

# Effects of Pulses of Higher Temperature on the Development of Enzyme Activity During Malting

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## ABSTRACT

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The increase of temperature at the beginning, in the middle and at the end of malting has been evaluated in terms of quality parameters (malting losses, index of acrospire development, friability, HWE, viscosity, SNR) and enzyme ( $\beta$ -glucanase and  $\alpha$ -amylase) development, in a good quality malting barley (Otis) and a higher protein–higher  $\beta$ -glucan content barley used for feed (Extra). A shift from 15 to 20°C at the beginning of malting was shown to increase acrospire development, friability, HWE and SNR and to reduce viscosity, without significantly affecting malting losses. This effect was related to higher  $\beta$ -glucanase and  $\alpha$ -amylase activities within each variety. However, the same enzyme activities were not directly related to a better malting quality when the two genotypes were compared. This confirms previous indications that diversity in malting performance between genotypes cannot simply be traced back to differences in enzyme activities; but, indeed, it suggests that, for a defined barley lot, changes in the levels of enzyme activities following different malting procedures may have a direct effect on malt quality.

**Key words:**  $\alpha$ -Amylase activity,  $\beta$ -glucanase activity, malting temperature, quality parameters.

## INTRODUCTION

The quality of malt relies upon both the intrinsic characteristics of the raw barley, and on the malting process. The potential of barley to produce good malt depends on a number of factors, comprising the structural characters of the barley grain (size and shape, mellowness,  $\beta$ -glucan and protein content), the intensity of modification during malting, and the speed and the level of hydrolytic enzyme development<sup>7,21</sup>. The latter trait can also be influenced by the malting process itself. It has been suggested that, in the 13–25°C range, a higher temperature at the beginning of the malting process produces malts with higher enzyme activity<sup>4,19</sup> and lower viscosity<sup>20</sup>, while using such higher temperature at the end of the process can reduce the level of soluble nitrogen<sup>7</sup>. On the other hand, if too high a tem-

perature is employed in the overall malting process, fast but not uniform modification occurs in the grain<sup>7</sup>,  $\beta$ -glucanase activity may be reduced<sup>19</sup> and, after an initial stimulatory effect<sup>1,4,19</sup>, a fall in  $\alpha$ -amylase and protease development is evident before completion of the process<sup>1,19</sup>, reducing HWE and total soluble nitrogen while increasing viscosity<sup>4</sup>.

Although a low  $\beta$ -glucan content in barley can be important in determining malting quality, the fast degradation of this component<sup>13</sup> and then a rapid increase in  $\beta$ -glucanase activity<sup>9,14</sup> have been stressed as key traits for malting barley<sup>3,23</sup>. Since  $\beta$ -glucanase production is a major factor determining modification during malting and extract yield relies on an adequate level of  $\alpha$ -amylase during saccharification<sup>7</sup>, in this paper these two enzymes have been considered together with other quality parameters to evaluate the effects of higher temperature pulses during malting.

## MATERIALS AND METHODS

In the first experiment, three varieties, Cherì, Airone and Amillis, with different malting quality potential, were tested for the effects of malting temperature on friability. Malting was performed with a manual method employing 100 g of barley. Briefly, samples were soaked in beakers filled to 0.35 L, then three 7-h steepings with two intervening 17 h air rests were used. At the end of each steeping, the grains were drained and blotted. The subsequent germination phase (113 h) was performed in 250 mL flasks whose caps had a 1 cm large hole. After 36 h germination, grains were removed and mixed before being reintroduced into flasks. Kilning proceeded for 24 h at 50°C and 24 h at 65°C; rootlets were removed, samples were weighed and moisture content measured for calculation of losses (due to leaching, respiration and rootlet removal<sup>7</sup>). Three temperature regimes, from soaking to the end of germination, were tested: 1) constant 14°C, 2) constant 16°C, and 3) an initial 55 h of imbibition at 16°C followed by 113 h of germination at 14°C.

