increase the efficiency of the enzyme conversion at a lower temperature.

Acidity, or pH, affects enzyme activity. Different enzymes, and even enzymes from different sources, have optimum pH ranges under which they are most active. This was previously discussed for different proteases, but also is true for amylases.
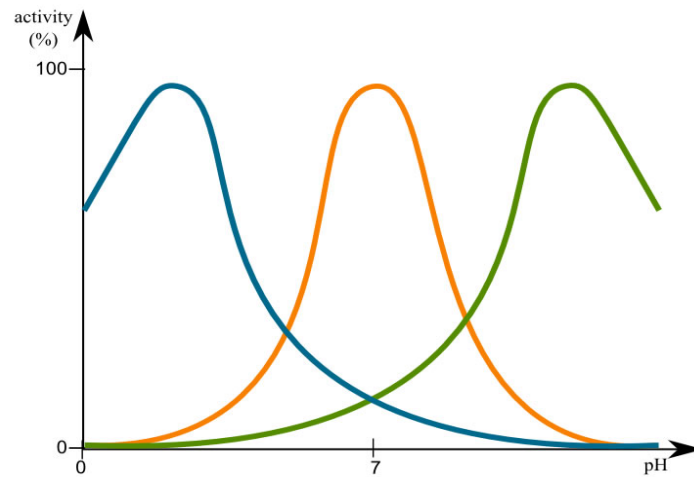
When formulating, designers must not only be aware of the starting pH of the formula, but how it changes over time. For example, as chemical leaveners are consumed, the overall dough pH may be altered out of the optimum range for the enzyme. The same is true for yeast-leavened products, as the pH can change dramatically as fermentation proceeds in products such as crackers and bread. So the usefulness of a given enzymatic activity depends in part on matching the pH optimum of the enzyme with the pH of the dough.

Care also is necessary when adjusting the formula pH. Cocoa powder and other chocolate-flavoured ingredients require an alkaline system for optimum flavour. Adjusting such a system to be more acidic for the enzyme can adversely affect the flavour. Colour development is strongly related to pH, and any alterations will affect a product's crust colour.

Salt level can affect the enzyme's activity because salt can help stabilize certain enzymes. The opposite is true, however, for proteases, which are inhibited by high salt concentrations. This could be the result of salt making gluten less available to the action of the enzymes.

If salt levels can't be adjusted, the order of addition can overcome this limitation. In a sponge-and-dough bread, for example, enzymes can be added to the sponge. Because salt won't be added until the dough stage, the enzymes will have more time to react uninhibited. Salt also can be added to later steps in multiple-stage mixing procedures for other products, but the time between stages isn't nearly as significant as it is between a sponge and a dough.

Certain enzymes may require ionic co-factors to be active. Many carbohydrases will not function without calcium ions. Zinc is necessary for neutral fungal proteases.

Enzymes are indeed a highly specific, useful collection of ingredients for bakery products. Enzyme activity itself is useful, and many enzyme applications offer clean-label advantages. Although the number of different enzymes and the cacophony of different activity measurement methods may seem intimidating, product designers can sort through this to determine the best enzyme and the conditions that enzyme requires in the formula and during processing for maximum effectiveness. All it takes is a new understanding of these old ingredients.

General effects of enzymes in bread making

positive influence on	gluten network	increased volume	improved flavour	crumb structure	shelf life properties
amylase		×	×	×	×
protease	×				
xylanase	×	×		×	

oxidase	×	×		×	
lipase	×	×	×	×	
transglutaminase	×	×			×

<u>Use of enzymes in biscuits and crackers</u>

Another application of enzymes in baking is in the production of biscuits and crackers. The requirements of the flour are altogether different from those in bread-making; a 'soft flour' which produces a dough with pronounced plastic properties is preferred. For this purpose, flour with relatively low protein content is desirable. The gluten protein structure should not be too strong, otherwise the dough will be too difficult to handle.

Unless flour with these properties is available, it is necessary to add an agent to weaken the gluten. Reducing agents (substances which have the opposite effect to oxidizing agents) have been used for this purpose, in particular sodium bisulphite. The bisulphite has the desired effect on the gluten, but unfortunately it affects other substances in the flour, including the content of vitamin B1 (thiamine). This vitamin is completely or partially destroyed. Sodium bisulphite has been banned in certain countries and is becoming less popular due to health risks. An alternative is the application of a protein-degrading enzyme. This softens the gluten without affecting the other constituents of the dough. Several fungal and bacterial proteases can be used for this purpose. Proteases can also be used when making bread with 'hard flour' i.e. flour high in gluten

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