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Impact of Low Hydration of Barley Grain on β-Glucan Degradation and Lipid Transfer Protein (LTP1) Modifications During the Malting Process

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Supporting Information

ABSTRACT: One of the objectives of the malting industry is to reduce the energy cost during kilning without major effect on malt quality. In this study, the impact of a low hydration steeping process on lipid transfer protein (LTP1) modifications and β-glucan breakdown was evaluated in low (LH) and high (HH) hydrated malts. LTP1 modifications analyzed by MS/MS revealed acylation, glycation, and disulfide bond breakage in both LH and HH malts. LTP1 free amine content measurement and fluorescence of Maillard protein adducts revealed no significant difference between LH and HH malts. Immunolabeling of LTP1 during malting highlighted the diffusion of the protein from the aleurone layer to the endosperm at the end of steeping in both LH and HH malts. By contrast, a significant higher amount of β-glucans was measured in LH malts after five days of germination, whereas no significant difference between LH and HH malts was revealed through immunostaining of β-glucans or evaluation of the endosperm integrity after seven days of germination. The possibility to reduce the effects of a low hydration steeping process on β-glucan hydrolysis by increasing germination time was discussed.

KEYWORDS: barley, malt, steeping process, β-glucan, lipid transfer protein

INTRODUCTION

In regard to the increased interest in economic and environmental issues, the malting industry is looking for processes that could reduce the cost of energy[1] and wastes.[2] In the malting process, steeping at low hydration level is one of the new insights that would allow not only significant economical benefits by reducing the energy necessary for kilning but would also reduce the water consumption and the amount of effluent to be treated. Indeed, a 5% reduction of green malt hydration level was estimated to allow a 20% economy in thermal and electric energy, corresponding to 0.25×10^9 kWh/year for the French malting industry (P. Boivin, unpublished result), but the critical point is to obtain sufficient modifications of the grain to provide a malt quality compatible with beer production.

In barley grains, β-glucans account for up to 75% of the cell wall polysaccharides of the endosperm [3] and efficient degradation of β-glucans has been described as a crucial element for the malt quality.[4−6] Actually, a high amount of nonhydrolyzed β-glucans in malt induces a lower friability[7−9] and a higher wort viscosity[5,10] that are deleterious for the mashing and filtration steps of brewing. In addition, an efficient degradation of β-glucans is determinant for the subsequent action of other enzymes such as α and β-amylases that need to diffuse and access to their substrate.[11,12] Altogether, this emphasizes the importance of β-glucan degradation during malting.

Besides, the modifications of barley grain during malting are determinant for the quality of beer, especially the formation of foam.[13] In this regard, the lipid transfer protein 1 (LTP1), abundantly expressed in the aleurone layer of barley endosperm, has been extensively associated with beer foam formation.[14,15] Indeed, LTP1 can account for about 5% of soluble proteins[16] but has been identified as a major protein of beer and beer foams together with protein Z.[13,17] While the native barley seed LTP1 displays poor foaming properties, this protein becomes a surface-active protein that concentrates in beer foam during the malting and brewing process.[18] In particular, it has been shown that during the germination, LTP1 has to be transferred in the endosperm to allow its glycation through Maillard reactions[19,20] during the kilning step. In addition, most of the LTP1 from malt and beer is acylated with one or two lipid adducts.[21,22] It has been demonstrated that this lipid adduction occurs during germination[23] and enhance the surfactant properties of the protein.[24] Finally, the foaming properties of modified LTP1 are acquired after its unfolding due to disulfide bond breakages on boiling during the brewing process.[22]

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Table 1. Quantification of LTP1 and Acylated LTP1 in HH and LH Malt Extracts[a]

	total LTP1 (μg of LTP1/g of malt)	total LTP1 (μg of LTP1/g of malt)
LH malt	1270 ± 169	1300 ± 180
HH malt	1510 ± 192	1550 ± 208

[a] ELISA analyses were conducted on triplicates. Mean values of ELISA triplicates ± standard deviation.

The aim of the present work was to determine the effects of a steeping process leading to low hydrated barley grains on these two markers of malt quality, i.e. the degradation of β-glucans as well as the physicochemical modifications of barley LTP1. Previous studies have shown that germination rate was sharply affected beyond 36% hydration level at the end of steeping.[25] Consequently, steeping diagrams of the micromalting process were designed to obtain green malt at 38% and 45% moisture.

MATERIALS AND METHODS

Plant Material. Barley (*Hordeum vulgare*, cv Esterel) grains were obtained from IFBM (Institut Français des Boissons, de la Brasserie et de la Malterie, Vandoeuvre, France). Malt samples were obtained by applying two steeping programs to barley grains. They allow 38% and 44% (fresh weight basis) hydration level at the end of steeping. To obtain malts at low or high hydration level, two distinct steeping programs were used. The steeping schedule used for 38% moisture was 8 h wet, 6 h dry, and 5 h wet, whereas the steeping diagram 8 h wet, 16 h dry, 7 h wet, 10 h dry, and 4 h wet was used for the 45% moisture malt. Germination was conducted in the same conditions for LH and HH malts at 16 °C. The samples were collected every day from the end of steeping to seven days of germination and were either stored at −80 °C before immunolabeling studies or used immediately for physiological and biochemical studies. At the fifth day of germination, green malt samples were kilned (50 °C, 8 h; 1 h to reach 64 °C, 7 h; 1 h to reach 80 °C, 3 h; 1 h to reach 85 °C, 3 h; ambient temperature reached in 2 h) in order to get malt with a classical malting duration.

