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# Quality attributes for barley malt: "The backbone of beer"

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**Abstract:** Malting is the process of preparing barley for brewing through partial germination followed by drying. This process softens the grain cell wall and stimulates the production of diastatic enzymes, which convert starch into malt extract. The suitability of a barley grain for malt production depends upon a large number of quality parameters that are crucial for the identification and release of high-quality malt varieties. Maintaining tight control of these quality attributes is essential to ensure high processing efficiency and final product quality in brewery and malt house. Therefore, we have summarized the basic malting process and various physiological and biochemical quality parameters that are desirable for better malt quality. This study may provide an understanding of the process, problems faced, and opportunities to maltsters and researchers to improve the malt efficiency by altering the malting process or malt varieties.

KEYWORDS
barley, diastatic enzymes, enzymes, malt, quality

## 1 | INTRODUCTION

Beer is one of the most widely consumed drinks available among various alcoholic beverages. Beer contains B-complex vitamins, trace amounts of certain minerals, such as Ca, K, and Mg and a wide range of polyphenols, which helps in maintaining health and have protective effects against cardiovascular disease. Nutritional properties of beer and its preference in social events motivate brewers to improve beer quality, which is influenced by malt quality. Malting is forced germination, performed to acquire a certain amount of proteolytic and amylolytic enzymes (Habschied et al., 2020). The production of hydrolytic enzymes is essential for the hydrolysis of endosperm cell wall carbohydrates and protein matrix. Malting is 5-7 days process that constitutes three steps steeping, germination, and kilning. In brief, the grains are immersed in water, allowed to germinate and then germination is terminated by blowing the hot air to lessen the moisture content, thus, halting metabolic activity. Barley (*Hordeum vulgare* L.) is the major gain of choice for malt production worldwide since it has high starch to protein ratio. Its husk assists in filtration, provides firm texture to grains and protects the sprout during the germination. The utilization of barley in the beer industry has given this crop the status of an industrial crop and crop of interest for entrepreneurs, farmers, and researchers (Kumar et al., 2013). The amount of malt extract produced relies upon several morphophysiological grain parameters like kernel size, shape, grain hardness, moisture, dormancy, microbial contamination, and biochemical parameters viz., protein content, $\beta$-glucan, starch content, hydrolytic enzyme activities which in turn influence diastatic power (DP), malt yield, Kolbach index (KI), malt friability, homogeneity, free amino nitrogen, wort viscosity, saccharification rate, germination capacity (GC), etc. The purpose of this review is to understand and correlate different grain and malt parameters with the malt quality, which can assist the maltsters in selecting the potential malt variety.

## 2 | THE MALTING PROCESS

Large and structurally complex compounds (starch, proteins, and nucleic acid) that are present in barley grain must be partially or fully degraded into simpler

  

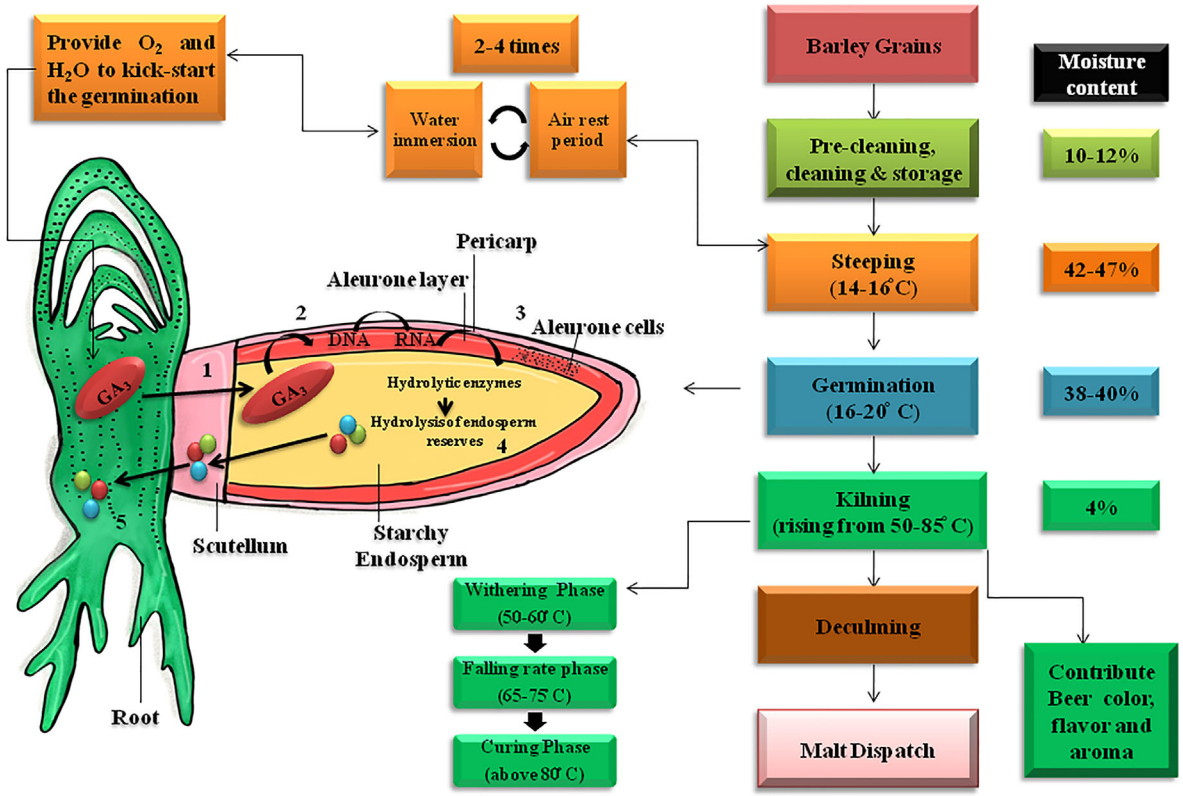


**FIGURE 1** Overview of malting process

components (sugars, amino acids, and nucleotides) by the hydrolytic enzymes produced during malting. Once the malt is prepared, it needs to be characterized for quality traits that ensure the proper completion of the malting process. Currently, there is a need for finding new methods to improve the malt quality by increasing germination ability and reducing the time of sprouting (Shintassova et al., 2019). Due to variations in equipment and kind of malt required, expenses and malting conditions may differ, but the basic malt process has remained the same for years. Advances in science and technology have ensured a constant quality of malt and enabled the shortening of production cycles. Malt production process begins with the cleaning of grains, steeping in water followed by germination and kilning (Figure 1).

## 2.1 | Effective cleaning and sorting of the grain

The adequate cleaning of grains is essential before entering the steep vessel so that the finished product contains the minimum contaminating matter which might affect the qualities and flavor of the beer. As the grains arrive for malting, they are rapidly precleaned to take away gross impurities. After the precleaning stage, the cleaning process extracts out the contaminants, which are of similar size as barley like undersized grains, weed seeds, and broken grains. Cleaners and separators are installed to remove the light material and destoners can be optionally installed to take out the stones from grains based on density differences. Finally, in the sieving equipment, barley for malting is graded by grain size into different categories, for example, grain sizes of more than 2.5 mm, 2.2 to 2.5 mm, and less than 2.2 mm. Broken grains and grain of less than 2.2 mm width are removed, may be used as animal feed. Barley grains that has been processed ready for malting are selected for malting and are preserved in circular steel bins with large capacity (Guido & Moreira, 2013). The recommended moisture content for storage of barley grains is 12% (Henry, 2016).

