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Application of Enzymes in Brewing

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Abstract

Brewing is one of the oldest food processes done by mankind. The technology of beer production took place six thousand years ago and has been done by the world's oldest civilizations such as the ancient Egyptians and Mesopotamians. Nowadays, brewing is one of the lead food industries in the world in general and especially the west. Enzymes utilization is one of the main pillars of brewing industry, and whether the enzyme is endogenous in the kernel itself or been added from external sources, a deep understanding and research of those enzymes is mandatory for the sake of better production and higher quality. The aim of this review is to explain the flow process of brewing and to discuss different enzymes used in brewery industry with the description of their mechanism of action.

Keywords: Brewing; Enzymes; Hydrolysis

Introduction

Enzymes are vital biochemical materials that have been proven to be not only critical for living organisms but also in the field of food science and technology. Enzymes are participating in many important reactions in human body such as signal transduction, cell regulation, generating movement, digestion, absorption, and metabolism [1]. Enzymes are mainly tertiary structured globular proteins and their primary structure is made up of up to thousands amino acids (Figure 1), which are linked together via peptide bonds in a linear chain [2].

In addition to their physiological characteristics, enzymes play a vital role in food industry. Since 6,000 BC and until today enzymes have been utilized enormously in multi different stages of food processing (e.g., brewing, cheese making, reduction of maturation time in wine making, meat tenderizing, etc.). List of enzymes and their food applications is given in Table 1 [4].

One of the main applications of enzymes in food industry is brewing. Brewing is defined as the process of beer production in which the sugars in starch are fermented to ethyl alcohol through the action of yeast [5]. It is one of the oldest food production techniques; it goes back to time even before Christ [6]. In Ancient Egypt women used to bake and brew as a house duty where beer during this era was called heqet [6,7]. Ancient Egyptians used bread, water, dates, honey, and yeast to produce their thick, dark red beer [7]. Nowadays, brewing industry is vastly growing and has shown an increased trend during the last decade (Figure 2) [8]. According to the 2015 statistical data China (460 bn hl) and USA (189.21 m hl) are the biggest producers of beer [8]. The total number of breweries in the United States from 1990 to 2016 was calculated to be 5301.8 [8]. The intent of this discussion is to explain the flow process of brewing and to discuss different enzymes used in brewery industry and describe their mechanisms of actions.

Brewing Process

The brewing process consists mainly of nine general steps which are, malting, milling, mashing, lautering, hopping, fermentation, conditioning, filtering, and canning or bottling (Figure 3) [9].

The process starts with malting, where the grains (usually barley or wheat) are dried into malt in order to convert complex carbohydrates into dextrin and maltose by three sequential processes which are, steeping (14–18°C), germination (16–20°C), and kilning (50–110°C) using water, heat, and action of enzymes (e.g. α -amylases and proteases) [9,10]. After malting, water is added to the malt before grinding in order to give the malt homogeneity and further hydrolysis to simple sugars by milling and mashing process [9,10]. The wort (resultant of mashing process) will then go through the lautering (from the German word *abläutern* which means to rinse off), where the grains are filtered from the sugar solution extracted during mashing in a lauter tank as it contains small filters that holds the hulls of the grains from the sweet solution [9,11]. During hopping, the wort is boiled (100°C) together with hops in order to sterilize the wort from any contamination and

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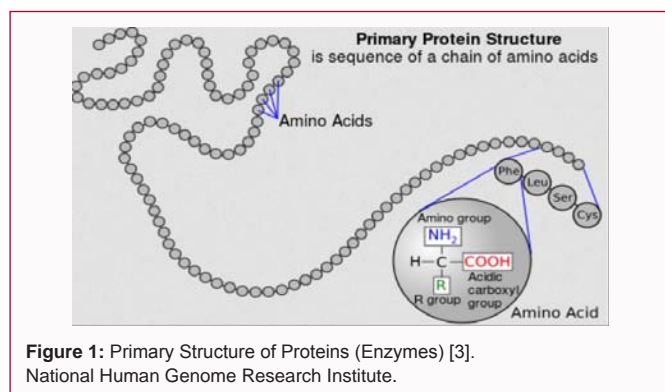
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Table 1: Enzymes and Their Applications in Food [4].

