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Abstract

Maximal water uptake and diffusivity were determined from experimental soaking curves using analytical solution of the second Fick's law in four barley samples displaying distinct hydrolytic activities during malting. Water diffusivities in the whole grains, were found to vary between 5.28 and 7.61×10^{-12} m^2/s, whereas those of the endosperms were 5 to 7-fold higher, ranging between 35.2 and 49.5×10^{-12} m^2/s. These differences highlighted the potential barrier role of the grain outer layers. The relationships between maximal water uptake, water diffusivity and grain biochemical and structural parameters (*i.e.* hardness and other less studied parameters such as vitreousness and porosity) was investigated. The lowest water diffusivity value was obtained for the barley sample displaying the lowest porosity, highest hardness and vitreousness but the highest enzymatic activity during malting, in contradiction with what is generally stated. Water diffusion was not found as the limiting factor for malt quality and, thus, its role during barley steeping has to be reconsidered.

Keywords: maximal water uptake, water diffusivity, barley steeping diagram, porosity, hardness, vitreousness

List of abbreviations

C, moisture concentration (kg/m^3)

C_∞, moisture content at equilibrium with pure water in grain (kg/m^3)

C_0, initial moisture content (kg/m^3)

C_{exp}, experimental moisture content values (kg/m^3)

C_{pred}, predicted moisture content values (kg/m^3)

C_t, total amount of water entering the sphere at time t, (kg/m^3)

D, diffusivity (m^2/s)

D_1, true density (kg/m^3)

D_2, apparent density (kg/m^3)

D_{eff}, effective diffusivity (m^2/s)

dw, dry weight

FI, friability index

fw, fresh weight

n, number of moisture content measurements

p, number of estimated model parameters

P, porosity (%)

PSI, particle size index

r, radial position in the sphere (m)

r_e, equivalent radius (m)

r_{max}, maximal radius (or equivalent radius) (m)

RMSE, Root Mean Squared Error

t, time (s)

v, volume of the whole grain or corresponding endosperm (m^3)

1-Introduction

The malting process includes three main steps: steeping, germination and kilning. Steeping results in water uptake by the grain, increasing the grain water content up to 43-46 % (fresh weight basis), and in radicle protrusion (Bewley and Black, 1982). During steeping (24 to 36 h), and mainly during the germination phase (4 d), barley grains produce hydrolytic enzymes, among which the β-glucanases and amylases, which lead to modifications of the grain structure. Therefore this simple step has an important impact on the final quality of malt (Schuster, 1962). Bryce et al. (2010) have shown that steeping barley for 24 h on a continuous basis or using a standard steeping diagram leads to equivalent results in terms of malting quality.

Different grain characteristics (*i.e.* starch or protein content, endosperm structure and cell wall composition) were already suggested to influence water uptake (Barber et al., 1994; Brookes et al., 1976; Ferrari et al., 2010; Molina-Cano et al., 1995; Molina-Cano et al., 2002; Molina-Cano et al., 2004; Ogushi et al., 2002). But, relationships between water uptake and biochemical composition was not clear. For example, some authors reported that low nitrogen content favors water uptake (Bathgate et al., 1978; Chandra et al., 1999; Molina-Cano et al., 2002), when others did not find any relationships between these two characteristics (Gamlath et al., 2008; Leach et al., 2002; Swanston et al., 2006).

The compactness of the endosperm structure seemed to impact significantly the water uptake of barley grain during steeping (Gamlath et al., 2008; Psota et al., 2007; Vejrazka et al., 2008). Hardness and vitreousness are two distinct characteristics commonly used to describe endosperm texture particularly in wheat. Hardness is a physical parameter defined as the degree of resistance to deformation and appears to be controlled by genetic factors (Turnbull and Rahman, 2002). Vitreousness also affects the rheological properties of the

endosperm and appears mainly determined by the environmental conditions during grain development (Vejrazka et al., 2008). It is usually estimated by visual inspection as vitreous kernels are somewhat translucent and characterized by more compact embedding of starch granules into the protein matrix whereas mealy kernels are opaque and floury. Experimental measurement of particle size index after grinding (PSI) or hardness scores measured by near infrared spectroscopy characterized an overall hardness taking into account both genetic and environmental factors. On the contrary, grain vitreousness, assessed by visual analysis of kernel cross-sections or light transflectance, could be more related to growing conditions (Parish and Halse, 1968). To our knowledge, distinction between hardness and vitreousness is scarcely examined for barley grain and often only merged in a single concept of steeliness/mealiness. Some authors however related a mealy structure to a higher water uptake and enzyme movements in the endosperm (Schildbach and Rath, 1994; Chandra et al., 1999; Gamlath et al., 2008). A mealy endosperm thus appears to be more easily degraded by hydrolytic enzymes during malting than a steely endosperm (Swanston et al., 1995). Porosity is also a potential interesting physical parameter, which was rarely taken into account even if it could well characterize the degree of compactness of the endosperm. Furthermore, moisture diffusivities in the whole grain or the corresponding endosperm were never measured in all of the previous studies. Therefore, it appears interesting to better establish relationships between water uptake and mainly water diffusivity in relation with malt quality and the barley grain biochemical composition and structure revealed through hardness, vitreousness and porosity.

