Development of enzymatic activity in wheat grain during germination process

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DEVELOPMENT OF ENZYMATIC ACTIVITY IN WHEAT GRAIN DURING GERMINATION PROCESS

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ABSTRACT

The aim was to determine the optimal germination time in order to achieve the best results for investigated indicators in relation to the recommended values. Wheat samples underwent germination process in controlled conditions, regarding temperature and air humidity, over a period of 7 days. Obtained results for total distatic power were in range from 240 °WK in starting sample to 420 °WK at the end of the germination process. α-amylase activity was in range from 0 DU/dm in starting sample to 60 DU/dm at the end of germination process. It was determined that germination time ranging from 158 to 168 h is optimal to obtain recommended values for wheat malt (diastatic power 250–420 °WK, α-amylase activity 40-60 DU/dm). Satisfying results were achieved on the half of the 4th day of germination. In conclusion, obtained results indicate that optimal germination time for synthesis of enzymes that affect the total diastatic power of malt (except β-amylase which is activated only during the germination process) and α-amylase synthesis, is between 156-168 h. In case of compromising these indicator values with the rest of important malt quality indicators, the optimal germination time ranges from 120-140 h.

Keywords: wheat, germination, α-amylase activity, diastatic power, malting optimization

INTRODUCTION

The development of enzymatic activity during the germination of wheat grain was determined. Malting consists of three major phases: steeping, germination and kilning. Germination is a crucial process where most of the targeted changes, such as malt modification and the synthesis of enzymes, take place. When the germination of kernels has reached a desired stage, germination is terminated by the controlled drying of the seeds, or in other words, kilning the green malt by blowing hot air through the grain bed. Malt wort fermentability is dependent on an adequate supply of the essential nutrients required by yeast. Levels of the nutrients will ultimately depend on other factors, particularly levels of enzymes such as starch-degrading enzymes, proteases, and β-glucanases. Starch-degrading enzymes play a key role in sacharrification of mash and

supply yeast with fermentable sugars. It is generally considered that the rapid and efficient conversion of starch to fermentable carbohydrates during brewing is dependent on a number of parameters including the level of the already mentioned starch degrading enzymes in the malt, the gelatinisation temperature of the starch and the actual temperature program of the mash. Starch hydrolysis is carried out by a number of malt enzymes working together (including α-amylase, β-amylase, limit dextrinase and α-glucosidase). Although α-amylase is able to hydrolyse intact starch granules, the rate of hydrolysis is very slow compared to that of solubilised starch [1, 2]. Effective hydrolysis by α-amylase, therefore, occurs only after the starch has been solubilised (or gelatinised). Effects of starch-degrading enzymes on fermentability became more significant in better modified malts with α-amylase showing stronger effects than total diastatic power [3]. The poorer fermentability of malt was predominately due to low levels of α-amylase, although, free amino nitrogen (FAN) also appeared to be an important factor. The reason why it is important to provide sufficient enzymatic activity in malt (neither too high nor too low, rather optimal) is because the relationships between fermentability and levels of starch-degrading enzymes. They have also been controversial but several fermentability studies have shown a need for adequate modification [4, 5]. Recently there has been some consensus on the importance of these enzymes but only when allowances were made for individual enzymes, such as α-amylase, β-amylase and limit dextrinase versus diastatic power [6], and when allowances were made for differences in β-amylase thermal stability [5-7]. Research has shown that positive effects of increased enzyme levels can be negated by other factors such as starch gelatinisation temperature, [8] (starch gelatinized at lower temperatures is more completely hydrolysed to fermentable sugars as starch degrading enzymes are still active), although, others have found this effect insignificant [5]. Gjertsen and Hartlev [9] also found enzyme levels had little effect on fermentability when enzymes were present at high levels, in which case modification was more important. Bathgate et al., [10] though, found fermentability could be limited by over modification because of increased levels of soluble protein and reduced levels of fermentable sugars due to elevated malting losses. MacGregor [11] indicated starch granules readily gelatinize during mashing in malts with good β-glucan breakdown but with only adequate, not complete protein breakdown because good modification also ensures adequate levels of amino acids for the yeast, although, in low gravity, or all malt worts, free amino nitrogen (FAN) is seldom limiting [12]. Achieving optimal enzyme activity in the grain can be considered very important for the proper conduct of the malting process. Suppression of excessive production of starch-degrading enzymes in the grain is particularly important in wheat because they are established varieties that have a diastatic power of up to 160 °L (about 600 °WK). Therefore the objective of this work was to determine minimum required time of germination, which is necessary for the development of optimal enzyme activity in grain.