Enzymes	Applications in Food
β-glucanase	Used in brewing industry.
Lipase	Used broadly in food industry. Ex: nutraceuticals as fat burners, infants' formulas, etc.; oil industry; dairy industry; and Pet food industry.
Chymosin	Used widely in cheese making.
Pectinase	Used widely in fruit juice industry and wine production.
Ficain	Meat tenderizers, usually in combination with papain and/or bromelain.



improve beer's flavor and stability [9]. The wort will be cooled down and left to be aired so that the yeast will be added for the fermentation process (6–25°C) in order to ferment the sugar to ethanol and carbon dioxide to produce the beer [9,10]. Later on, the beer is conditioned by lowering the temperature to (-2–0°C) in order to get rid of the harmful and unwanted particles that may affect the carbonation, aroma, and taste of the beer [9,10]. Later on, the beer is filtered with sheets (pads) or kieselguhr (diatomaceous earth) filters in order to get rid of the solid suspended particles such as hops, barley grains, yeast, etc [10,12]. Finally the beer is packaged in cans, kegs or bottles, one of the most critical steps during packaging is to remove oxygen from the product in order to avoid spoilage and deterioration. Through all the steps that were mentioned above in the brewing flow chart, enzymes are key factors in maintains and improving those steps. Below each enzyme will be mentioned and their role in brewing will be discussed [13].

Barley Kernel

The brewing process is based on the soaking starch from plant

source such as barley in water then the fermentation of the product by yeast [10]. For this process to occur, enzymes are used for their critical role in catalyzing different steps. Barley is a great source for starch and widely used for beer production [10,14]. The barley kernel contains most of the brewing enzymes (Figure 4) that is secreted when the kernel is soaked in hot water and when protease and glucanase enzymes are used [14]. As mentioned before malting consists of three steps which are steeping, germination, and kilning. During steeping the barley is steeped in water for around two days to absorb moisture then germination takes place for 3-7 days where the barley is converted to malt [10,14]. Germination stops by the starting of kilning where the malt is dried from moisture and color and flavor of the malt is changed [10]. The stored malt after malting undergo mashing where hot water is applied and starch gelatinization occurs [10]. The starch in brewing is from two main sources the starch from the barley itself and adjunct starch [17]. Adjunct starch is an outer source of starch that is added as an extra source of fermentable glucose and nutrients for fermenting yeast [17]. The starch is gelatinized breaking it's granules exposing the amylose and amylopectin chain to the enzyme actions. Alpha-amylase start to act on the starch hydrolyzing it to dextrins in a process called liquefaction [17]. After liquefaction, saccharification amylolytic enzymes further hydrolyze the starch and dextrins into glucose that is used for fermentation by the yeast [14]. The usage of saccharification mashing enzymes is depending on the quality and type of beer, for example the enzyme glucoamylase is usually recommended and well adopted in the production of light beer [18]. The barley kernel can be sometimes mixed with malt when commercial enzymes are used as this combination sometimes leads to a desirable attributes in the final product [14].

Enzymes in Brewing

Enzymes utilized in brewing industry are diverse their action and properties. The main enzymes used in beer brewing industry can be divided into four main processes which are germination, mashing, fermentation, and clarification [13]. Brewing process required a great knowledge of enzymology as each enzyme has its own temperature point, where it can be in active when the medium is far away from that point (Figure 5) [14]. The four most common enzymes used in brewing are beta glucanase, protease, alpha amylase, and beta amylase. Enzymes can be ether endogenous or external (commercial) [9,13,14]. Commercial enzymes can be used for extra quality attributes such as, clarification, color, texture, or flavor and sometimes it is mandatory to use the enzymes from external source when the barley mashing

Table 2: Brewing Enzymes and their Effects [14, 18-20].

Enzyme	Sources	Process	Function
α-amylase	Endogenous in the barley kernel. Bacillus licheniformis Bacillus subtilis	Malting Mashing	Starch hydrolysis Improve Clarification
β-amylase	Endogenous in the barley kernel. Wheat Kernel Bacillus licheniformis	Malting Mashing	Starch hydrolysis Improve malting Improve Saccharification Increase fermentation yield
β-glucanase	Endogenous in the barley kernel. Trichoderma sp. Orpinomyces sp.	Malting Mashing Fermentation	Improve malting Lower Viscosity Improve Clarification Aid in production of a clear wart
Fungal α-amylase	Aspergillus sp.	Fermentation	Increase fermentation yield
Protease	Endogenous in the barley kernel. Aperguillus sp. Pineapple latex	Malting Mashing Storage	Improve malting Improve fermentation Improve clarification Improve chilling and storage quality
α-acetolactate-decarboxylase (ALDC)	Bacillus subtilis recombinant	Fermentation	Reduce fermentation time
Amyloglucosidase	Aspergillus niger	Mashing	Increase the amount of glucose in wort