## 2.2 | Steeping

The malting process begins with the steeping process, in which dried grains are imbibed in water in order to elevate the moisture level to around 42-47% (Schwarz & Li, 2010). Steeping comprises a series of imbibition in water (2-4 days) each followed by an air-rest period, during which the water is drained and carbon dioxide ($CO_2$) is evacuated using fans. Sufficient aeration of the steep vessel with water under controlled temperature (12°C for partly dormant and 16-18°C for less dormant grains) with efficient

$CO_2$ removal is necessary during steeping process as lack of oxygen brings about microbial development, anaerobiosis, and souring while excessive aeration results in unwanted growth and starch loss (Briggs et al., 2004). De Carvalho et al. (2018) and Burger & LaBerge (1985) observed that ultrasound application decreased the time period needed to acquire the necessary moisture content for germination of barley grains, that is, 29 and 44% at 20 and 25°C, respectively.

The time period between immersions and air rest cycles varies with respect to grain variety, malt house, crop year and seasonal changes, but generally, it lies between 4 and 12 hr (Schwarz & Li, 2010). The first immersion washes the grain, extracting out any persisting dust or debris and also leaches out tannins which are husk components with an astringent taste. During the immersion period, embryo imbibes water rapidly via the micropyle and redistributes it throughout the nonrespiring endosperm while the air-rest period allows the water to redistribute slightly faster. Once the hydration level is attained, the embryo resumes metabolic activities and initiates the biosynthesis of gibberellic acid (GA), that acts as a catalyst for the production of hydrolytic enzymes (glucanases, proteases, and amylases) which depolymerize embryonic starch, initiating root and shoot development.

Exogenous $GA_3$ (0.1-0.5 ppm) can be added at the end of steeping, immediately before germination to accelerate the germination process or to enhance the impact of the naturally produced GA (Bamforth & Barclay, 1993). However, maltsters do not prefer malting additives because they leave unwanted residues in the malt extract. Recently, treatment with strong oxidants; Ozone ($O_3$) and hydrogen peroxide ($H_2O_2$) after 2 hr of first and second steeping were reported to activate hydrolytic enzymes to different levels by inducing the gene expression of abscisic acid (ABA) catabolism (*ABA8'OH*) and GA biosynthesis (*GA20ox1*) without contributing any unwanted residues in the malt (Ma et al., 2020). In a recent study conducted by Pater et al. (2020), steeping water treated with low pressure glow plasma and low temperature in the air or under nitrogen had been observed to improve the malting process, resulting in good quality malt. Once the steeping process is complete, the grain should have attained high moisture levels with a high (%) chit count, which is a white colored visible protrusion of the growing cotyledon/rootlet from the embryo of the grain.

## 2.3 | Germination

The process of germination is marked by embryo development, manifested by the growth of the rootlets and increment in shoot length, with the concomitant modification of endosperm. In this, steeped grains are spread on the floor and the grain bed is manually turned over to keep the grains loose, allow adequate airflow, avoid matting and prevent overheating from respiratory heat of grains. Currently, this process is conducted using a pneumatic system in the vessels of different sizes and shapes such as drums with circular germination vessels or rectangular Saladin boxes.

Germination occurs under aerobic and humid conditions at 16-20°C for 3-6 days depending on the process conditions and raw material (Poutanen, 2020). Grain moisture must be about 45-46% on day 2 and 42-43% on day 5 (Palmer, 2018). During the germination stage, GA diffuses to the aleurone layer and promotes the biosynthesis of hydrolytic enzymes, which are released into the inner endosperm for depolymerization of endosperm cell wall polysaccharides and proteins; the process is referred to as grain modification. This process transforms the hard endosperm of the grain into the soft (friable). Hydrolyzed products are then translocated to the embryo via scutellum to provide energy and building blocks for its respiration and growth. It was recently indicated that $GA_1$ is the most abundant bioactive form synthesized as a glycosyl conjugate in the scutellum which moves to the aleurone and activates de novo synthesis of $GA_3$ conjugate and $GA_4$ (Betts et al., 2020). Ideal modification is considered when the first shoot (acrospire) has developed to around three of fourth of the grain length (Schwarz & Li, 2010). The germinated grain is known as green malt after which it is moved toward the kilning process.

## 2.4 | Kilning

The green malt is shifted to the kilning chamber where hot air is blown to kill the embryo and terminate the germination (Oser, 2015). The kilning process aims to discontinue the internal modifications, reduce the moisture content of malt below 5%, and to ensure the product stability for storage, transport, and to prevent enzyme denaturation. Kilning also promotes the formation of melanoidins via the nonenzymatic Maillard reaction between amino acids and sugars (Howe, 2020). During curing phase of kilning, this browning reaction can be utilized to add color to the malt. Usually, kilning is performed in a double-deck kiln. It is conducted in a stepwise manner with a gradually rising temperature from 50 to 85°C for around 21 hr (Poutanen, 2020). Three steps involved in the kilning process are described as follows:

(a) *Free drying/withering phase*

During this stage, kiln temperature is relatively low (50-60°C). Moisture is freely present on the surface of the grain, on heating moisture level drops from 44 to 12%. This

phase takes about 12 hr to go in the single-deck kiln and 24 hr in double-deck kiln bed.

(b) *Intermediate/falling rate phase*

During this phase, the temperature is raised slowly over several hours in a stepwise manner.

The moisture tends to move from the interior of the grain to its surface. This movement restricts the rate of drying. Due to the slow rate of drying this phase is referred as the falling rate phase. The temperature in this phase ranges from 65 to 75°C with final moisture content of 4%.

(c) *Bound water/curing phase*

In this phase, the temperature is increased above 80°C for 1 or 2 hr after which malt is cooled to attain the temperature suitable for storage and transport. If the temperature is raised while grains are wet, it would cause denaturation of enzymes and malt quality would be affected. Curing phase enables the development of ideal aroma and color of malt (Mallett, 2014).

After kilning process, the dried rootlets or culms are removed owing to their extremely hygroscopic nature. Briggs et al. (1981) observed that about 3 to 5% of the original barley is retrieved as culms in the traditional malting processes. Before being placed in storage bin, malt is aged up to at least 3 weeks to allow uniform distribution of residual moisture throughout the grain. Careful regulation of time, moisture and temperature is necessary throughout the malting process to repress the formation of undesirable chemical compounds and denaturation of heat-sensitive enzymes. The Kilning step is the dominant energy-requiring process as it consumes 85-90% of the energy utilized in the malting process (Guido & Moreira, 2013).

## 3 | GRAIN: STRUCTURE AND MORPHOPHYSIOLOGICAL CHARACTERISTICS

### 3.1 | Grain structure

Physical structure of barley grain comprises three main parts, namely the starchy endosperm, the germ or embryo, and the outer protective layer. The endosperm is around 77 to 82%, the embryo is approximately 2 to 3% whereas protective layer has husk (10 to 12%), the pericarp and testa (2 to 3%) and the aleurone layer (4 to 5%) (Palmer, 2018). The husk is having palea on the ventral and the lemma on the dorsal side of grain (Fox, 2009). The husk protects the grain against abrasion during handling and its damage causes loss of filtration potential. In hulled varieties, the palea and lemma are attached tightly to the pericarp whereas in hull-less varieties, they are loosely attached and gets detached during threshing (Duffus & Cochrane, 1993). The seed coat and husk together protect the grains from desiccation and microbial contamination.

Directly below the testa lies the aleurone layer. Unlike maize and wheat, the aleurone layer in barley is made up of three cell layer layers (Shewry & Ullrich, 2014). GA formed in the germinated embryos can induce hydrolytic enzymes in aleurone cells which contain about 60% pentosans and 30% $\beta$-glucans that are degraded in the process of malting. During malting, this degradation process may help in releasing endosperm-degrading enzymes. The lytic enzymes produced by aleurone layer hydrolyze the endosperm reserves to smaller molecules for the transport through the scutellum to the embryo to provide energy and building blocks for its respiration and growth. The embryonic tissues consist of the axis and the scutellum. The scutellum serves as shield between endosperm and embryo and mediates the release of hormones to the aleurone and starchy endosperm (Walker-Smith & Payne, 1984).