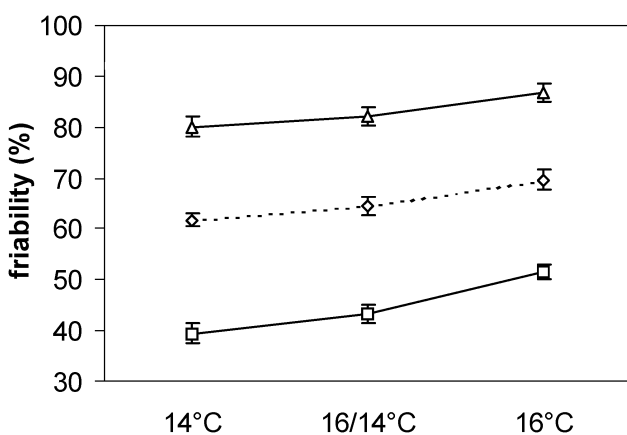
For the following experiment, two spring barley varieties, Otis, of good malting potential, and Extra, a feeding barley, were grown in Fiorenzuola (North Italy) in 2000. The barley samples were characterized for protein content<sup>15</sup>, with Otis having 8.3% and Extra 11.4%, for  $\beta$ -glucan content<sup>11</sup>, with Otis at 3.4% and Extra at 4.8%, and for germinative energy<sup>7,11</sup>, with Otis showing 90 and 99%

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and Extra 94 and 98% germination respectively after two and three days (4 mL test, 19°C). Malting was performed as described above, except for varying temperature regimes from soaking to end of germination for roughly equivalent periods of malting. These comprised first, maintaining the temperature at 15°C for the whole period (treatment A); then a higher temperature pulse (20°C) was applied for the 55 h imbibition phase (treatment B) or for the first 51 h of germination (treatment C) or for the final 62 h of germination (treatment D). Four 100-g replications for each genotype and treatment combination were employed, with two of them used for determination of malt parameters, while the others for the daily removal of 15-g sub-samples each, for enzymes analysis. All the daily replicated 15-g sub-samples were immediately frozen in liquid nitrogen and stored at -20°C. When the 15-g sub-samples for the whole malting process were collected, they were lyophilized, milled and stored at -20°C pending enzymatic determination.

On the dry malt, an index of acrospire development, measuring acrospire elongation relative to kernel length, was calculated as a weighted average after the ASBC classification of acrospire growth (Gianinetti et al., in preparation), friability was determined with a friabilimeter<sup>11</sup> (Pfeuffer GmbH, Germany), hot water extract<sup>15</sup> (HWE) was established as indicated by Gothard et al.<sup>12</sup>, viscosity was measured with a rotational viscometer<sup>15</sup> (Brookfield, USA), SNR was calculated by Kjeldhal determination of nitrogen in malt and wort<sup>15</sup>. Enzyme activities in barley, dry malt and samples removed during malting, were assayed with the Ceralpha method<sup>18</sup> ( $\alpha$ -amylase assay kit, Megazyme, Ireland) and the azo-barley glucan method<sup>17</sup> ( $\beta$ -glucanase assay kit, Megazyme, Ireland). Enzyme activities from samples removed just before kilning were considered as green malt final activities; areas beneath the curves of enzyme activity development were calculated and considered as integrated activities (e.g., activity of an enzyme multiplied by the time it can act). Statistical analysis (ANOVA and Principal Component Analysis) was performed by using Systat 9.0 software.



**Fig. 1.** Changes in friability for three barley varieties with different malting quality, Cheri ( $\Delta$ ), Airone ( $\diamond$ ) and Amillis ( $\square$ ), following malting with increasing temperatures (see Materials and Methods for details). Bars represent standard error ( $n = 2$ ) for each measurement.

## RESULTS AND DISCUSSION

The temperature during the malting process influenced the friability of malt (Fig. 1). Although secondary in relation to the effect of genotype, higher temperature regimes (an increase of 2°C at the beginning or for the whole malting process) produced higher friability. This could be expected if a simple increase in metabolic activity and speed of embryo development were the underlying changes. However, higher temperatures are known to cause negative effects on quality if extended beyond the initial phase of malting, due to a faster decline of hydrolytic activities<sup>4,19</sup>.