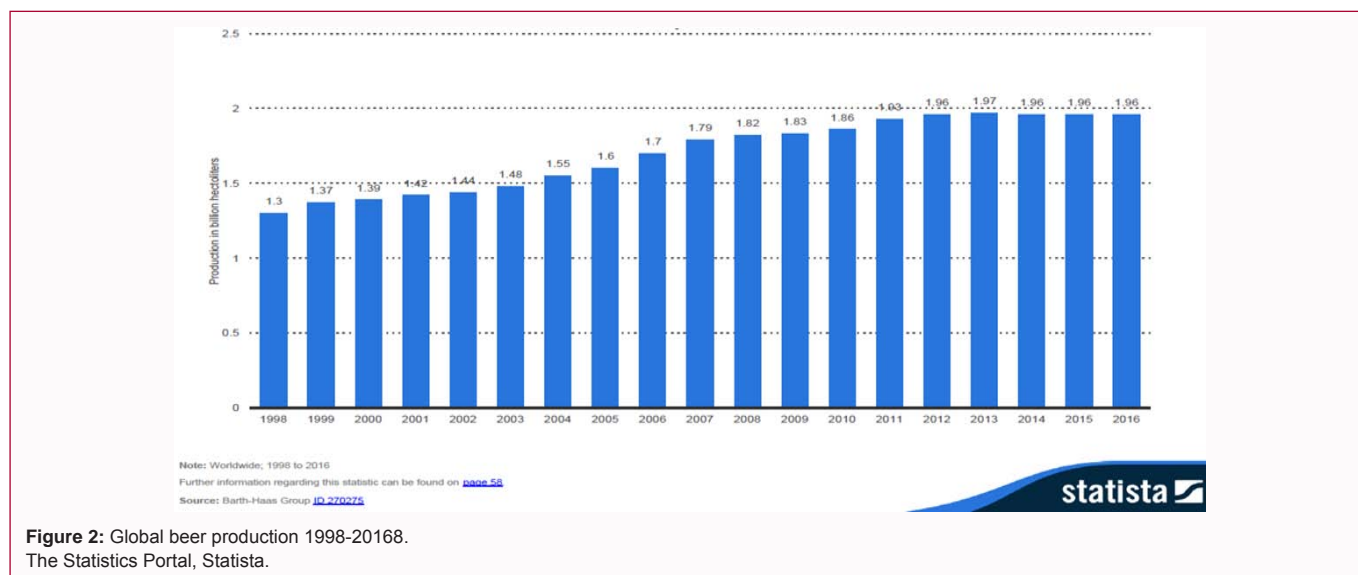


Figure 2: Global beer production 1998-20168.
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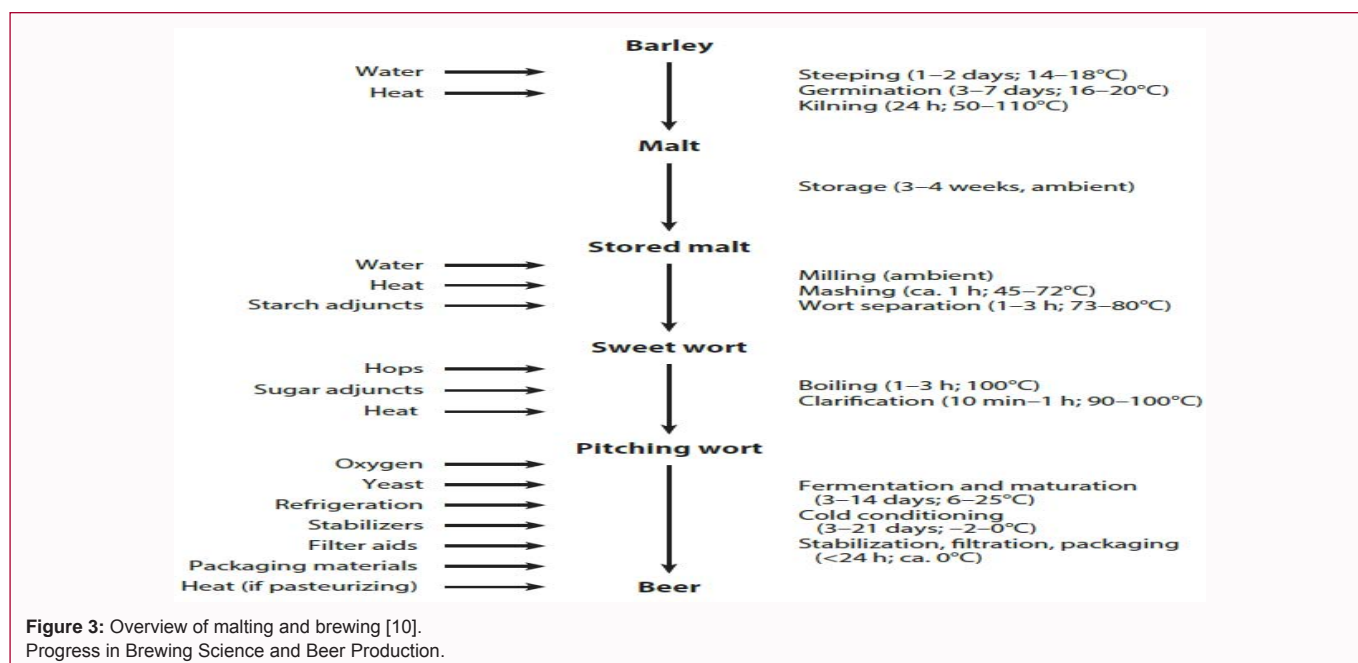