LTP1 Purification. LTP1 purification was realized as previously described.[26] Briefly, 1 g of malt flour was mixed with 5 mL of deionized water for 30 min and then heated to 100 °C for 30 min in a water bath. After a centrifugation of 10 min at 8000*g*, 100 μL of iodoacetamide 0.5 M was added to the recovered supernatant and the mixture was incubated in the dark for 30 min, the latter reaction was stopped by adding 0.1% of trifluoroacetic acid. The sample was dialyzed against water and lyophilized before purification by RP-HPLC using a column (25 cm × 4 mm) packed with Nucleosil C18 (Macherey-Nagel, France) coupled with a UV detector (205 and 218 nm). The fractions containing LTP1, assessed by SDS-PAGE with a 15% acrylamide gel, were pooled and freeze-dried.

Fluorescence of Advanced Maillard Compounds. Fluorescence emission spectra were recorded from 350 to 500 nm with an excitation wavelength at 330 nm by using a FluoroMax fluorescence spectrophotometer (Spex−Jobin-Yvon, France) to follow advanced compounds of Maillard reaction [27] in purified LTP1 samples.

LC-MS Analysis. Liquid chromatography mass spectrometry (LC-MS) analyses of purified LTP1 were performed using a Waters 616 system (Waters, Manchester, UK) coupled to a LCQ Advantage ion trap mass spectrometer (ThermoFinnigan, USA). Chromatographic separations were conducted on a reverse-phase column (Nucleosil C18, 4 mm i.d., 25 cm length, Macherey-Nagel) at a flow rate of 0.3 mL/min and maintained at 62 °C. The gradient consisted of a linear increase from 20% to 50% of solvent B (acetonitrile containing 0.08% formic acid; solvent A consisting in H_2O containing 0.12% formic acid) in 35 min, followed by a rapid increase up to 100% of B within 1 min. Mass data acquisitions were piloted by the Xcalibur software. The instrument operated in the positive ion mode. The spray voltage was 4.5 kV, and measurements were recorded on the m/z range (400−2000).

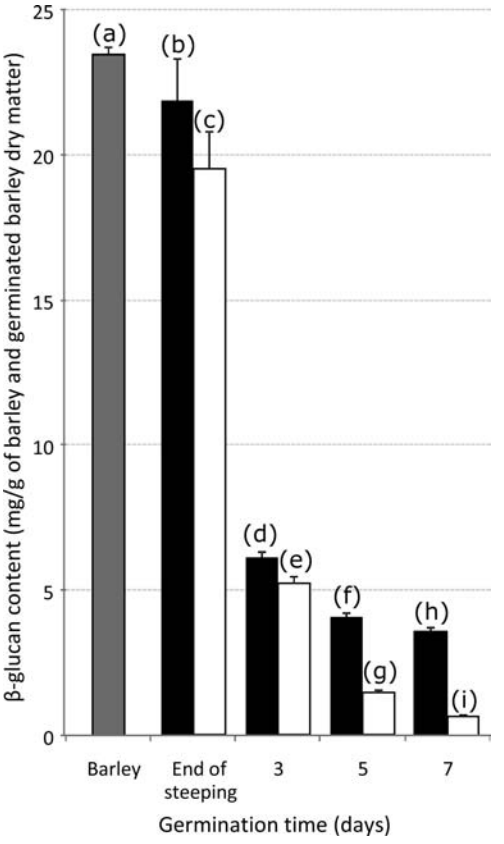

Figure 1. Nonhydrolyzed β-glucan content in barley (gray solid square) and green malts obtained at 38% (black solid square) or 45% (open square) hydration level. Means of three measurements ± standard deviation. Vertical bars represent standard deviation. Letters on the bars indicate whether the values are significantly different at $P < 0.001$.

Determination of LTP1/LTP1b Content by Indirect ELISA (Enzyme-Linked ImmunoSorbent Assay). The assay was conducted as previously described.[23] Microtiter plates (Maxisorp; Nunc, Denmark) were coated with 100 μL of LTP1 standard (LTP1b or total LTP1) or assay solution. The specific detection was obtained using purified monoclonal antibodies (MAbs) anti-LTP1b or antitotal LTP1. A standard curve was generated by using pure barley LTP1. The amount of LTP1 in samples was estimated by reference to the standard curve .

Paraffin Embedding for Immunolabeling. Transverse sections sampled from half grains were transferred into plastic molds, fixed in a formaldehyde solution for 20 h at 20 °C, and processed for paraffin inclusion as described by Jamme et al.[28] Microtome sections of 10 μm (Microm HM340E Microtech, France) were used for immunolabeling.

Immunolabeling. Sample sections were saturated with 0.01 M Na-phosphate buffer saline (PBS), pH 7.2 containing 4% of fat-free milk powder (PBS-milk) during 30 min prior to incubation for 1 h with monoclonal antibodies anti-(1→3,1→4)-β-glucans[3] (1:250; Biosupplies Pty Ltd., Australia) and monoclonal anti-LTP1 (1:300; produced at the laboratory). After extensive washing in PBS, the sections were incubated for 1 h with the goat antimouse IgG coupled to Alexa Fluor-546- (1:100; Molecular Probes, Invitrogen USA). Immunostained