### 3.2 | Morphophysiological characteristics of grain

Selection of a barley cultivar for malting depends on the physical structure and biochemical traits of grain that should meet industry standards. Detailed information of the key grain traits associated with malt quality is provided below:

#### 3.2.1 | Kernel shape/size

Barley grain with large size, thin cell walls, and loose packing of endosperm take up water rapidly and allows uniform distribution of water and hydrolytic enzymes (Deme et al., 2020). One of the important factors affecting the uniformity and shape of barley kernels is the arrangement of kernels on the barley spikes which can be of two types as illustrated in Figure 2. In two-rowed type barleys, only two of the six rows of grains develop whereas in six-rowed types, all six rows of grains develop. In six-rowed barley, there are more grains but the size is too small for malting (Palmer, 2018). Conversely in two-rowed barleys, grains are larger, heavier, have higher test weight and low protein content. They usually need longer steeping and germination period to become fully modified, but produces higher malt yield. Six-rowed barley modifies more rapidly and tends to have higher enzyme activity but have lower malt yield (Burger & LaBerge, 1985). Thus, two-rowed barley grains are preferred over six-rowed for malting. Six-rowed barley is

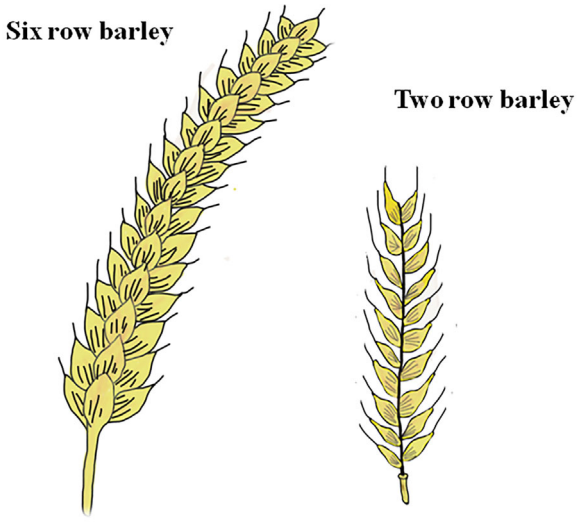


**FIGURE 2** Types of barley based upon arrangement of kernels on spikes

frequently brewed with adjunct grain (rice, corn, wheat) to account for the reduced sugar content. For assessment of the desired shape and size of barley grains for malting, following parameters are used:

(a) *Hectoliter weight (HLW)/test weight*

Hectoliter weight is a measure of density (weight per unit volume) and is commonly expressed in kilograms per hectoliter (i.e. kg/hl or kg/100 L) (Verma et al., 2008). Anything that affects the transport of nutrients to the kernel during grain filling or degrades the integrity of the kernel is believed to reduce HLW of grain. High hectoliter barley samples indicate sound grain which performs well in the malting process (Fox et al., 2007). According to European Brewery Convention (EBC), HLW of brewing barley must range from 65 to 75 Kg/hl (Galano et al., 2008). Since HLW shows little predictive values, so it is rarely considered as a reliable parameter for evaluating malt quality (Kunze, 2004).

(b) *Thousand kernel weight (TKW)*

Since moisture content affects the malt quality, thus, thousand kernel weight (TKW) is measured as the average kernel weight on dry weight basis and is usually expressed as grams per thousand kernels (g/1000 kernels). Kernels with high TKW are plumper, malt and mill more evenly and have more proportion of endosperm than small kernels, thus, TKW is a valuable parameter to maltsters and millers (Armstrong et al., 2002). TKW (g) should be greater than 45 g for two-rowed barley and more than 42 g for six-rowed barley (Anonymous, 2012). Recently, an automated system for evaluation of gain TKW has been developed by Wu et al. (2018), which combines an android device and a weighing module. The Android application provides more accurate information and is less tedious and time-consuming as compared to traditional methods.

(c) *Uniformity and plumpness*

A larger uniform grain size enables homogenous water uptake and grain modification. High plump kernels are desirable as they indicate high starch density and will lead to the consistent processing and high malt yield. Grain plumpness is determined by starch, nonstarch polysaccharides (NSP), lipids and proteins in the endosperm (Fox et al., 2003) and is highly influenced by the growing environment and genotype (Fox et al., 2006). Grain plumpness is the % weight of grains retained over a 2.5 mm sieve (% w/w) (Walker et al., 2009). Two-rowed barley should have at least 85% plump kernels while six-rowed barleys should have at least 70% plump kernels (Burger & Laberge, 1985).

### 3.2.2 | Impact of dormancy in malting

Many plants have evolved the ability to retard germination, even under ideal conditions, a phenomenon termed as seed dormancy. An acceptable level for barley grain germination during the malting process is >96% (Kumar et al., 2013). However, for malting purpose, the grain should undergo dormancy before harvest as this inhibits preharvest sprouting (PHS) during raining, a condition known as "precocious germination." Although PHS grain is alive, but it produces low malt quality with high wort $\beta$-glucan and low malt extract due to reduced germinative energy during storage. Some dormancy is preferable like in areas where PHS damage is high but extended dormancy is undesirable as it requires large storage times and poses problems in achieving nondormant barley early after the harvest period. For producing high-quality malt, maltsters need to evaluate grains for its GC and germination energy (GE) (Briggs, 1998). GC measures the percentage of grains that would normally germinate under optimal conditions while GE is the number expressing the percentage of fast-germinating seeds (Domin et al., 2020). The germination ability is affected by the quantity of water added, such that grains that do not germinate well in excess water are said to be water sensitive. The difference between the germinative capacity and germinative energy of a sample of grain is a measure of dormancy (Briggs, 1998). That is,

$$\text{Dormancy}(\%) = \text{GC}(\%) - \text{GE}(\%)$$

Various genetic (variety), environmental, such as temperature, water status, bacteria in soil and biochemical

factors, are associated with dormancy (Shu et al., 2015). One of the biochemical factors is the antagonism between ABA and gibberellins (GA), GA overcomes the dormancy by upregulating the expression of genes encoding proteases and α-amylases in aleurone layers while ABA induces the protein kinase (*PKABA1)* which downregulated α-amylase expression, deepens the seed dormancy, and inhibits premature seed germination (Gómez-Cadenas et al., 2001). Along with endogenous phytohormones, an enhanced level of total phenolic acids, free phenolic acids (sinapic and ferulic acid), and $H_2O_2$ have been also observed to affect seed dormancy. Many other agents sometimes reduce dormancy including brief steeps in hot/warm water, steeps in dilute potassium hydroxide, sodium hydroxide or lime water, acid ammonium fluoride, brief exposures to nitrates, formaldehyde, sodium metabisulphite, hydroxylamine, azides, carbon monoxide, cyanides, and so on (Briggs, 1998).

### 3.2.3 | Grain hardness and malt quality

The hard grain is the one that is resistant to any deformation by crushing, cutting, scratching, or penetration of foreign matter whereas, the soft grain is that which easily breaks down under pressure (Posta et al., 2007). Endosperm structure of good malting varieties can be easily modified and does not limit water uptake and passage of hydrolyzing enzymes. Therefore, varieties to be used for malting are characterized as soft whereas varieties to be used for feed are characterized as hard. Grain hardness is associated with compositional and structural characteristics of grain endosperm such as starch, β-glucan, proteins, and their interactions and packing of starch granules during grain filling. Grain hardness is also linked with the thickness of cell wall and the amount of protein-bound to the starch granules. Nair et al. (2011) documented that hard grains had a continuous protein matrix with higher starch-protein binding indicating that the binding ability of the protein-starch interface affects grain hardness.