Figure 3: Overview of malting and brewing [10].
Progress in Brewing Science and Beer Production.

is not producing enough enzymes to breakdown and hydrolyze the starch which can lead to low quality beer and less yield [9,14]. During malting β -glucan, fibers and proteins are broken down by the action of proteases, glucanases, and xylanase [14]. After the gelatinization of starch by the hot water the amylases enzymes start to act on the wort producing sugars through the liquefaction and scarification processes, yielding sugars to be fermented [14]. Later on, fermentation process takes place where α -amylase and β -glucanase can be reused again in order to improve the fermentation yield and filtration [13-15]. Usually other enzymes can be used for extra quality such as alpha acetolactate decarboxylase (ALDC) which improve fermentation by reducing the fermentation time and other commercial types of proteases that keeps the beer clear during cooling and chilling of beer [14,15]. Different types of brewing enzymes and their sources are summarized in Table 2.

Beta glucanase

Beta glucanase is one of the important enzymes in the brewing

flow-chart especially during malting and mashing processes [13]. Cellulase enzymes are the first line of action against the starch granules as during the gelatinization step they digest the outer layer of the starch granules and with the effect of hot water the starch granules are opened, letting the internal starch to be an optimum substrate for the other hydrolyzing enzymes to act on [14]. β -glucanase generally hydrolyzes the 1-3 β -glycosidic bonds between glucose molecules in glucans (Polysaccharide made of molecules of D-Glucose). This reaction is desirable in beer brewing as it lowers the viscosity of the warts, and support proteases in the hydrolysis of the matrix of the starch granules in order to make the kernel soften during germination. Usually, β -glucanase are found endogenously in barley itself and is called endo-b1, 3-1, 4-glucanases, however recently commercial β -glucanase from microbial organisms is used for the standard production of light beers (less carbohydrate vale) and shorter maturation time in addition to its original functions in malting and mashing filters for an improvement in texture and light quality. Although, beer turbidity might be desirable in some beers

such as craft beers, commercial β -glucanase is sometimes added as an extra step for beer clarification, as β -glucanase helps in breaking the turbidity system by hydrolyzing the beer haze (proteins bonded to polyphenols, β -glucans, and starch) [10]. The amount of the enzyme used commercially is usually 0.3-1 kg per ton of the wort material [18]. β -glucanase has an optimum pH and temperature values of 6.0 and 45 - 50°C; and denaturizes at 60°C which is common as 45°C is known to be the lowest optimal temperature for enzymes that hydrolyzes cell walls [16].