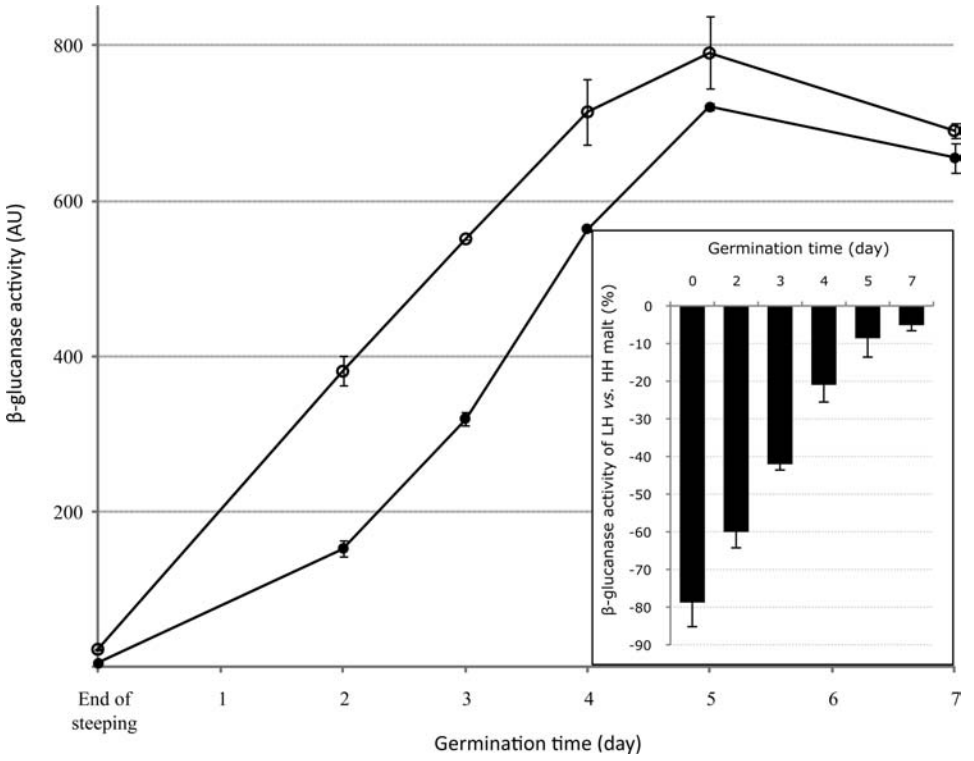

Figure 2. β-Glucanase activity of HH (empty circles) and LH (full circles) malts during germination. Insert indicates the proportion of β-glucanase activity of low hydrated malt compared to that in high hydrated malt during germination. Mean value of three measurements ± standard deviation (vertical bars).

sections were then thoroughly washed in PBS and deionized water and mounted in deionized water to be visualized by fluorescence. The immunofluorescence sections were analyzed by a A1 Nikon (Japan) confocal laser scanner microscope. The resulting images were acquired, stored, and visualized with a Nikon NIS-Elements software program.

Evaluation of Endosperm Integrity of Green Malt by Light Microscopy. For albumen texture analysis, 20 μm thick longitudinal cryosections of grain/malt at different stages of germination were obtained using a cryotome (Microm HM 500 OM, France). Light microscope observations of longitudinal cryosections were used to evaluate the endosperm texture of malts during germination. The method was adapted from Turnbull et al.[29] Longitudinal 20 μm cryosections of malt were realized and washed through PBS and water before observation with a A1 Nikon (Japan) confocal laser scanner microscope.

Biochemical Analyses. *O-Phthaldialdehyde Assay.* *O*-Phthaldialdehyde assay was realized with a modified method of Rowlett and Murphy.[30] Briefly, 50 μL of purified LTP1 samples of 2 mg/mL in 50 mM pH 7.8 phosphate buffer were added to 1 mL of OPA solution (*O*-phthaldialdehyde 0.8 g/L in 50 mL containing 1 mL methanol; 25 mL tetraborate 0.1 M pH 9.3; 2.5 mL SDS 10%; 100 μL 2-mercapto-ethanol and H_2O) and incubated 2 min. The absorbance was measured at 340 nm, and free amine content was determined from a standard curve obtained from lysine.

β-Glucanase Activity was measured using the azo-barley glucan procedure (Megazyme, Ireland) on the green malts. The enzyme was extracted during 15 min from 0.5 g of a green-malt flour with 8 mL of extraction buffer (40 mM acetate/phosphate, pH 4.6). The supernatant of the extract was then preincubated 5 min at 30 °C as the azo-barley glucan substrate solution (in 0.5 mL aliquots). Substrate and enzyme were mixed by adding 0.5 mL of extract in the aliquots, and the reaction was incubated for 10 min at 30 °C. A precipitating solution (sodium acetate 300 mM, zinc acetate 20 mM, pH 5.0, in methoxyethanol 80%) was then added, after 5 min at room temperature. After centrifugation, absorbance at 590 nm of the supernatant was measured.

Determination of Nonhydrolyzed β-Glucans. the content of non hydrolyzed β-glucans was determined by following the instruction of the mixed-linkage β-glucan assay procedure (Megazyme, Ireland). Briefly, malts obtained at various hydration levels and at different times of germination were crushed in liquid nitrogen. The β-glucans were extracted in ethanol 50% at 4 °C for 15 min, and then the enzymes were inhibited with 5 min of incubation at 100 °C in boiling water. Samples were centrifuged 1 min at 14500*g*, and the pellets were washed twice in ethanol 50%. The final pellets were resuspended in 20 mM sodium phosphate buffer pH 6.5 and heated to 40 °C before incubation with lichenase during 1 h at 40 °C. The samples were then centrifuged 1 min at 14500*g*, and three fractions were prepared from the supernatant: 50 mM sodium acetate pH 4 were added to one of them while β-glucosidase was added to the others. After 15 min incubation at 40 °C, the GOPOD reagent (glucose oxidase plus peroxidase and 4-aminoantipyrine) was added and the samples were further incubated 20 min at 40 °C. The amount of glucose released was evaluated through 510 nm absorbance.