The terms "mealiness" and "steeliness" are often used to describe soft and hard endosperm of barley grain, respectively. Mealy endosperm is characterized loosely packed starch granules in the protein matrix and is easily degraded by hydrolytic enzymes during malting while steely endosperm has larger numbers of starch granules with compact embedding in the protein matrix and is not degraded easily by hydrolytic enzymes (Palmer, 1989). The steelier barley samples had smaller grain size, darker wort color, lower friability, higher protein content, higher free amino nitrogen, and higher soluble nitrogen content than the mealy barley samples (Holopainen el al., 2005).

Various methods are used for measurement of grain hardness include Single Kernel Characterization System (SKCS), Particle Size Index (PSI), and Milling energy (ME). The SKCS measures grain hardness by determining the force required to crush a grain. SKCS value is 40 units for hard barley and <30 units for soft barley (Fox et al., 2003). PSI measures the percentage particle size of a ground sample passed through a sieve, the particle size of a ground grain rises with hardness. Fox et al. (2007) used 75 μm sieve and found that PSI units varied from 18.4 to 27.2 for hardest to softest barley among different breeding lines and commercial varieties. ME is the amount of energy required for crushing or grinding of the sample.

### 3.2.4 | Moisture content: A key determinant of storage life

Barley grain must be stored after harvesting to preserve its quality and germination capacity and simultaneously must be protected from high moisture. At physiological maturity, barley grain contains about 35% moisture, which is high for safe storage. Thus for reducing the moisture level, harvested grains can be transferred into drying bins, where a fan blows the natural air in at the bottom of the bin but this way is not satisfactory for reducing moisture content. The recommended temperature for short exposure time in barley is 45°C for malting/seed barley, grains must be cooled down to about 15°C (Arinze et al., 1994), Moisture level should be 14% moisture for short time storage (<6 months) and up to 12% for long time storage (>6 months) while storage can be at higher moisture content if it is stored at low temperature (Hellevang, 1994). However, moisture content of grain should not be below 10% to avoid the removal of metabolic water, which may cause embryo damage and reduce germination. To store the malt under appropriate conditions for several months, the moisture content of malt should remain below 4% (Kreisz, 2009; Kunze, 2014).

### 3.2.5 | Microbial contamination

Brewers are highly concerned about possible contamination by microbial attack as it severely affects the quality and yield of malt by interfering with respiration, causing uncontrolled breakdown of grain components and producing toxic secondary metabolites, mycotoxins. During the steeping high moisture (45%) and low temperature (10-14°C) enables the germination of fungal spores and mycelium growth (Mastanjevic et al., 2018). Some microorganisms proliferate by the end of the steeping phase (Douglas & Flannigan, 1988), but proliferation is at peak during

germination (Petters et al., 1988). However, during kilning, when temperature rises and moisture reduces (4%), conditions become unfavorable, the total number of different microorganisms present before this stage decreases as well as their respective microbial load. Selecting a reasonable genotype that is least susceptible to microbial attack and optimization of the malting conditions could be an effective measure in preventing infection and maintaining overall malt or beer quality. Different approaches have been proposed for inhibiting fungal growth and decreasing mycotoxin formation including preharvest methods, physical methods, chemical methods, enzymatic methods, biological methods, and microbial decontamination method (Misra et al., 2019). In spite of the progress attained by using these strategies, they have many demerits including low efficacy, requirement of expensive chemicals, and sophisticated equipments. Recently, Feizollahi et al. (2020) reported that atmospheric cold plasma treatment for 6 and 10 min reduced deoxynivalenol (DON) concentration by 48.9 and 54.4%, respectively, which is one of the major trichothecene mycotoxins generally produced by *Fusarium graminearum* and *Fusarium culmorum* in barley grains.

## 3.3 | Biochemical traits influencing malt quality

### 3.3.1 | Protein composition

Grains with high protein content are often undesirable because it limits the enzymatic breakdown of starch by obstructing access of hydrolytic enzymes and, thus, leading to poor malt extract (Brennan et al., 1996). Further, grains with high protein content require longer steeping time and, hence, modify harder. Higher protein content in malt contributes positively by enhancing foam stability and influences negatively by increased haze formation in beer (Fox et al., 2003). However, lower protein content impairs brewing through poor amino acid nutrition to yeast. Therefore, to meet maltsters' quality requirements, barley grain must have suitable protein content. For all-malt beers, protein values exceeding 12% is the indication of haze formation in beer or present mash runoff problems (Deme et al., 2020). Taking into consideration of the significance of protein in the brewing process, four protein quantification methods, that are, combustion methods—carbon, hydrogen, and nitrogen elemental analysis and Dumas and digestion ones— Kjeldahl and flux injection analysis—were compared and concluded that all quantification methods are valid for determining protein content in barley (Cenci et al., 2020). However, the authors suggested the usage of combustion methods due to their low health and environment hazards and the rapid analysis which takes up to 6 min.

Based on solubility, barley proteins can be divided into four types; globulin (salt-soluble), albumin (water-soluble), hordein or prolamin (alcohol-soluble), and glutelins (not extracted by either solvent). Based on biological function, barley seed proteins fall into two fractions; seed storage proteins (SSPs) and nonstorage proteins. The major storage proteins are prolamins termed as hordeins, accounting for nearly 30-50% of the total nitrogen content in barley grains (Shewry & Tatham, 1990). Depending on their amino acid composition and molecular mass, five types of hordeins have been reported in barley seed; low molecular weight (A), sulfur rich (B + $\gamma$), sulfur poor (C), and high molecular weight prolamins (D). Low molecular weight (A) hordeins do not seem to be true storage proteins. Together B and C hordeins constitute over 95% of the total hordein content (Balakireva & Zamyatnin, 2016). Subaleurone cells of barley are enriched in B and C hordeins, while D hordein is found only in the inner part of the starchy endosperm. Hordein polypeptides are negatively correlated with malt quality. Among all, D hordein was documented to have the strongest negative correlation with the malt quality (Howard et al., 1996). Šimić et al. (2007) observed that B and D hordein are significantly associated with lower malt yield. Hordeins had also been found to be involved in postpackaging haze formation in beer due to protein-phenol interactions especially in proline-rich protein. Various substances are commonly used in brewing to remove these haze active proteins like silica, tannins, and prolyl endoprotease. Hordeins, are toxic to gluten-sensitive beer consumers (i.e. people suffering from coeliac disease). Gluten-free beers must contain hordein content below 10 mg/kg (Taylor et al., 2006). It was also reported that beer produced from malt germinated for 7 days had 44% reduced hordeins than that germinated for 3 days. Therefore, a significant impact of malting conditions was found on beer hordeins (Taylor et al., 2018).

Albumins constitute another important type of barley grain protein found in beer account for 11% of the total barley protein content and include $\alpha$-amylase enzyme, lipid transfer protein 1 (LTP1) and protein Z (a serpin proteinase inhibitor) (Steiner et al., 2011). Both Z4 and LTP1 are very resistant to high temperatures and proteolytic modification, which contribute to their survival and resilience through the brewing process (Hejgaard, 1977; Jegou et al., 2001). Protein Z is bound with $\beta$-amylase, which can be cleaved by endopeptidase resulting in $\beta$-amylase activation. The Z protein family includes the ZX, Z4, and Z7 proteins, among which Z4 occurs in large amounts (80%) (Evans & Hejgaard, 1999). Using ELISA, it was identified that protein Z is the most prevalent protein in beer foam

and the addition of its purified form into the finished beer could enhance the stability of beer foam (Niu et al., 2018).

LPT1 is a structural protein involved in binding of starch and lipid inside the endosperm. It also contributes to the stability and formation of beer foam. The native form of LTP1 has poor foaming properties but its altered form accounts up to 40% in beer foam formation by interacting with protein Z and hordeins (Vaag et al., 1999).