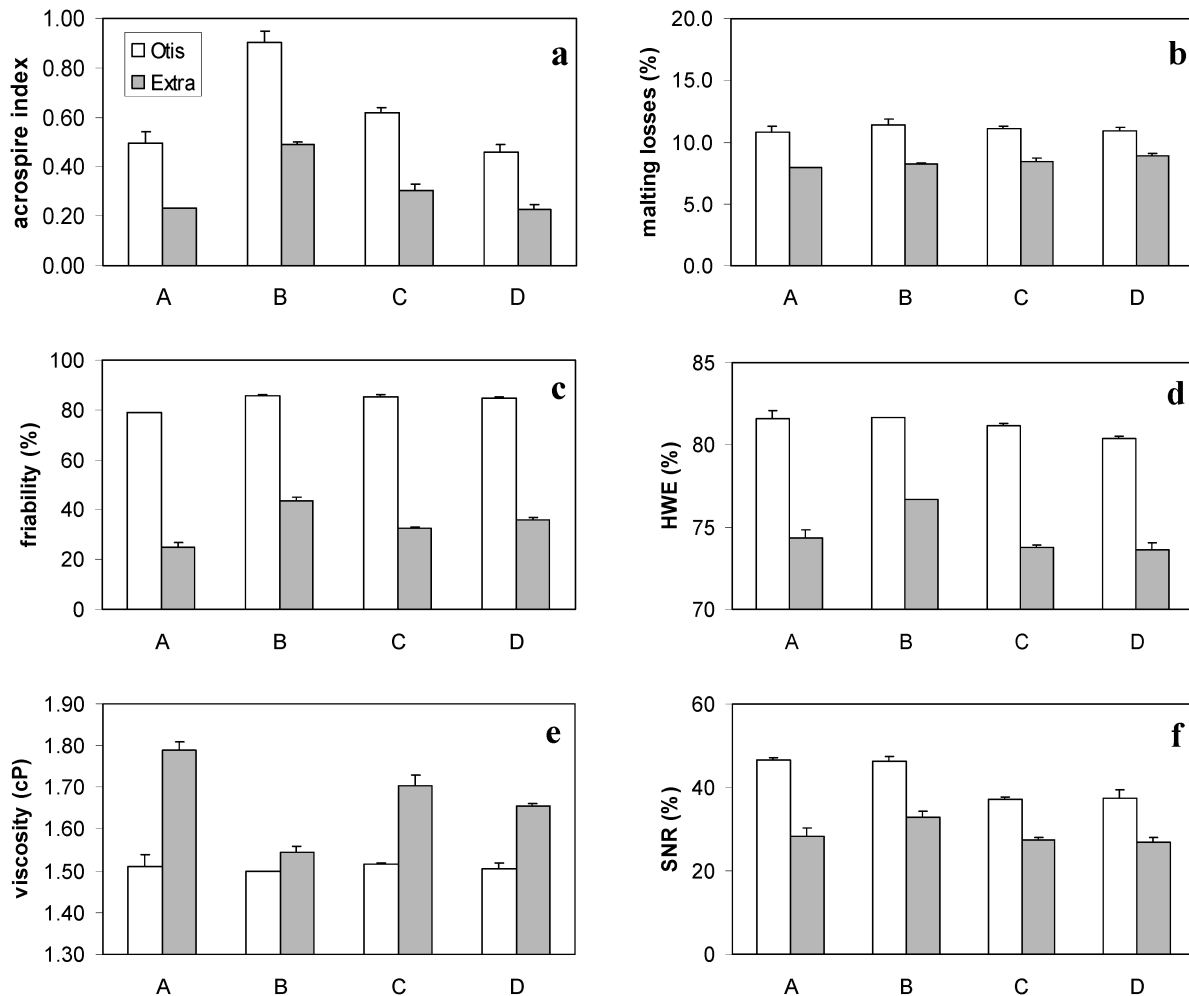
The wider temperature shift (15 to 20°C) adopted in the following experiment to verify the effect of pulses of higher temperature on malt parameters and, particularly, on the development of the two most significant enzymes,  $\alpha$ -amylase and  $\beta$ -glucanase, is in the range commonly used by many industrial maltsters<sup>7</sup>. The rationale of this experiment was to correlate changes in quality parameters to speed of development and/or final levels of these enzymes, comparing the genotype effect with the effect of higher temperature pulses during malting.

Considering malt parameters, we observed that both genotype and temperature regime during malting had highly significant effects (Table I), with the malting variety (Otis) showing higher friability, HWE and SNR, but lower viscosity under all conditions (Fig. 2c-f). This was linked to a greater growth of acrospire, with higher malting losses in Otis (Fig. 2a,b). Correspondingly, a higher germination percentage at the end of malting was observed for Otis (94%) than for Extra (88%) over all the treatments (not shown), suggesting the latter also had a slightly higher water sensitivity. The timing of the higher temperature pulse had an influence on the malt parameters: switching from 15 to 20°C in the middle and at the end of malting had similar effects (increasing friability but decreasing SNR, viscosity and HWE, compared with 15°C constant), while restricting the higher temperature to the beginning

Table I. Analysis of variance of genotype (Otis, Extra) and treatment (malting temperature: regimes A, B, C, D) effects for malt quality parameters and enzyme activity-related parameters<sup>a</sup>.