MATERIALS AND METHODS

Ten kg of wheat Golubica from trial fields of Agricultural Institute Osijek was obtained during season 2010. Grain samples were collected as untreated and conditioned grain, scaled and packed into in double-walled paper bags (1 kg). Until micromalting, the material was stored for two months in a dry and cool place (20 °C) to overcome post-harvest grain dormancy. To avoid the influence of microbiological contamination on malt quality, raw material control concerning *Fusarium graminearum* and *Fusarium culmorum* contamination was conducted, according to MEBAK procedure. Micromalting was conducted in steeping vessel and at drier Seeger micromaltery according to MEBAK [13], and germination was conducted in Climatic test chamber (Climacell 222, Medcenter Einrichtungen GmbH). Degermination was performed manually. Malt was stored for one month in order to stabilize. α-amylase activity and total diastatic power were determined in the samples. Other amylolitic activity indicators in grain such as extract, extract difference, viscostiy of laboratory mash, filterability of laboratory mash were also measured. All indicators were determined according to EBC (methods 4.5.1.; 4.5.2.; 4.8.; 4.12.; 4.13.) [14].

Table 1. Micromalting scheme of wheat samples

1st day	Immersion steeping for 5 hr, t = 14,0 °C; Dry steeping for 19 hr, t = 14,5 °C
2nd day	Immersion steeping for 4 h, t = 14,0 °C; Dry steeping for 20 hr, t = 14,5 °C
3rd day (*)	Immersion steeping for 2 hr, t = 14,0 °C;
3th day to 7th day	germination was carried out at 14,5 °C during 3-7 day on relative humidity of air in each procedure: r.H. = 85%; sampling during germination was performed daily
8th day	duration of kilning was 19 hr, performed according to standard procedures for pale malt (MEBAK) after last hour of germination, draying finished, malt degermination, measuring and packing in paper bags and stored

(*) control of the degree of soaking at the beginning of the third day and every hour of soaking under the water, when it was found that the grain does not tolerate any further soaking under water, moisture content of 44,5% was adjustment with sparging (spray steeping) in germination box (1st day of germination)

The determination of the combined activity of -α and -β amylase of germinated grain was performed under standardized reaction conditions was carried out as follows: malt

enzymes were extracted with water at 40 °C. Standard starch solution was hydrolysed by the malt enzyme extract, the amount of reducing sugars formed by amylolytic action was estimated iodometrically. The result was expressed as grams of maltose produced under the specified conditions by 100g of malt (EBC 4.12.). The determination of α-amylase activity of germinated grain was performed as dextrinization time of standardized starch solution in the presence of excess β-amylase. Malt α-amylase is extracted with 5 g/L Na-chloride solutions at 20 °C. Buffered limit dextrin is hydrolysed by the grain enzyme extract. The amount of α-amylase is estimated visually using a colour standard which corresponds to the concentration of starch remaining in solution at the specified end point of the dextrinization process. The result is calculated as the quantity of α-amylase which will dextrinize soluble starch, in the presence of an excess of β-amilase, at the rate of 1g per hour at 20 °C (EBC 4.13.). Micromalting of the wheat samples was carried out according to the procedure shown in Table 1.

RESULTS AND DISCUSSION

Wheat varieties appropriate for malting have not yet been commercialized. For that reason, local bread varieties are being used for malting. In accordance to analytical quality parameters, optimizing of process conditions for malting has been performed only for Northeuropean varieties (mostly German) that genotipically differ from domestic varieties. This is not appropriate since agro climatic conditions during cultivation are significantly different from country to country. Some researches indicate that most domestic varieties (especially bread varieties) can be sorted into B quality group which is characterized with higher N share in grain and relatively low mash viscosity (caused by lower share of soluble pentosanes) [15, 16]. Enzymes synthesis is extremely energetically demanding set of anabolic reactions. For that reason it is necessary to determine duration of that process so it would not harm the malt quality, and cause huge economic losses during malting. Total diasatstic power should include α-amylase activity and activity of other amylolitic enzymes in the grain, especially β-amylase. α-amylase is synthesized during germination process and it is genotipically determined. Expression of its potential depends on process parameters during germination [17, 18]. Total diastatic power implies activation of other amylolitic enzymes that are present in wheat grain, and are hugely dependent on agro climatic conditions to which the crop was exposed. Stated facts indicate that duration times of these processes are of most importance to gain favorable α-amylase activity and total diastatic power, without damaging the rest quality indicators. Connection of activation time duration and enzyme synthesis (total diastatic power) to other quality indicators is shown in fig. 1. α-amylase synthesis, and its correlation to other malt quality indicators is shown in fig. 2.

In amylolitic complex activation, also known as diastase, a significant activity has been observed from the very beginning (240 °WK) and also even activity increase till sixth day. After that the curve shows that saturation has been achieved. From technological point of view, total diastatic power should not exceed 250 – 420 °WK, so it is obvious

that optimal values have been achieved between third and fifth day. The malting process cannot be stopped at that time because α-amylase activity is not fully developed (fig. 2). Stopping the process would inevitably lead to fermentability extract decrease because α-amylase activity is an important precursor for formation of limit dextrinases who are important for β-amylase activity. Seventh day of germination, saturation of diastaze activity occurs, and that is what Sacher noticed during his experiment [17, 18] and he enrolled this to β-amylase activity decrease (fig. 1). In case of α-amylase synthesis during germination (*de novo*), it is extremely important to achieve optimal values for enzyme activity since, due to the nature of starch in raw materials intended for brewing, it is not possible to achieve enough fermentability extract and that can be detrimental to economic aspects of malt and beer production.