### 3.3.2 | Structure and composition of carbohydrates

Carbohydrates comprise up about 80% of barley grain and includes starch and NSP. Starch is the most abundant polysaccharide in barley kernel and is exposed to two physical processes (malting and mashing). At germination stage, various starch hydrolyzing enzymes are produced and released from bound state and subsequently activated. During mashing (approximately 65°C), the crushed malt is mixed with hot water, the shorter amylopectin chains and the long amylose chains are degraded quickly to glucose, maltose and maltotriose (Quek et al., 2019), which will further be fermented to produce ethanol by yeast.

In plants, the proportion of amylose and amylopectin varies, based on amylose content, the starch in barley grain can be classified into waxy (95-100% amylopectin and non-detectable to <5% amylose), normal (~25-27% amylose), and high amylose type (>35%) (Shu & Rasmussenm, 2014). Generally, waxy barleys have high $\beta$-glucan level, low cell wall modification levels, and low hot water extract (HWE) values. Barley amylose and amylopectin exist in two forms; large and small granules. The large granules are lenticular (lens-like) in shape and have a diameter of 15-25 μm. The small granules are typically under 10 micrometers and contain slightly more proportion of amylopectin (75-77%) by weight as compared to large granules (which contain 71-76% amylopectin). On percentage basis, the small starch granules are much more abundant than the large starch granules. Due to higher surface area to volume ratio, small granules are rapidly hydrolyzed during malting than large granules, but barley malt still contains 17-27% of small starch granules (De Schepper et al., 2020). Barley varieties that have starch granules that are loosely packed into the protein matrix of the endosperm have more open spaces in which water can diffuse more freely (Bamforth & Barclay, 1993). This increases the rate of hydration in such varieties (i.e. less compacted endosperm) and reduces the steeping time, which is considered a desirable characteristic in malting (Palmer & Harvey, 1977).

Since starch molecular structure is a determinant of starch hydrolysis, various studies have been conducted to predict the changes in barley starch structure during malting. For example, Kano et al. (1981) documented that during malting amylopectin was preferentially attacked, resulting in increased amylose content after malting. Wenwen et al. (2019) concluded that barley cultivars with lower protein and amylose level, smaller amylose molecular sizes, and lower amount of amylopectin with long branches produces more fermentable sugars after mashing.

NSPs are essential structural elements of different components of barley grain. The cell wall of barley starchy endosperm constitutes 75% $\beta$-glucan, 20% Arabinoxylans (AX), 2% glucomannan, and 2% cellulose, while aleurone cell wall constitutes about 71% AX, 26% $\beta$-glucans, and 3% cellulose and mannose polymers (Fincher & Stone, 1986). Based on genetic and environmental factors total NSP content in barley varies from 23 to 41% (Holtekjølen et al., 2006). As compared to hull-less barley varieties, hulled varieties contain more total NSP, probably due to the presence of AX and cellulose in the hull. $\beta$-glucan and AX can cause filtration issues in beer such as high wort viscosity, decreased filtration rate, low extract yield, and haze formation. Thus, $\beta$-glucan and AX should be hydrolyzed during malting for efficient brewing. Walker et al. (2001) indicated that by the second day of germination, the $\beta$-glucan level indicates if the variety has desirable extract potential. Three main methods employed to estimate $\beta$-glucan content in barley are as enzymatic, calcofluor, and colorimetric methods (Nishantha et al., 2018). Enzymatic method is the most commonly used method (McCleary & Codd, 1991) in which $\beta$-glucan polymers are hydrolyzed into monosaccharide units by consecutive action of enzymes using endo-(1→3,1→4)-$\beta$-D-glucan-4-glucanohydrolase (lichenase), followed by $\beta$-glucosidase, and finally linked to develop color with glucose oxidase utilizing a chromogenic substrate (Schmitt & Wise, 2009).

### 3.3.3 | Hydrolytic enzymes: A key to successful brewing

One of the essential applications of enzymes in the food industry is brewing. The aim of germination during malting is the hydrolytic enzyme production in the grain, which may be classified into three major groups: cell wall hydrolases (arabinoxylanase and $\beta$-glucanase); starch hydrolases {$\alpha$-amylase, $\beta$-amylase, limit dextrinase [LD] [debranching enzymes], and $\alpha$-glucosidase} and proteolytic enzymes (Figure 3). The amount of endogenously synthesized enzymes depends upon the levels of GA and the responsiveness of the aleurone layer to the hormone. Different types of hydrolytic enzymes and their activities are discussed below:

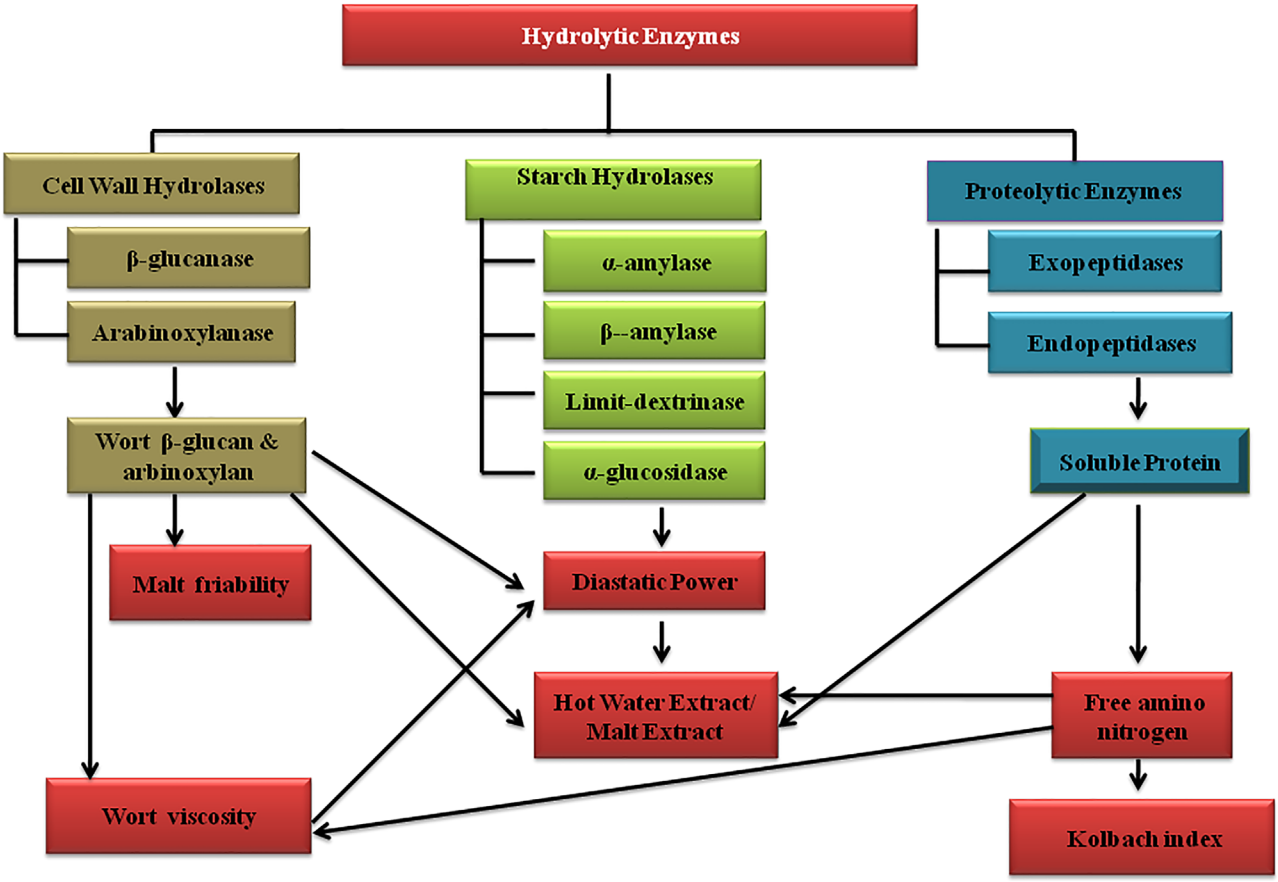


**FIGURE 3** Hydrolytic enzymes controlling malt quality

### 3.3.4 | Cell wall hydrolases

Better malting performance is associated with lower level of β-glucan and higher level of β-glucanase in grain (Narzis, 2012). The high concentration of β-glucan leads to incomplete hydrolysis of cell walls, which in turn impede the diffusion of enzymes produced during the mobilization of kernel reserves and disrupts various quality parameters of finished malt (Habschied et al., 2020). Therefore, degradation of β-glucan is essential. It can be solubilized directly by endo-β-(1-3,1-4)-glucanases or it can be first solubilised by several enzymes that remove the outer layers of cell wall. The term "solubilase" is used to represent the enzymes involved in this process. These enzymes comprised of β-(1-3)-glucanase, carboxypeptidase, phospholipases, endo-β-(1-4)-glucanase, xyloacetyl esterase, feruloyl esterase, and arabinofuranosidase (Georg-Kraemer et al., 2004; Jin et al., 2004; Kuntz & Bamforth, 2007). After solubilisation, the next step is the attack by endo-β-(1-3,1-4)-glucanase (EC 3.2.1.73).