RESULTS AND DISCUSSION

Impact of Low Hydration on β-Glucan Content and β-Glucanase Activity of Green Malts. The steeping programs used in this study led to grains with high (44.5% fresh weigh basis) and low (37.5% fresh weight basis) hydration level. The 45% hydration level was used as a standard malt hydration level, whereas 38% was chosen as a low hydration level that would not hinder the germination.[25] The germination and kilning steps were conducted in the same conditions for the two samples and resulted in green malt with 43.7% and 39.9% fresh weight basis water

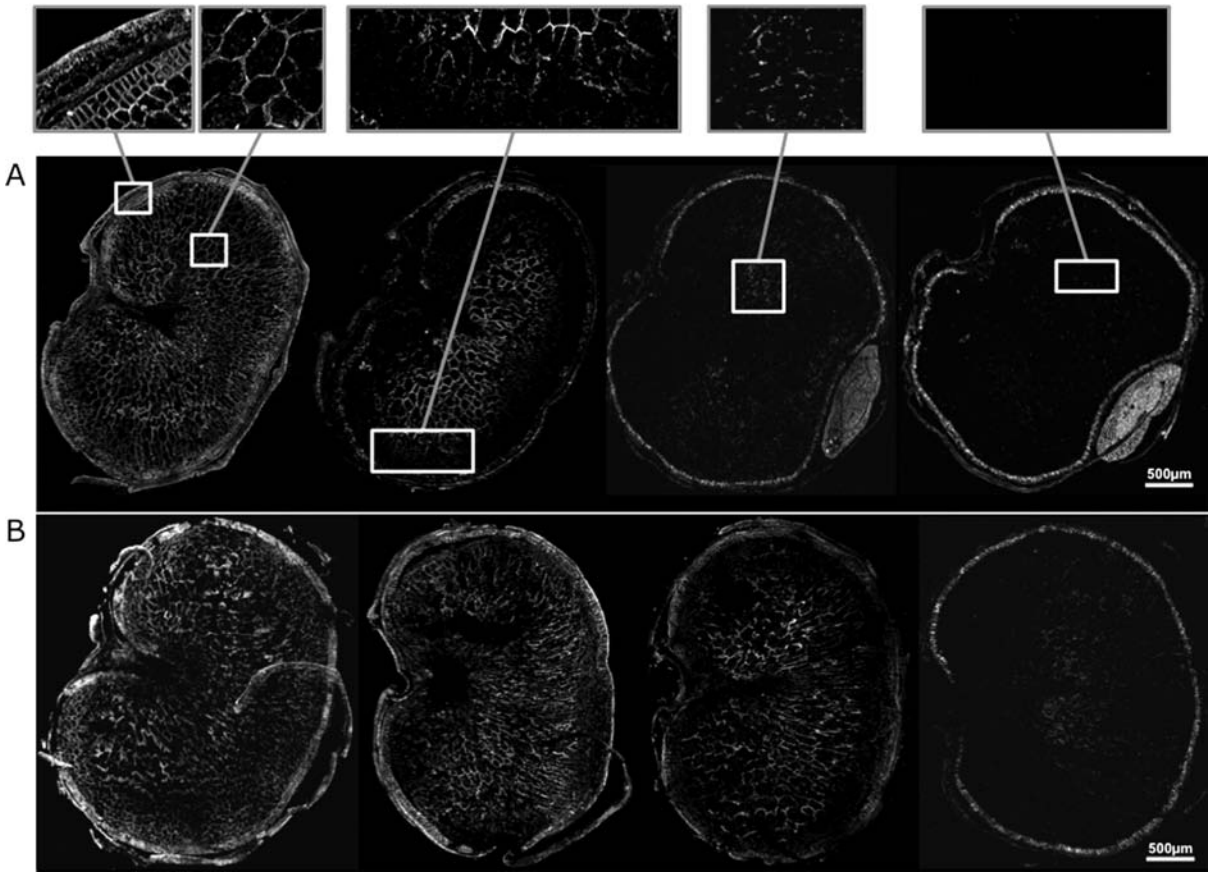

Figure 3. Immunolocalization of reticulated (1−3)(1−4)β-D-glucan in green malt transversal sections during the germination steps of malting. (A) and (B) represent high hydrated and low hydrated malts, respectively, after 0, 3, 5, and 7 days after steeping, from left to right. The boxes on top are zooms.

content, respectively. These malts were called hereafter high hydrated (HH) and low hydrated (LH) malt, respectively. In both malt types, germination occurred and grains starting to germinate quickly after steeping.

Nonhydrolyzed β-glucan content was determined in the HH and LH samples at the end of steeping and during germination (Figure 1). After steeping, the β-glucan content decreased from 23.5 mg in barley to 19.5 mg/g in HH and 21.9 mg/g in LH grains. These results confirmed that β-glucan degradation was significant during the steeping step, as shown by Bamforth et al.[6] and in accordance with Sungurtas et al.,[31] who showed that (1−3, 1−4) β-D-endoglucanase activity is already significant at the end of the steeping process. During germination, the majority of β-glucan hydrolysis occurred in the first three days in both samples in agreement with previous reports.[7,30,32] However β-glucan hydrolysis was further observed until seven days of germination in the green malts. The amount of nonhydrolyzed β-glucans was significantly higher in LH green malt than in HH green malt. Indeed, after seven days of germination, 3% of the initial barley β-glucans remained in HH green malt and 15% in the LH green malt. Nevertheless, for both malts, the remaining amount of nonhydrolyzed β-glucans (4 mg/g) was comprised in the range value described for standard steeping conditions.[7,31]

To complete these results, β-glucanase activity of HH green malt and LH green malt extracts were compared during germination (Figure 2). At the end of steeping, β-glucanase activity was low for both LH and HH programs. During germination, β-glucanase was significantly ($p < 0.01$) lower in LH green malt compared to HH green malt. However, it is noticeable that this difference in β-glucanase activity decreased along germination, only 10% and 6% of difference activity between LH and HH green malt being measured at five days and seven days of germination, respectively (Figure 2 inset). These results showed that the LH steeping program did not impact the production of β-glucanase enzyme but rather affected the kinetics of the enzyme biosynthesis.