Amylase

Amylases are utilized mainly in breaking down the starch (Polysaccharide made of large number of alpha-glucose molecules) into dextrins, oligosaccharides, maltose, and glucose molecules as a part of the malting process. During brewing both amylatic enzymes alpha and beta amylases are utilized to convert the starch to its fermentable form [10,14]. Alpha amylase (α -amylase) is a hydrolyase enzyme that catalysis the hydrolyses of starch's two large macromolecules amylose (the linear chain of the starch in the granule) and amylopectin (the branched chain of the starch in the granule) into dextrins by breaking the internal α (1-4) glycosidic bonds between the α -glucose molecules whereas beta amylase (β -amylase) catalysis the hydrolysis of amylose and amylopectin into maltose by breaking the external α (1-4) glycosidic bonds [16]. In the malting process, α and β -amylase are released from the starch granules after the outer layers have been already hydrolyzed by the β -glucanases and xylanases enzymes. Both Alpha and beta amylases continue to hydrolyze the starch solution within wort during the mashing phase to simple sugar that will be ready to be fermented [16,17]. Alpha amylase is also used after malting and mashing during fermentation process in light beers as it known to help in increasing of the yield of carbohydrate that can be fermented [14]. Controlling amylases can also play a very important role in beer quality, as depending on the amount of amylases acted and amount of starch available the beer alcohol amount is determined [17]. The high the concentration of sugars in wort, the higher the ethanol content in the beer which can occur by using commercial amylases (from *Bacillus Subtilis*) accompanied by the endogenous one and addition of an adjuncts sugar or starch whereas low sugar or amylases wort leads to low alcohol beer content [17,18]. The two amylase enzymes have different optimum temperature values, β -amylase has an optimum pH and temperature of 5.5 and 62.78°C, while α -amylase has a higher optimum temperature 73.89°C and lower pH 5.2 [16]. The two enzymes can work at the same temperature, however not in a high efficiency as they would do in each optimum temperature and accordingly affects the ratio of the fermented and non-fermented sugar at the end [16]. Whenever commercial α -amylase is used in addition to the endogenous one, the manufactures recommend using 1-2kg of the enzyme per ton of barley for a better starch liquefaction [18]. However, for β -amylase it is recommended to use an external dose depending on the source of starch as β -amylase is mostly vital in the saccharification process [18].

Protease

Proteases are class of enzymes that catalyze the hydrolysis of peptide bonds in proteins. Proteases are used in brewing for many benefits and the most important two are digestion of protein for clarification and facilitation of malting [9]. Protease increase the degree of solubility of the proteins and accordingly lowers the viscosity of beer, in addition protease develops a good conditions for yeast growth by satisfying the availability of free amino nitrogen

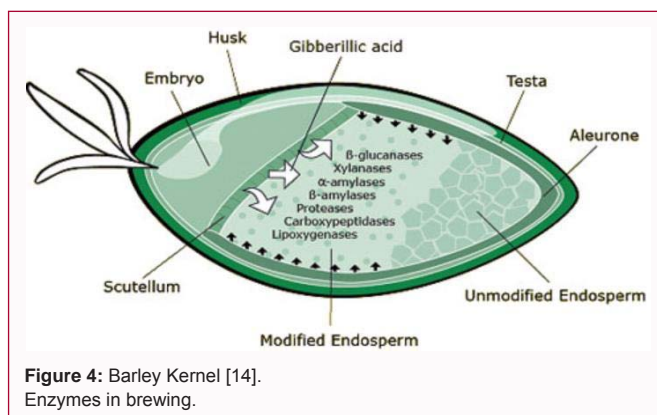


Figure 4: Barley Kernel [14].
Enzymes in brewing.

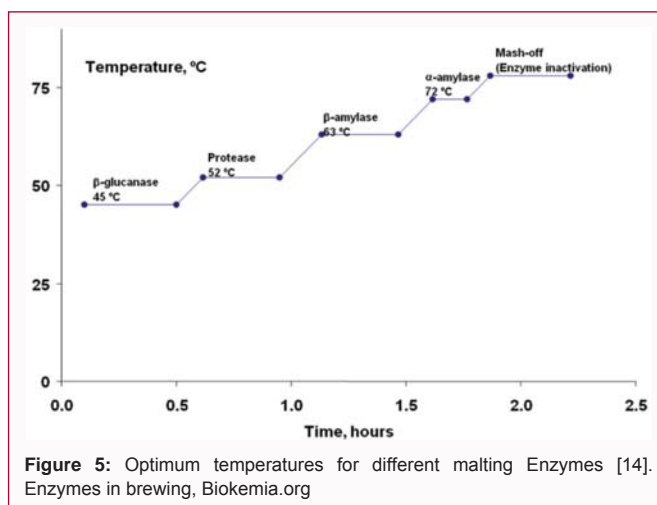


Figure 5: Optimum temperatures for different malting Enzymes [14].
Enzymes in brewing, Biokemia.org