Endo-β-(1-3,1-4)-glucanases are the main hydrolases responsible for digesting high-viscosity β-glucans. The activity of these enzymes is turned on after2 days of malting and rises quickly up to the fourth day (Jamar et al., 2011). They cleave specifically the (1-4)-linkages on the reducing end of β-(1-3)-glucosyl residues and release oligosaccharides that contain a variable number of β-(1,4)-glucosyl residues at their nonreducing end and a single β-(1,3)-glucosyl residue at their reducing end. Barley endo-β-(1-3,1-4)-glucanases have two isozymes, EI (expressed in the scutellum layer and increases in young tissue during seedling development) and EII (exclusively synthesised in the aleurone layer during germination) (Slakeski et al., 1990; Wolf, 1992). These enzymes are thermolabile as they lose 40% activity during kilning while during mashing (i.e. 65°C) endo-β-glucanase is inactivated rapidly. Therefore, if β-glucans are not hydrolyzed during malting, they will not be hydrolyzed during mashing resulting in poor beer quality. The β-(1-3, 1–4)-D-oligoglucosides released by β-(1-3, 1–4)-glucanases can be further hydrolyzed by β-glucan exohydrolase. Two isozymes of β-glucan exohydrolase, Exo I and Exo II are present and their activity increases three to four times after germination (Fincher & Stone, 1993). The genes encoding the exohydrolases are transcribed in the scutellum of germinated grain, but mRNA is also abundant in elongating coleoptile (Jamar et al., 2011).

Exo-β-glucanase is more sensitive to kilning than endo-β-glucanase (Fincher & Stone, 1993). Since it removes only one glucose from nonreducing end every time it attacks, it has less impact on reducing β-glucan content. Therefore, rather than exo, endo form of β-glucanase plays a major role in β-glucan degradation (Jin et al., 2004). Usually, β-glucanases are endogenously found in barley grain itself, however, commercial β-glucanase from microorganisms is utilized additionally for the standard production of light beers and shorter maturation time.

In concert with β-glucanases, the arabinoxylanases are also thought to digest the endosperm cell walls. Arabinoxylanases are often produced later in germination. First, acetyl (Humberstone & Briggs, 2000a), ferulic acid esterase (Humberstone & Briggs, 2000b), and β-glucan solubilases release AX from the endosperm cell walls (Egi et al., 2004), then α-arabinofuranosidase, endo-β−1,4-xylanase, exo-β−1,4-xylanase, and β-xylopyranosidase hydrolyze AX from the cell walls (Hrmova et al., 1997). α-Arabinofuranosidase catalyzes the separation of α-L-arabinofuranose residues linked α-(1,2) or α-(1,3) to the xylan backbone. As these residues are removed, exo-β−1,4-xylanase and endo-β−1,4-xylanase degrade arabinoxylans into smaller xylose oligosaccharides and xylobioses. β-xylopyranosidases, the hydrolyses of β-(1-4) xylosidic linkage within xylooligosaccharides. It has been suggested that hydrolysis of β-glucan is hindered by AX and, to an extent, by the attached acetic acid and ferulic acid moieties (Bamforth & Kanauchi, 2001), thus, co-ordinated synthesis of cell-wall hydrolyzing enzymes during germination of grain is important for mobilization of endosperm reserves and may influence the quality of malt for beer production.

### 3.3.5 | Starch hydrolases

*Amylases*

During malting, α- and β-amylases are the principal enzymes responsible for hydrolyzing starch into fermentable sugars. These enzymes are released from the starch granules after the outer layers have been already hydrolyzed by cell wall hydrolases. The two amylases have different optimum temperatures, α-amylase has higher optimum temperature at around 73.89°C and lower pH of 5.2 and while, β-amylase pH of 5.5 and optimum temperature at 62.78°C (Sammartino, 2015).

α-Amylases (E.C 3.2.1.1) are the most abundant proteins synthesized in response to GA during germination. This enzyme is not detectable in barley grain; however, it increases once germination commences (Bathgate & Palmer, 1973). It hydrolyzes long-chain saccharides and belongs to glycoside hydrolases family 13. α-Amylase possesses an endoattack mechanism by randomly hydrolyzing α−1-4 glucosidic bonds. This random attack results in a production of fermentable sugars which acts as substrates for β-amylase, α-glucosidase, and LD. α-Amylase causes a fast reduction of the viscosity of the medium due to which it is also called as liquefying amylase (Guerra et al., 2009). The α-amylase family constitutes two groups based on isoelectric points (pIs): AMY1 (the low-pI group) and AMY2 (the high-pI group), the latter being more abundant, that is, 80-90% (Cu et al., 2013). Among various hydrolytic enzymes involved in malting, α-amylase is the most heat stable enzyme, with only 4-18% of its activity lost in kilning process (Sjöholm et al., 1995). Exogenous amylases can be added to reinforce the action of the endogenous enzymes by complete hydrolysis of starch before the enzyme deactivation takes place by exposure to high temperature during the mashing stage. This approach has many advantages such as reducing the need of malt for mashing, raising the wort fermentability of poorly modified malts, and allowing the use of other noneasily maltable starchy sources (Guerra et al., 2009). One of the examples of exogenous source is α-amylase extracted from thermostable bacteria like *Bacillus licheniformis* and engineered strains of *Escherichia coli* or *Bacillus subtilis* (George & Georrge, 2020). Sheng et al. (2018) treated barley aleurone with exogenous gamma-aminobutyric acid (GABA) and reported that it induces the expression of α-amylase gene and activates many signaling cascades regulating starch degradation.

β-Amylase (E.C 3.2.1.2) are exo-acting amylases belonging to glycoside hydrolases family 14. This enzyme can hydrolytically cleave amylose with an even number of glucose moieties to maltose but not glucose moieties with an odd number, due to its inability to use maltotriose as substrate. β-Amylase hydrolyzes maltose from the nonreducing ends of amylopectin until it reaches near 1,6-D-glucosidic bond, leaving β-limit dextrin. Synthesis of β-amylase occurs during the development of grain and accumulates in quiescent grain in two major forms: a soluble form and an insoluble protein complex associated with the periphery of starch granules (inactive) (Hara-Nishimura et al., 1986). The insoluble form is predominant in mature barley kernels and on liberation from the bound state, it becomes completely active (Grime & Briggs, 1996). The release of β-amylase is accomplished in vitro using reducing agents like dithiothreitol or β-mercaptoethanol and in planta by cysteine endoproteases such as malt endopeptidase.