In Situ Hydrolysis of β-Glucans in LH and HH Green Malts. The structure of barley endosperm and in particular the distribution of the β-glucans has been described as a key parameter of the malt production.[33,34] In this regard, the diffusion of the β-glucanases within the endosperm and the extent of endosperm cell wall degradation are critical points. We therefore followed along germination, the degradation of β-glucans in the endosperm by immunolabeling (Figure 3). In barley grain before steeping or at the end of steeping (stage 0 of Figure 3), the β-glucans were evenly distributed in the starchy endosperm. Strong labeling of the aleurone layer and of the pericarp was obtained in barley and steeped grains as previously observed in wheat grains.[35,36] The production of β-glucanase takes place in the aleurone layer and in the scutellum.[37,38] As expected, upon germination, the nonhydrolyzed β-glucans were progressively restricted to the central region of the endosperm as observed in the cross sections of germinating barley grain. The same pattern of degradation was observed in both HH and LH green malts, which indicated that the diffusion of the enzyme occurred. After five days of germination, cell wall degradation was almost completed in the starchy endosperm of HH green malt but less extended in LH green malt.

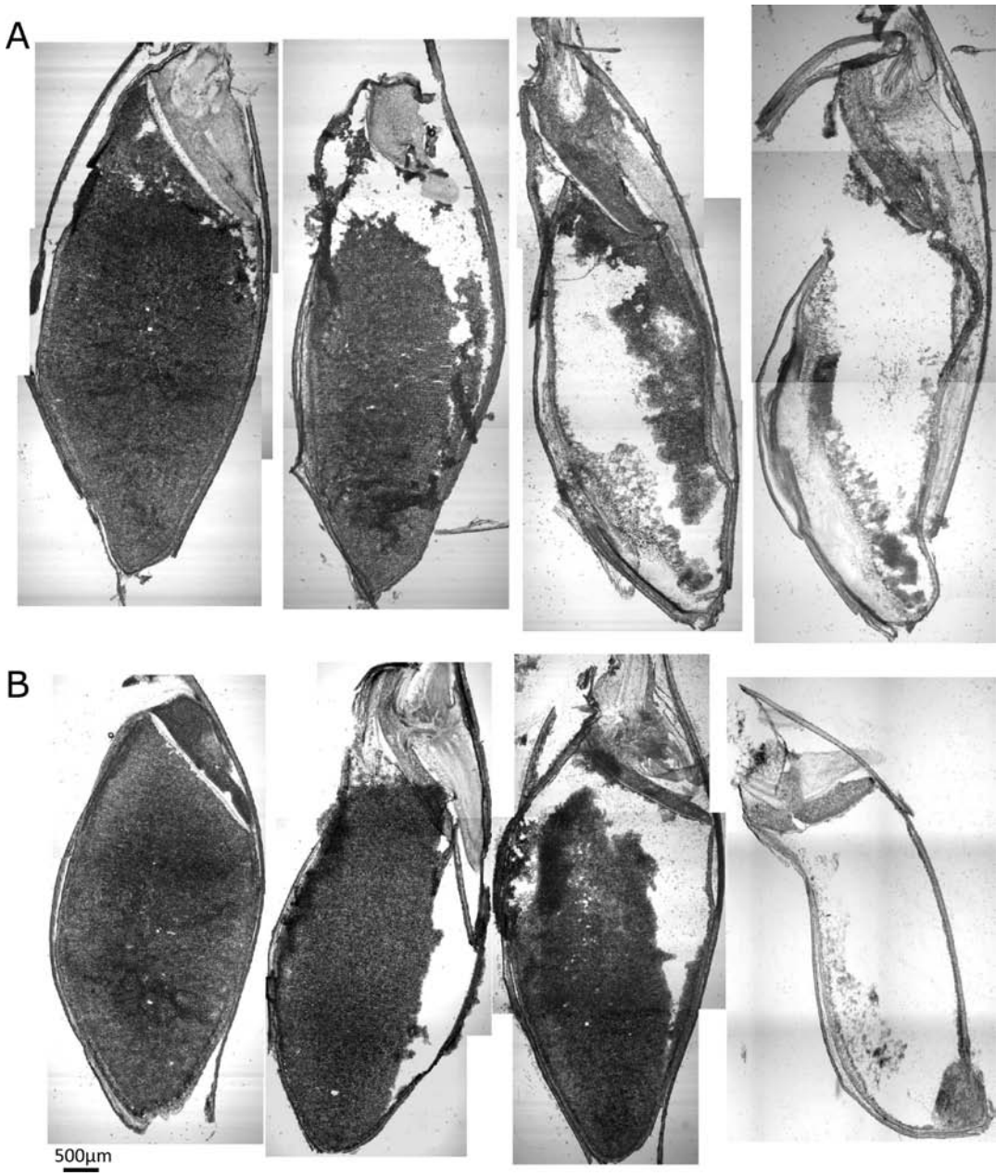

Figure 4. Light microscopy images of cryosection of germinating barley. (A) and (B) represent high hydrated and low hydrated malts, respectively, after 0, 3, 5, and 7 days after steeping, from left to right.

A comparable amount of nonhydrolyzed β-glucans was revealed after three days of germination in HH malt and five days of germination in LH malt (Figure 3). This suggested a two-day lag when germination was performed at lower hydration. Indeed, after seven days of germination, β-glucans could not be anymore detected in LH malt, as observed after five days of germination for HH samples.