Parameters	Genotype	Treatment	Genotype $\times$ Treatment
Acrospire index	***	***	n.s.
Malting losses	***	n.s.	n.s.
Friability	***	***	**
HWE	***	***	*
Viscosity	***	***	***
SNR	***	***	***
$\beta$ -Glucanase activity (green malt)	n.s.	***	n.s.
Integrated $\beta$ -glucanase activity	*	***	n.s.
$\beta$ -Glucanase activity (dry malt)	***	**	**
$\alpha$ -Amylase activity (green malt)	**	**	n.s.
Integrated $\alpha$ -amylase activity	***	***	n.s.
$\alpha$ -Amylase activity (dry malt)	**	**	*

<sup>a</sup> Significance: \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*  $P \leq 0.001$ .



**Fig. 2.** Quality parameters for Otis (white) and Extra (black) at four malting temperature regimes: A, 15°C for the whole process; B, the initial 55 h (imbibition phase) at 20°C, then 15°C; C, 15°C base, with the first 51 h of the germination phase at 20°C; D, 15°C base with the last 62 h of germination at 20°C. Bars represent standard errors.

of the process resulted in overall better malting performance (high friability, SNR, HWE, and low viscosity for both genotypes; Fig. 2c–f). Again, this was connected to a strongly higher growth of acrospire (which was the parameter most sensitive to malting conditions, for both genotypes), but with a very small and not significant increase in malting losses (Fig. 2a,b). These responses were the same for both the good quality malting variety (Otis) and the higher protein–higher  $\beta$ -glucan content feeding barley (Extra), although interaction effects (genotype  $\times$  treatment) were significant for the four quality parameters due to a clearly stronger effect in the feeding variety than in the malting one. This means that, although the effect is in the same direction, it is relatively less in the malting quality genotype, presumably because it is already near the upper limit of quality potential, so that the scope for further improvements may be restricted.

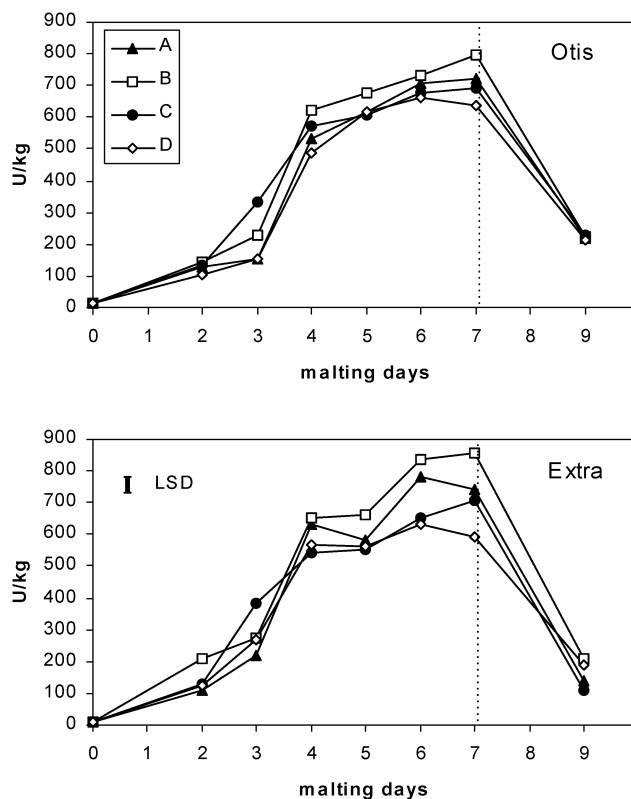
Similar maximum levels of  $\beta$ -glucanase activity during germination can be observed both in malting and feeding varieties, but with malting barley reported to achieve a quicker increase of activity resulting in a larger area beneath the time/activity curve, i.e. a more than doubled integrated activity within the central endosperm<sup>9</sup>. While  $\alpha$ -amylase and  $\beta$ -glucanase activities are well correlated<sup>5,14</sup>,

such a rapid increase should really be meaningful for  $\beta$ -glucanase, the action of which is required for modification during the malting process, while it is the final level of  $\alpha$ -amylase activity which is relevant to starch degradation in the mash. So we were specifically interested in looking for a correlation between malting parameters and integrated activities, particularly in the case of  $\beta$ -glucanase. When we consider the effects of genotype and treatment on quality parameters (Fig. 2) and on time/activity curves (Figs. 3 and 4), a direct relationship (i.e. enzymes development accounting for quality differences) is evident between treatments (treatment B produced the stronger acrospire development, the higher enzyme activities and the best malt quality, within each genotype), but not between genotypes. Specifically, integrated  $\beta$ -glucanase activity appears to be consistently associated to better quality of malt when each genotype is considered individually for the effects of the temperature regimes (compare Fig. 2 and Fig. 3), with, for example, a severe reduction in viscosity for Extra following treatment B (Fig. 2e), possibly also due to the promotion of early  $\beta$ -glucan solubilization which results in a subsequent more extensive degradation. Instead, these curves indicate that, though a significant effect exists for genotype and treatment on most activity-