Barley grain contains two active forms of β-amylase; *Bmy1* (specific to endosperm) and *Bmy2* (the ubiquitous β-amylase) (Vinje et al., 2011). Among both *Bmy2* is more thermotolerant (Clark et al., 2005) but, this form is not prominent in the mature grain (Vinje et al., 2011). Expression of *Bmy 2* is high early during seed development

whereas, *Bmy1* is expressed in bound form with other barley SSP later in seed development and is stored in the mature grain. Georg-Kraemer et al. (2004) studied the developmental expression of amylases during malting and observed that β-amylase activity was high on the second day while α-amylase activity began to rise only from the third day of germination. β-Amylase is the principal contributor of DP among all hydrolytic enzymes (Arends et al., 1995), because its turnover number is considerably more than the other starch hydrolyzing enzymes, thus, barley lines with a high content of β-amylase are accepted for micromalting.

## 3.4 | Limit dextrinase

LD, also referred to as isoamylase, amylopectin 6-glucanohydrolase, R-enzyme or pullulanase, belongs to the glycoside hydrolase family 13. It is an endogenous hydrolytic enzyme responsible for cleaving the α-(1→6)-glucosidic branch points in branched dextrins formed by the action of amylases on starch. The enzyme requires at least one α -1,4 linkage at each side of the susceptible bond, it does not break α-1,6 bonds linking a single glucosyl residue to a maltosaccharide. Its activity in malt has been observed to range from 50 to 160 U/Kg (Duke & Henson, 2009). However, LD activity is not usually measured during malt analysis. There are three forms of LD in barley: active free form, insoluble bound, and soluble inactive (latent). The "Free" form is extractable in buffer itself and the "bound" form is brought into solution as an active enzyme using proteases or reducing agents (MacGregor, 2004). Since "latent" form is bound with an inhibitor, thus, the enzyme-inhibitor complex is cleaved by proteases, reducing agents or high temperature. Degradation of amylopectin occurs only by free form.

Biosynthesis of LD begins around 6 days after anthesis and is stored in the mature grain as a bound protein but is present in a low amount. During the initial 5 days of germination, there is a quick increment in the synthesis of bound LD. As germination continues, conversion of the bound enzyme to an active free form occurs by cysteine endoproteases (Longstaff & Bryce, 1993). Cho et al. (1999) have shown that the level of LD expressed in barley is enhanced if thioredoxin is overexpressed in the barley, which suggests the occurrence of a specific reducing event occurring during germination that activates LD.

Thermostability of LD in free form is poorer than other forms. Stenholm and Home (1999) reported that when purified LD is heated, it loses all its activity in less than 10 min at 65°C. However, if crude extracts of malt are heated, some 60% of the activity is still present after an hour at conversion temperatures. Recently, Du et al. (2020) applied molecular dynamics (MD) simulation tool to study key factors involved in thermostability of LD and identified several salt bridges that are responsible for thermostability and various thermally sensitive regions of LD in barley.

## 3.5 | α-Glucosidase

α-Glucosidase (EC 3.2.1.20) is one of the least active and most thermolabile amylolytic enzymes of barley malt resulting in reduced production of fermentable sugar during mashing. Like α-amylases, synthesis of α-glucosidase occurs during the germination stage. Although α-glucosidase activity is considered to be unimportant during mashing due to its destruction during kilning (Muslin et al., 2000). However, it may be important for the normal postgerminative growth of the grain. Exo-acting enzymes are the major forms of α-glucosidase found in germinating grains which belongs to glycoside hydrolase family 31 (Frandsen & Svensson, 1998; Naested et al., 2006). Two isoenzymes of α-glucosidase have been purified from malt named as G1 (high pI) and G2 (low pI) (Sissons & MacGregor, 1994). α-Glucosidase acts on maltose and other short maltooligosaccharides produced by LD and amylases, converting them to glucose. Sun and Henson (1990) found the synergistic effect of α-glucosidase and α-amylase in starch solubilization, results in higher extract levels. The optimum pH for α-glucosidase is 5 for starch while it is 4.5-4.6 for maltose as substrate (Agu & Palmer, 1997).

Muslin et al. (2000) reported that recombinant barley α-glucosidase expressed in *Pichia pastoris* was thermolabile, with half of its activity being lost at 58°C and pH 5. Malt containing more thermostable α-glucosidase showed significantly higher fermentable value, this motivated various researchers to enhance thermostability of α-glucosidase. Various studies have been conducted to increase the thermostability of α-glucosidase. Chen et al. (1994) enhanced the thermostability of α-glucosidase by substituting alanine for asparagine. In another study, thermostability of α-glucosidase from four plant species (barley, spinach, sugarbeet, and *Arabidopsis*) was investigated by comparing their amino acid sequence and reported the occurrence of proline residue in three most thermostable α-glucosidase that was not present in α-glucosidase of barley (Muslin et al., 2002). Clark et al. (2004) investigated that α-glucosidase from sugar beet has higher thermostablity than barley and observed considerable variation in the position and number of *N*-glycosylation recognition sites after the alignment of the deduced amino acid sequences of both the crops.

### 3.6 | Proteolytic enzymes

A large complex of proteolytic enzymes carries out the breakdown of storage proteins during the grain germination and subsequently malting. Ungerminated barley contains little proteolytic activity, but it rises rapidly during the early stages of the germination step (Harris, 1962; Zhang & Jhones, 1995a). During mashing, proteolytic enzymes hydrolyze cell wall protein resulting in the exposure of the starch to the hydrolytic enzymes. Unlike other hydrolytic enzymes, proteases are not specific to a particular substrate (protein) but they are specific to certain structural features of the peptide chain. They are categorized into two groups depending upon cleavage site in the proteins: exopeptidases and endopeptidases. During the germination process, proteins are firstly digested by endoproteases and subsequently by exopeptidases (Bensova et al., 2018).

Exopeptidases mainly have oligopeptides and polypeptides as their substrate and break the terminal amino acid (Swanston et al., 2016). Based on specificity, exopeptidase can be classified into carboxypeptidases (have optima are in the range 4.8-5.7 and attack the *C*-terminus of the peptides) (Mikola & Kolehmainen, 1972) and aminopeptidases (have higher pH optima than the carboxypeptidases and attack from the *N*-terminus of the peptides). Unlike exopeptidases, endopeptidases do not attack at the ends of the chain attacks but at certain sites in between the chain (Rao et al., 1998). Therefore, they mainly use proteins and higher polypeptides as their substrates. Endopeptidases are classified into four groups; serine proteinases, cysteine proteinase, aspartic proteinases, and metalloproteinases (EC 3.4.24). Among malt endoproteases, cysteine endoproteases are the most effective (Koehler & Ho, 1990; Zhang & Jones, 1995b).

Among proteolytic enzymes, most thermostable is carboxypeptidases. The optimal temperature of their activity is 40-50°C, gets inactivated above 70°C. Their optimal pH is close to that of mashing, therefore, 80% of the released amino acids are subsequently cleaved during mashing by these enzymes (Benesova et al., 2017). At germination, about 35-40% of the proteins are broken down into low molecular compounds, such as oligopeptides and amino acids, which are essential for yeast nutrition. Insufficient breakdown of storage proteins causes problems with beer filterability, while excessive protein degradation is the cause of the development of undesirable flavors and beer color (Benešová et al., 2018).

## 4 | ROLE OF ADJUNCTS IN BREWING

Brewing adjuncts are the materials other than malted barley that bring additional sources of carbohydrate and protein into wort. Adjuncts are used by brewers worldwide to produce less expensive malt and modify beer quality (foam stability, color, flavor) (Kok et al., 2019). Adjuncts can be solids (mash vessel adjuncts) which comprised of unmalted cereals such as barley, rice, corn, rye, wheat, sorghum, oats, triticale, and unmalted pseudocereals (amaranth, buckwheat, quinoa), teff, cassava and granulated sugar (sucrose) (Annemüller & Manger, 2013; Goode & Arendt, 2006). Liquid adjuncts are also used by dissolving in wort during the hop-boil and include sucrose-based syrups, derived from sugar beet or sugar cane and hydrolyzed starch syrups and syrups obtained from hydrolyzed cereals.