In this study, equilibrium water uptake and diffusivity in barley whole kernel and corresponding starchy endosperm were measured in samples displaying contrasted level of hydrolytic activities after a standard steeping diagram and consequently distinct malting values. These values were compared and related with the biochemical composition and structural parameters of the different barley samples.

2-Experimental

2.1. Plant material and grain pearling for endosperm isolation

Four barley grain samples from distinct cultivars were grown in France in 2007 and were provided by IFBM (French Institute of Brewing and malting, Vandoeuvre-les-Nancy, France): one (2-row) spring barley (*Sebastian*) and three winter (6-row) barleys (*Azurel*, *Esterel* and *Cervoise*). Experiments were performed with grains sized between 2.2 and 2.5 mm in diameter in order to avoid possible influence of the grain size on hardness, porosity and moisture diffusivity measurements. The grain samples were packed in sealed polyethylene bags and stored at 4 ± 1°C until experiments started.

Endosperm samples were obtained by grain pearling (80 g), at a constant speed of 800 rpm, using an abrasive laboratory mill (Satake Model TM-05C, Stockport, UK) equipped with a sieve of 1.4 mm. The pearling process was monitored according to time until a mass loss of around 50 % is obtained in order to assume a complete outer layers removal (pearling time *Sebastian*: 35 min, *Azurel*, *Esterel* and *Cervoise*: between 28 to 30 min). Purity of the starchy endosperm samples was checked with monitoring of the decrease content of biochemical markers (alkylresorcinols, phytic acid) specific to, respectively, the testa and the aleurone layer and the increase in starch content (Antoine et al., 2004; Hemery et al., 2009).

2.2. Production of green malt and enzyme activity measurements

Malt quality was evaluated on green malts obtained after a typical steeping diagram (8/14/6: 8 h steep, 14 h air-rest and 6 h steep at 15°C) followed by a 3 d germination phase at

15°C in a closed container with 100% relative humidity. Usual malting quality parameters such as fine extract, friability and viscosity were evaluated by IFBM (French Institute of Brewing and Malting) using standard methods from the American Society of Brewing Chemists (ASBC Methods of Analysis14th edition).

To complete the malting quality study, hydrolytic activities (α- and β-amylases, β-glucanase) and β-glucan content were measured in dry grains and green malt. β-glucanase, α- and β-amylase activities were measured using enzymatic assay kits purchased from Megazyme (MegaZyme International Ireland Ltd., Ireland). Each analysis was carried out at least in triplicate.

2.3. Biochemical analysis

Initial water content was determined on whole grain and endosperm samples after grinding (Cyclotec^{TM} 1093, FOSS) and drying the sample at 130°C for 2 hours (method 44-19, AACC, 2000). Each analysis was carried out at least in triplicate.

Major compounds in whole grain such as starch, proteins, β-glucans and arabinoxylans, were measured. Starch was measured according to AACC Method 76-13 (AACC, 2000) using a commercial assay kit purchased from Megazyme (MegaZyme International Ireland Ltd., Ireland). Protein content was determined using Kjehldal method (normalized method NF method V03-050, 1999 with N x 6.25). β-glucans were evaluated either in grains and in green malt using a commercial assay kit from Megazyme (AACC method 32-23, AACC, 2000), and total arabinoxylan content in grains was measured as already described (Greffeuille et al., 2006).

164

165 **2.4. Physical analysis**

166 The apparent whole kernel volume was estimated by toluene displacement using a 25 mL
167 pycnometer after addition of 75 barley grains (triplicate measurement) as previously
168 described in Roca et al. (2006). The apparent volume of one grain (v) was deduced from the
169 volume of 75 grains and the equivalent radius of the whole kernel (r_e) was then obtained
170 using the following equation:

171 $$r_e = \frac{1}{2}\left(\frac{6v}{\pi}\right)^{1/3}$$ **Equation 1**

172 The geometry of the obtained endosperm samples was closed to that of a sphere. The
173 equivalent radius was therefore calculated as the arithmetic average of length and breadth of
174 the endosperm sample measured using a digital caliper (= average value of 25 samples'
175 measurements).