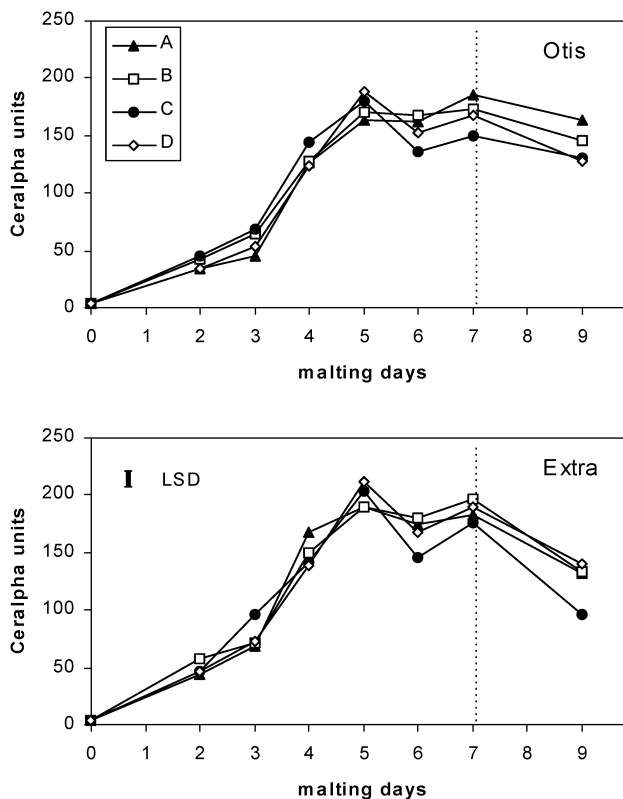
related parameters (Table I), the four describing accumulation, i.e. integrated (over time, from beginning of soaking to the end of germination) and green malt (just before kilning) activities for both  $\alpha$ -amylase and  $\beta$ -glucanase, are higher in the feeding variety (or not significantly different, in the case of green malt  $\beta$ -glucanase activity), confirming that, notwithstanding the essential role they play in determination of malt quality, these activities are not consistently higher in malting barleys<sup>6,16</sup>; indeed, they are also strongly dependent on the environment<sup>2</sup> (Gianinetti et al., in preparation). In the case of  $\beta$ -glucanase, this has been explained with the necessity to consider the levels of enzyme activity in relation to  $\beta$ -glucan content in barley, so that low activity may be enough if barley is low in  $\beta$ -glucan<sup>10,16,23</sup>. Instead,  $\alpha$ -amylase activity in most malting and feeding varieties is usually above the optimal level, at least when adjuncts are not required, so that differences above such a threshold have no effect on starch degradation during mashing<sup>7</sup>. Conversely, comparing activities of these enzymes in the kilned malt indicates a greater susceptibility of Extra enzymes to warm drying (stronger declines between 7 and 9 days), suggesting a diverse combination of differentially-thermostable isozymes was present<sup>8</sup>, which would justify the significance of the interaction effect (Table I). In regard to the effect of treatment, an initial higher temperature induced the higher  $\beta$ -glucanase activities, either integrated or in the green malt (Fig. 3), while increasing temperatures at the end of germination

provoked a precocious decline of  $\beta$ -glucanase activity, reducing both integrated and green malt activities. Extra also showed the wider differences in integrated and green malt  $\beta$ -glucanase activities among different treatments (Fig. 3, bottom).