The antibody used for revealing nonhydrolyzed β-glucans has a high binding with the (1−3, 1−4) β-D-glucans with a degree of polymerization (DP) of 6−7 and higher.[3] For instance, the affinity of the antibody for the DP6 oligosaccharides is 66 times higher than that for the DP4 oligosaccharide.[3] This could explain the apparent discrepancy between biochemical analyses (Figure 1) and immunolabeling results in LH malt (Figures 3a,b). Furthermore, these results also suggested that the remaining β-glucans in the LH green malt after 7 days of germination had a low degree of polymerization. This point is noticeable, as previous studies have suggested that an increase of wort viscosity is correlated to the presence of residual β-glucans with DP 500 and higher.[39−41]

Incomplete or inhomogeneity in β-glucan degradation is often associated with malt friability.[9,12] Microscopy observations of longitudinal cryosections were then used to evaluate the starchy endosperm texture of LH and HH green malts during germination. Indeed, at each germination stage, the cross sections were washed with aqueous buffer. While barley starchy endosperm remained intact at the end of steeping, a progressive extraction of starchy endosperm material occurred during germination. These results highlighted the germination-induced modifications in the

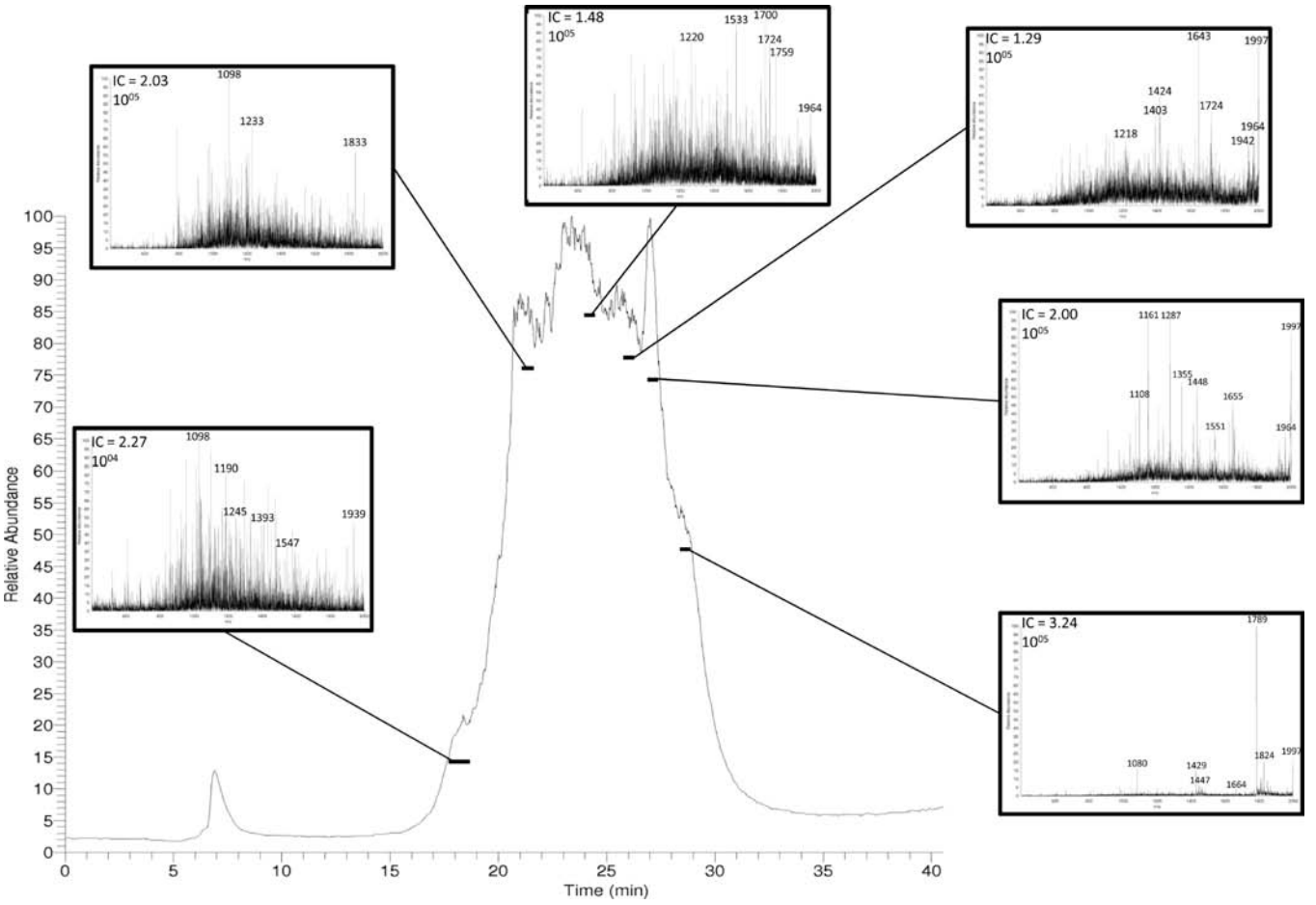

Figure 5. LC-MS chromatogram of LTP1 extracted from high hydrated (HH) malt with associated mass spectra. The same chromatogram has been observed with low hydrated malts (Supporting Information Figure 1). The experimental ions corresponding to the modified forms of LTP1 in LH and HH malts are presented in Supporting Information Tables 1 and 2. IC = ionical current.

endosperm integrity that occurred during malting as illustrated by light microscopy images (Figure 4). In accordance with β-glucan disappearance, more than half of the endosperm of HH green malt was extracted after five days of germination, whereas complete disaggregation of the endosperm was obtained after seven days of germination in LH and HH green malts.

Altogether, these results showed that a lower hydration of barley grains at the end of the steeping process delayed cell wall degradation in LH green malt compared to a HH one. These differences were obviously weakened after seven days of germination.