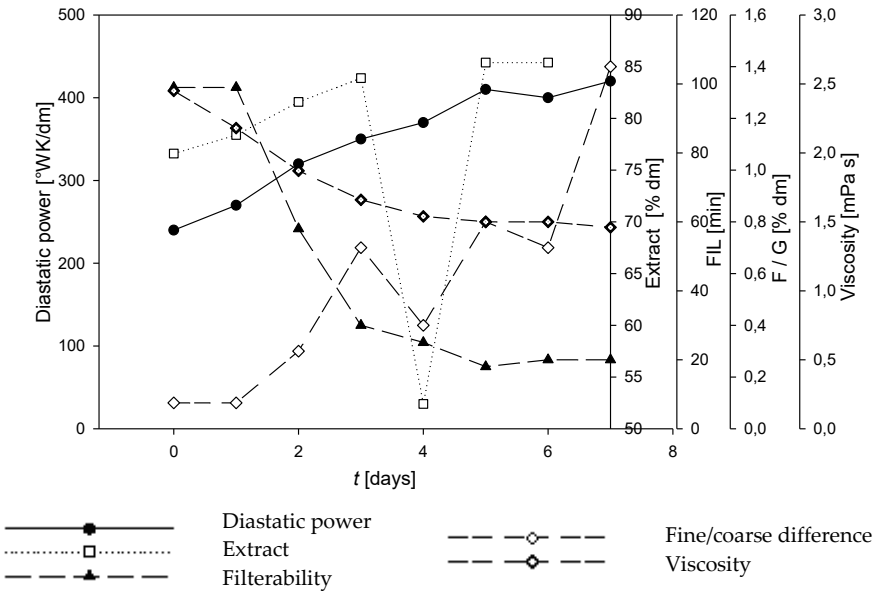

Figure 1. Development of malt grain total diastatic power during germination and its relation to other indicators of quality

In fig. 2 it is noticeable that α-amylase activity shows decreased growth velocity during first three days and sudden increase during fourth, fifth and sixth day. After the sixth day a saturation and decrease of activity occurs and this can be an attributed to decreased β-amylase activity (and total diastatic power). Also a surplus of limit dextrines in wort appears (product inhibition). Optimal α-amylase activity has been

achieved between fifth and sixth day (fig. 2). Considering the rest observed indicators (extract, extract difference, viscosity of laboratory mash, filterability of laboratory mash) and recommended values for listed parameters, it can be concluded that during malting wheat by standard procedure, optimal values for total diastatic power and α-amylase activity ranges from 120 - 140 h.

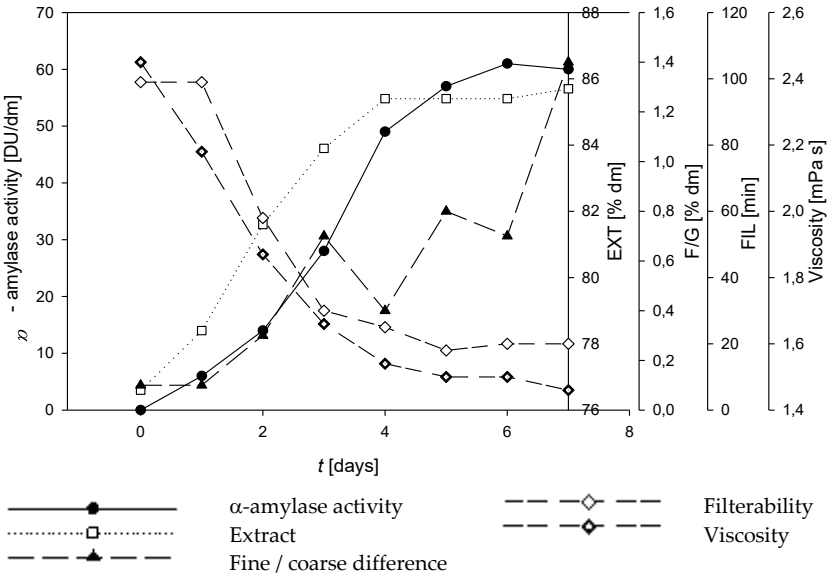

Figure 2. Development of malt grain α-amylase activity during germination and its relation to other indicators of quality

CONCLUSIONS

In conclusion, obtained results indicate that optimal germination time for synthesis of enzymes that affect the total diastatic power of malt and α-amylase synthesis is between 156-168 h. In case of compromising these indicator values with the rest of important malt quality indicators, the optimal germination time ranges from 120–140 h.

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