It has been reported that the use of 30% of corn adjunct may result in 8% reduction in total production cost (Baca, 2001). Goode and Arendt (2003) used unmalted sorghum as an adjunct in 50% barley malt and observed that a good quality beer was obtained as compared to beer obtained from 100% barley malt. Agu (2002) reported that brewing with red rice has a unique aroma and flavor. Mehra et al. (2020) found that use of hydrolyzed red rice as an adjunct with barley malt provides enough nutrients for yeast to convert sugar into alcohol. Zhang et al. (2017) reported that use of extruded rice as an adjunct produced a higher level of flavoring compounds than traditional beer. Currently, rice is the most common adjunct of beer, but as the demand for rice has risen severely, the quest for more adjuncts to replace rice is ongoing (He et al., 2018). The amount of adjuncts used for brewing depends on various factors including mashing technology, fermentation time, required beer quality, and exogenous enzymes. Although adjuncts have various advantages but the amount of nitrogen present in most types of adjuncts is less as compared to malt, and hence, excess use of adjuncts can reduce free amino nitrogen (FAN) content in wort, which can result in longer fermentation time. Therefore, the levels of moisture, FAN, and total nitrogen should be maintained within desired amounts when adjuncts are used by brewers.

## 5 | MALT EVALUATION

The brewing industry has enforced necessities and assumes malthouses to distribute materials with high stability of basic seed components and performance characteristics. Final quality of malt and beer can be evaluated by following test or parameters:

### 5.1 | Diastatic power (DP)

DP is the most critical parameter of malt evaluation. It represents the aggregate activity of four starch-hydrolytic

enzymes; β-amylase, α-amylase, limit dextrinase, and α-glucosidase that are activated during malting and mashing (Arends et al., 1995; Qi et al., 2006). In general, high DP indicates better malt quality and a higher malt extract. DP is significantly positively correlated with activity of β-amylase. Variation in DP of malt is affected by the complex interaction of genetic variation and environmental factors (Arends et al., 1995). In EBC, DP is analyzed in Windisch Kolbach units (°WK) and in Institute of Brewing (IOB) in degrees Lintner (°L). Two are related by the equation, Lintner = (°WK+16)/3.5 (Howe, 2020).

## 5.2 | Hot water extract (HWE)

HWE commonly known as malt extract is another crucial trait while choosing promising malt variety. HWE generally determined by specific gravity, measures the total soluble materials obtained from the malt that is ground in a specified manner at a fixed temperature for a specific time. This process broadly mimics the mashing process. Around 90 to 92% of the total wort soluble materials constitute carbohydrates. The remaining constitute peptides of various size, nucleic acid hydrolytic products, amino acids, low amount of phenolic compounds, various lipids, vitamins and minerals (Burger & Laberge, 1985). Various standard procedures are used by internationally for measuring HWE which includes; IOB-Methods of Analysis (1997); Analytica–EBC (1998); and ASBC-Methods of Analysis (1992). IOB method uses a constant temperature infusion mashing profile, while ASBC and EBC procedures use a multitemperature programmed mashing profile (Harasymow et al., 2003). According to Fox et al. (2003), the quality HWE is influenced by various factors including, environmental factors, biochemical and genetic factors, malting process and mashing conditions. HWE values is 79 to 81% in western two-rowed types and 77 to 79% in six-rowed type barley (Burger & Laberge, 1985).

## 5.3 | Kolbach index (KI)

KI measures the ratio of soluble nitrogen in the wort to the total nitrogen present in the malt (Verma et al., 2008). It is an important indicator of proteolysis occurred during malting and mashing, as greater the hydrolysis of proteins during malting, the more nitrogenous compounds will be soluble. When protein hydrolysis is low, KI is also decreased, resulting in wort filtration problem, lower malt extract, and protein turbidity. When protein hydrolysis is high, KI also increases and the normal proportion of protein components is compromised, resulting in accelerated yeast aging and thin beer taste (Fang et al., 2019). KI may be determined by the combustion analysis, Kjeldahl method, or UV spectroscopy (Haslemore & Gill, 1995). KI values of elite malt used in the brewing industry should be within 35 and 41%.

## 5.4 | Free amino nitrogen (FAN)

FAN is the determination of the total nitrogen compounds that are metabolized or assimilated during fermentation by yeast which includes α-amino acids (with the exception of proline, which is not an α-amino acid), ammonia, and small peptides (Hill & Stewart, 2019). During the fermentation process, amount of FAN needed for the optimum yeast growth is around 130 mg FAN/L (Stewart et al., 2017). Traditionally, Dumas and Kjeldahl methods were used to measure wort nitrogen, these methods do not give true protein measurement. An alternative to these methods is Kaiser or Ninhydrin test is utilized for the estimation of free amino acids (ASBC, 2009). For the quantification of specific amino acids, separation may be performed using liquid chromatography and revealed using the ninhydrin test.

## 5.5 | Malt friability

Friability is used to determine the modification of cell wall of malt grains (measures how hard a malt kernel is). Friability determination relies on the principle that malt that is poorly modified is steely and difficult to grind (Schwartz & Li, 2010). The friabilimeter crushes malt between a metal sieve and rubber roller and measures the percentage (by weight) of crushed malt that can pass through the sieve (Bamforth & Barclay, 1993). Friability value for malt is considered >90%. This method is beneficial for identification of malt that may yield problems in wort clarification, lautering, or beer filtration.

## 5.6 | Malt homogeneity

Along with malt friability, another trait determined by the friabilimeter is malt homogeneity. It is measured by collecting the sample retained in malt friabilimeter mash during friability analysis and passing it over 2.2 mm sieve. The nonhomogenous fraction is that which is retained on the 2.2 mm sieve and remaining is homogenous malt (Verma et al., 2008). Malt homogeneity is measured by deducting the amount of material retained by the sieve from the original sample weight and then changing to percentage (Swanston et al., 2016).

### 5.7 | Wort viscosity

Wort viscosity determines the gumminess of wort when compared to water. Higher viscosity values show inadequate hydrolysis of cell wall that results in poor wort and beer filtration. $\beta$-glucan and AX are primarily responsible for highly viscous solutions. Viscosity is expressed in cP (centipoise unit; 1 cP = 1 mPa•s) (Deme et al., 2020). The optimum viscosity for 8% wort should be around 1.6 cP (Briggs et al., 2004).

### 5.8 | Saccharification time

Mashing process consists of gelatinization, liquefication, and saccharification (Kunze, 2004). Time taken by the starch to transform into sugars during mashing is called saccharification time. Iodine test is used to verify the complete transformation of starch in wort. A good malt saccharifies in less than 10 min (when enzymes initiate hydrolysis of starch), a longer duration is caused by a bad disintegration of the starch (Kumar et al., 2013).

## 6 | CONCLUSIONS

Poor malt extract results in long processing times in the brewhouse, unreliable fermentations, haze formation, problems in filtration, and off-flavors. To enhance brewing efficiency and yield, malts with high enzyme activities and high extract values are essential. The quality of malt depends upon multiple grain parameters, including kernel shape, size, uniformity, hectoliter weight, grain hardness, grain protein, carbohydrate, moisture content, etc., which affects the malt parameters, that is, DP, malt yield, KI, free amino nitrogen, friability, homogeneity, wort viscosity, saccharification rate. Therefore, while buying malt, the brewers prefer a high-quality product, which will provide the economic wort and will operate adequately throughout the beer production process. This review provides the knowledge of various quality and quantity attributes for malt production as well as understanding of several biochemical and physical changes occurring during this process which will benefit maltsters and brewers to attain the high beer quality.

## AUTHOR CONTRIBUTIONS

Writing-original draft, writing-review & editing: Heena Rani. Conceptualization, supervision, writing-review & editing: Rachana Bhardwaj.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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