LTP1 Modifications in LH and HH Malts. The different modifications of LTP1 during malting were assessed through LC-MS analysis (Figure 5 and Supporting Information Table 1). To monitor the LTP1 modifications during brewing, these analyses were conducted on LTP1 extracted from boiled malt extracts. Indeed, previous studies [26] have demonstrated that the protein content of a boiled malt extract is nearly identical to that of the corresponding beer extract.

MS analyses revealed the occurrence of acylation (with one and two lipid adducts with a mass of 294 Da). These modifications were due to the covalent adduction of the α-ketol 9-hydroxy-10-oxo-12(*Z*)-octadecenoic acid. This oxylipin is formed from linoleic acid through the combined action of lipoxygenase and allene oxide synthase.[23] Glycations (with 1 up to 5 hexoses adducts with a mass of 162 Da) were also highlighted through MS analyses as well as cysteine alkylation in relation to disulfide bond disruption upon LTP1 denaturation. All these modifications were observed in both LH and HH malts (Supporting Information Tables 1 and 2).

By using specific antibodies, total LTP1 and lipid adducted LTP1 were quantified by ELISA in LH and HH malts (Table 1). As previously observed,[22,23] almost all the LTP1 was lipid adducted in malts without significant ($p > 0.01$) differences between LH and HH malts. It seemed therefore that LTP1 acylation was not affected by the reduced hydration level during the steeping process. Therefore, the acylation-related foaming properties of LTP1 should not be affected by the reduction of the hydration level.

During malting, LTP1 is also glycated through Maillard reaction, which includes the initial condensation reaction between amine groups of the protein (N-terminal and ε-NH_2 of lysine residue) and reducing sugars.[19,20] As a consequence, the measurement of free amine groups within the protein was used to evaluate the degree of LTP1 glycation in the LH and HH malts (Figure 6A). As the Maillard reaction mainly occurs during the kilning step of malting, the relative proportion of free amine contents was evaluated on purified LTP1 extracted from LH and HH kilned malts (Figure 6A,B). A significant decrease of free amine groups was observed between barley LTP1 and malt LTP1 which contain four lysine residues. Indeed, we measured a 20%

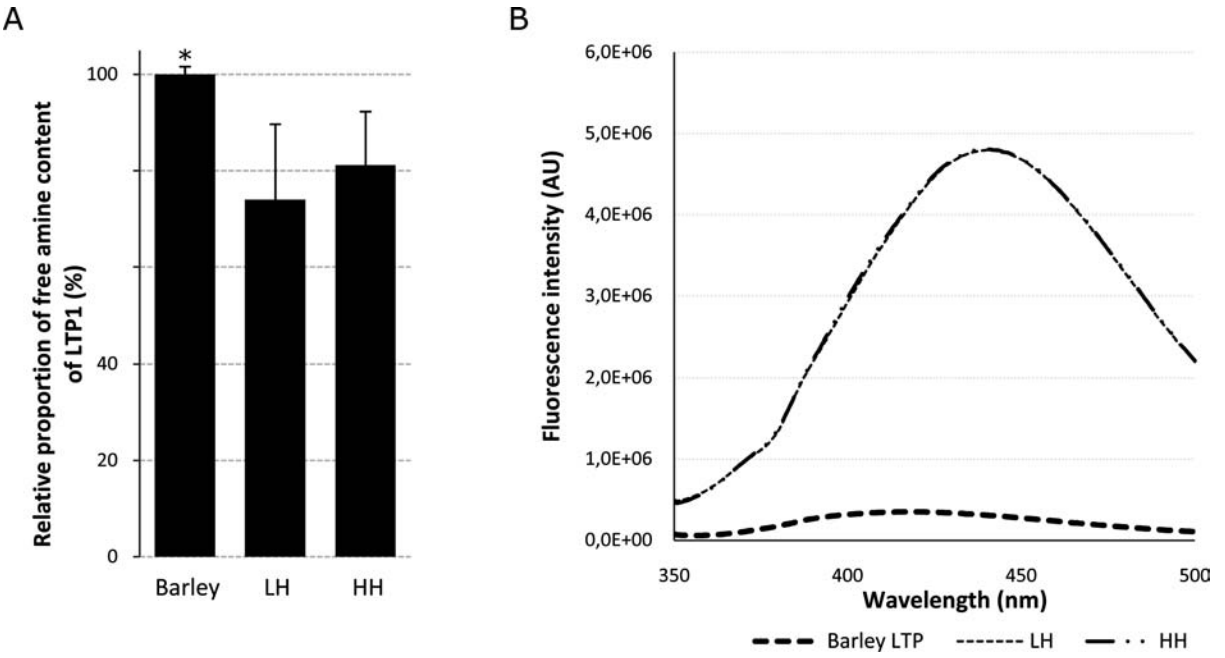

Figure 6. Analyses of LTP1 glucosylations. (A) Relative proportion of free amine content of LH and HH malts LTP1 extracts compared to barley LTP1 extracts. (B) Emission spectra of advanced maillard products of LH and HH malts were obtained with an excitation fixed at 330 nm. Mean values of 3 measurements ± standard deviation (vertical bars). * = significantly different ($p < 0.005$).