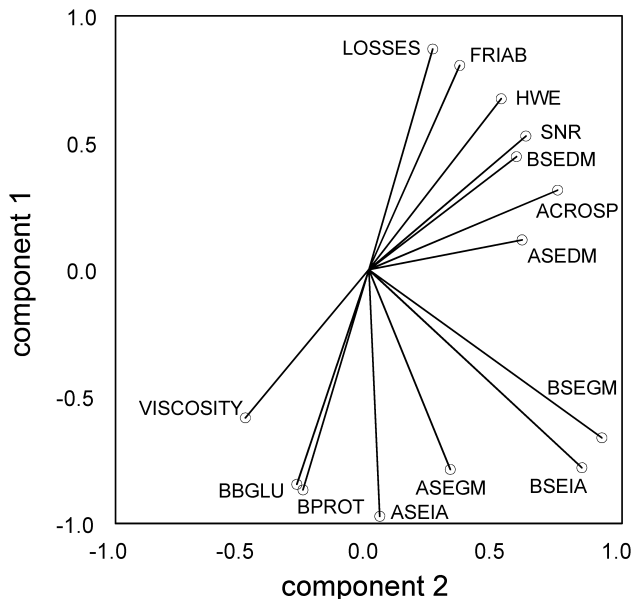
Finally, a Principal Component Analysis plot (Fig. 5) provided a visual presentation of the correlations between the parameters studied. As expected and confirmed by other experiments (Gianinetti et al., in preparation), HWE, friability, SNR and acrospire growth are negatively correlated with barley protein and  $\beta$ -glucan contents and viscosity; however, they positively correlate with malting losses, although this does not appear to be true when a larger number of samples is considered (Gianinetti et al., in preparation). Moreover, they positively correlate with  $\alpha$ -amylase and  $\beta$ -glucanase activities in kilned malt, do not correlate with integrated and green malt  $\beta$ -glucanase activities and negatively correlate with integrated and green malt  $\alpha$ -amylase activities. While the former, positive, relationships can be explained by the degradative role of these enzymes in the mash, the second ones attest that  $\beta$ -glucanase activity during malting is not necessarily associated *per se* with malting quality but should be evaluated in the context of barley  $\beta$ -glucan content<sup>10,16</sup> and, eventually,  $\beta$ -glucan solubilization<sup>24</sup>; so that higher enzyme development during germination does not necessarily distinguish malting and feeding barley quality<sup>6</sup>. The negative relationships between quality and  $\alpha$ -amylase ac-



**Fig. 3.** Development of  $\beta$ -glucanase activity during malting, in a malting barley (Otis, top) and in a feeding barley (Extra, bottom), at four temperature regimes (treatments A, B, C, D as in Figure 2). The dotted line at 7 days indicates start of kilning (see text for details). Least significant difference (LSD) for this assay is reported as a bar.



**Fig. 4.** Development of  $\alpha$ -amylase activity during malting in a malting barley (Otis, top) and in a feeding barley (Extra, bottom), at four temperature regimes (treatments A, B, C, D as in Figure 2). The dotted line at 7 days indicates start of kilning (see text for details). Least significant difference (LSD) for this assay is reported as a bar.



**Fig. 5.** Principal Component Analysis plot visualizing overall correlation among studied parameters (the closer the segments the higher the correlation). Abbreviations as follows: LOSSES, malting losses; FRIAB, malt friability; HWE, hot water extract; SNR, soluble nitrogen ratio; ACROSP, index of acrospire development; BSEGM,  $\beta$ -glucanase activity in green malt; BSEDM,  $\beta$ -glucanase activity in dry malt; BSEIA, integrated  $\beta$ -glucanase activity; ASEG,  $\alpha$ -amylase activity in green malt; ASED,  $\alpha$ -amylase activity in dry malt; ASEIA, integrated  $\alpha$ -amylase activity; BPROT, barley protein content; BBGLU, barley  $\beta$ -glucan content; VISCOSITY, viscosity of hot water extract.

tivities should be due to an association between high  $\alpha$ -amylase development capability and high protein content<sup>22</sup>, so that higher protein content acts more negatively on quality than the associated higher  $\alpha$ -amylase activity can do positively, in absence of adjuncts in the mash.

Our results support the beneficial effects of keeping a slightly higher temperature during the initial stages of malting to increase enzyme activities and improve malt quality. In fact, while a better malting performance between genotypes is not necessarily linked to higher enzyme activities, variations of malt quality induced by changes in the malting process (e.g. different temperature regimes) appear to be mediated, within each genotype, by corresponding changes in enzyme levels (particularly considering the activity integrated over the malting process, in the case of  $\beta$ -glucanase), because of the identical characteristics of the starting sample. This suggests that in further studies aimed to obtain a better comprehension of the role of the enzymes in modification (and then improving the efficiency of practical approaches to tuning of the malting process), other factors, like structural characters of the barley grain in different lots, have to be considered at the same time.

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