which is necessary for yeast growth [9,14,21]. During mashing protease softens the kernel layer by hydrolyzing the cell wall protein leading to the exposure of the starch to the mashing enzymes and accordingly better mashing and wort fermentability [16]. Protease must be carefully monitored during administration as protease may affect the quality of the foam which is very desirable in most beers [21]. Brewing protease optimum temperature is 52°C and deactivated at temperature range 70 - 75°C. Although protease is known to have a tendency for an acidic pH, it was found that it still catalyze even in high pH maybe even up to 10 [16,22]. Although, protease is present naturally in the barley kernel it is mandatory to use external commercial protease in order to maintain an acceptable beer quality, especially when low degradation quality starch is used [23]. Protease overdosing can cause undesirable effects such as enzyme degradation and foam instability [9,16]. As most of the proteolytic mashing enzymes, manufacturers recommend protease dosage to be 0.3 - 1 Kg per one tone of barley [18].

Additional enzymes used in brewing

In the modern era and at the zenith of enzyme technology, other enzymes have been used in brewing industry for to achieve and maintain an optimum quality of beer during processing, storage, and transportation [14]. Some of the enzymes that are additionally utilized are from the same groups of enzymes that are typically used such as ficin and papain which belongs to the protease family and other enzymes that are being used from another groups of enzymes as alpha-acetolactate decarboxylase (ALDC) which belongs to the lyases family [24]. Proteases such as ficin and papain are sold commercially as they are extracted from plants such as figs and

Pawpaw latex [24]. Ficin and papain are used as chill-proof enzymes, where they hydrolyze the proteins that cause the chilling haze [18]. The chill-proof enzymes mode of action based on the removal of high molecular weight polyphenols and polypeptides by hydrolysis leading to the vanishing of the haze material [10,13,24]. The thermo-stability of those enzymes is usually between 60 – 65°C, while their typical pH of proteases [14,24]. Chill-proof enzymes dosages differs according to the source of the enzyme and the substrate used, however the common dose for barley brewing is 1 to 2 grams per one hectoliter of beer either storing vessels or before beer filtration [18].

Another enzyme that is utilized in the flowchart of beer production is alpha-acetolactate decarboxylase. On large scale production of beer it is mandatory to lower the time of production for more yield, alpha-acetolactate decarboxylase is used for that purpose as it reduce the fermentation time resulting in faster beer production without disturbing the quality [19]. Alpha-acetolactate decarboxylase catalyze the conversion of α -acetolactic acid to acetoin directly by eaving the carbon-carbon bonds between the α -acetolactate [14,19]. The optimum temperature of α - acetolactate decarboxylase falls between 25 to 40°C whereas the pH is within 5 and 7 [25]. The administration of α - acetolactate decarboxylase as the manufacture states is 1 to 5 grams per hectoliters of the wort when administered during initial fermentation or 0.4 to 1.0 grams per hectoliter of wort when administered after fermentation [25].

Conclusion

In the light of what was mentioned above, it can be assumed that brewing in the modern days cannot continue without the utilization of enzymes. The enzymes are biological catalysts that catalyze the biochemical and molecular reaction which is necessary for maintenance and production of many food products. All of the main enzymes such as amylases, proteases, glucanase, and cellulase are crucial for the beer production process. The administration of α - and β -amylase is vital for malting and mashing of barley in addition to its role in increasing the sugar yield for fermentation. The application of other carbohydrate enzymes such as glucoamylase and pullulanase is complementary for the producers; however it is wisely used to ensure faster and larger glucose production and accordingly more beer yield. So the production levels of dextrins and fermentability of wort are depending mostly on the action of amylases enzymes. Beta-glucanase is a key enzyme for malting as it disrupts the cell wall of the kernel and adjunct starch leading to the secretion of the other enzymes that result in malting. Also glucanase and cellulase are responsible for the reduction of viscosity in beer and aids in wort clarification. Proteases functions can be summarized into three points which are improved yeast growth, enhanced malting, and reduction of haze during storage of beer. It is crystal clear now that brewing is a complicated process that includes a couple of crucial steps such as malting, mashing, and fermentation. For instance, without malting and no commercial enzymes to compensate malting there would be no enough endogenous or external enzymes for starch hydrolysis, malt filtration, and proper fermentation. Moreover, these steps are very sensitive and any under or over-dose of enzymes administration can lead to a high number of unwanted and undesirable effects.

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