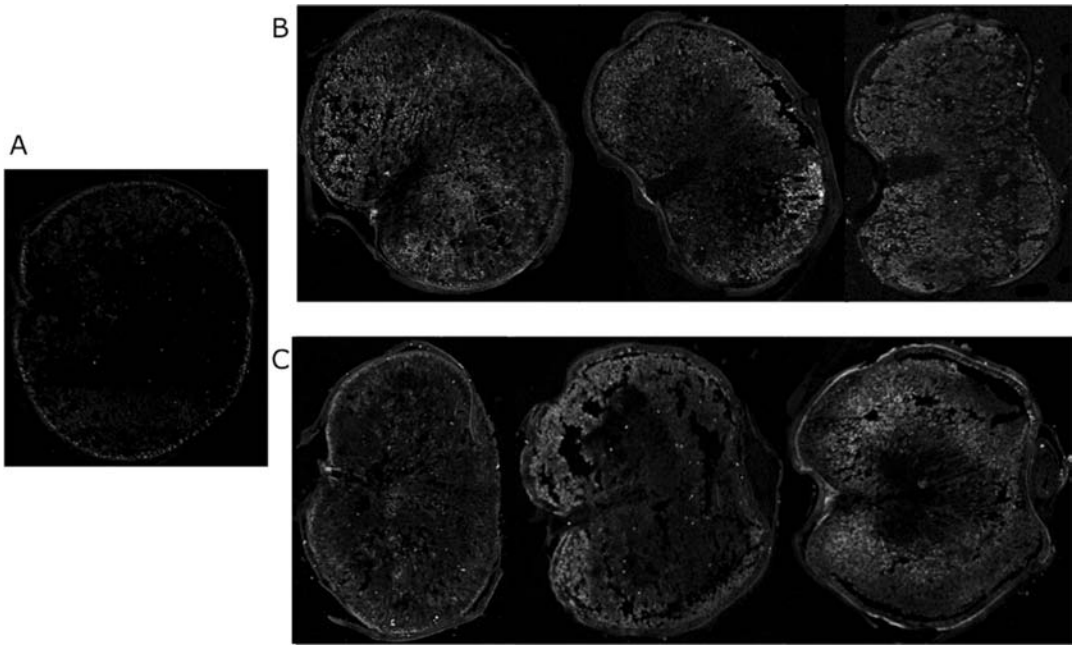

Figure 7. Fluorescence observations of LTP1 diffusion by immunostaining in green malts during the germination step of malting. (A) represents a section of barley before steeping. (B) and (C) represent high hydrated and low hydrated malts, respectively, after 0, 3, and 5 days after steeping, from left to right.

reduction of free amine in malt samples compared to barley LTP1. In addition, no significant difference was observed between LH and HH malt LTP1. Nevertheless, glycation of other amino acid residues could not be ruled out. In addition, LTP1 modifications with advanced Maillard products were observed. These advanced Maillard products consist of a broad range of compounds formed from carbohydrates and lipids as well.[42] For instance LTP1 adduction with N^{ε}-(carboxymethyl)lysine, N^{ε}-(carboxyethyl)lysine, pyrraline and argpyrimidine were observed (Figure 5 and Supporting Information Tables 1 and 2) in both LH and HH malts. Adduction of LTP1 with other advanced products of Maillard reaction was possible and could contribute to the noisy zone of the LC-MS peak of LTP1 (Figure 5). Consequently, to further compare the chemical LTP1 modifications induced in LH and HH malts, we chose to monitor

the formation of advanced Maillard compounds through fluorescence analysis of the purified protein (Figure 6B). The fluorescence intensity of barley LTP1 from 350 to 490 nm was sharply lower than those of malt LTP1, which fit the formation of advanced Maillard reaction products.

In accordance with free amine group measurement, no significant difference in fluorescence was observed between LTP1 isolated from HH and LH malts.

LTP1 Diffusion in LH and HH Green Malts. In mature cereal grains, LTP1 was located in the aleurone layer (Figure 7) as previously observed.[43,44] It has been hypothesized that a diffusion of LTP1 during germination would partly account for the modifications of the protein widely described during malting.[17,20,22,45]

In the present study, specific immunostaining of LTP1 confirms such a diffusion of the protein within the endosperm and also clearly demonstrated that this diffusion occurs during the steeping step in barley (Figure 7). Because any significant difference in the LTP1 diffusion was observed in LH and HH malt, it can be concluded that a low hydrated steeping process would not hinder LTP1 diffusion within the endosperm in full agreement with the LTP1 modifications observed in LH and HH malts. The diffusion of LTP1 within endosperm coupled to a significant β-glucan hydrolysis was probably sufficient to ensure LTP1 glycation during kilning of LH malt.

In summary, our results clearly showed that malting at low hydration level, i.e. 38% water content of steeped grains, did not significantly impact LTP1 diffusion and the physicochemical modifications necessary for the expression of its foaming properties. Indeed, all the modifications undergone by barley LTP1, i.e. acylation, glycation, denaturation, and adduction with advanced Maillard reaction products, were observed in both LH and HH malts. By contrast, after five days of germination, a steeping process leading to low hydrated grains induced a delay in β-glucanases diffusion and therefore in β-glucans hydrolysis, another key parameter of malt quality. Nevertheless, β-glucan degradation was homogeneous and the amount of glucose released by β-glucanases in LH malts was sufficient for LTP1 glycation. Moreover, our results indicated that the impact of the low hydration steeping process could be sharply reduced after seven days of germination. In the attempt to reduce water use in the steeping process and consequently the energy necessary for kilning, the extent of the germination time could therefore be further investigated at industrial scale as an alternative to achieve the endosperm modifications necessary for malting and brewing industries. The increase of germination time would result in a 2.5% increase of the energy cost (P. Boivin, unpublished results), whereas the LH malt processing would induce a 20% reduction of the energy cost of malting.

■ ASSOCIATED CONTENT

Supporting Information. LC-MS chromatogram of LTP1 extracted from low hydrated (LH) malt with associated mass spectra. List of the modified forms of LTP1 with the corresponding experimental ions observed in LH malts. List of the modified forms of LTP1 with the corresponding experimental ions observed in HH malts. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

DP, degree of polymerization
HH, high hydrated
LH, low hydrated
LTP1, lipid transfer protein 1

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