176 *Particle Size Index.* Relative hardness of barley grains was estimated through
177 determination of the particle size index (PSI) according to the AACC method 55-30 (AACC,
178 2000) modified by Le Brun and Mahaut (1988). A known mass (m) of each sample, with
179 grain moisture comprised between 11 and 13 % was milled, and sieved on a 0.075 mm sieve
180 to measure mass of the particles passing through the sieve (m_1). PSI was then calculated using
181 the following equation:

182 $$PSI(\%) = \frac{100 \times (m - m_1)}{m}$$

183 **Equation 2**

184 Low PSI values corresponds to harder endosperm. Three PSI measurements were done on
185 each barley samples and mean value was used for statistical evaluation.

186 *Vitreousness.* Grain vitreousness was assessed by visual analysis of kernel cross-sections
187 (obtained with a Pohl kernel cutter, Versuchs und Lehranstalt für Brauerei, Berlin, Germany)
188 and expressed as a percentage of vitreous grains (modified method of the European legislation
189 n°824/2000). Each grain was ranked in one of the fifth class of vitreousness from 0-100 %.
190 The number of grains in each class was taken into account after being multiplied with a
191 coefficient factor depending on its class. Average value calculated on 100 kernels (two
192 batches of 50 kernels) was used as the vitreousness index.

193 *Porosity.* Density was measured using a gas pycnometer (Quantachrom Corp., Syosset,
194 NY). True density (D_1) was defined as the ratio of the grain sample weight to the true volume
195 of the sample, which is obtained after grinding of the sample with a laboratory mill
196 (Cyclotec^{TM} 1093, FOSS). The apparent density (D_2) of grain was determined from the grain
197 kernel weight and the apparent volume of the kernel, including all pore spaces inside the
198 kernel. Measurements (5 distinct assays) were carried out on 5g of whole kernel or 2g of
199 ground kernel. Porosity (P) was determined as previously described (Roca et al., 2006):

200 $$P = (1 - \frac{D_2}{D_1}) \times 100$$

201 **Equation 3**

202

203 **2.5. Water uptake during grain soaking**

204 Around five g of barley grains, precisely weighted with a digital balance, were put in a hand-
205 made basket tightly closed and soaked in a water bath at 22°C. The basket was removed from
206 the water bath at predetermined times, quickly wiped with filter paper to remove residual
207 water at the grain surface and reweighed (Becker, 1960; Verma and Prasad, 1999). The
208 increase in sample mass during soaking was considered to reflect the increase of water
209 uptake. Water absorption curves were thus determined until equilibrium was reached. All

soaking experiments were carried out in triplicate. This soaking experiment was also applied to the endosperm samples.

2.6. Water diffusivity at 22°C: mathematical model and simulations

The second Fick's law was used to model the internal moisture transfer within the sample assuming an homogeneous material.

In the case of a spherical geometry the second Fick's law can be written as:

$$\frac{\partial C}{\partial t} = D_{eff}\left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r}\right) \quad \textbf{Equation 4}$$

where r is the radial position in the sphere (m), C the water concentration (kg/m^3), D the diffusivity (m^2/s) and t the time (s). In the soaking experiment carried out in this study, the initial and boundary conditions are, respectively:

$$t = 0,\ 0 < r < r_{max},\ C = C_0 \quad \textbf{Equation 5}$$

$$t > 0,\ r = r_{max},\ C = C_\infty \quad \textbf{Equation 6}$$

where r_{max} is the equivalent radius, C_0 is the initial moisture content (kg/m^3) and C_∞ is the moisture content after infinite time.

Grain swelling after 50 h of soaking was evaluated by measuring the initial and final volume by toluene pycnometry. Grain volume was found to increase about 1.6 (*Esterel*) to 1.8 fold (*Sebastian* and *Cervoise*) and could be considered identical for the four selected samples. Therefore grain swelling was neglected in the mathematical modeling of the soaking curve and the moisture diffusivities identified was considered as apparent diffusivity values or effective diffusivities (D_{eff}).

231 *Numerical solution (finite element method).* In the finite element method, water content
232 was described by a discrete set of functions defined over a finite number of sub-domains or
233 element allowing to take into account a 2D ellipsoid geometry for the whole kernel. In this
234 case, only half of the kernel domain was discretized with triangular elements. The moisture
235 diffusivity value was assumed to be constant whatever the moisture content. Discretisation
236 and the solving of equations (4) to (6) were realized using COMSOL MULTIPHYSICS®. The
237 effect of mesh density on the average moisture content was first studied, placing different
238 nodes along the short and long axis of the ellipsoid. The minimal coarser mesh, which does
239 not change the quality of the prediction compared to a finer mesh was chosen in order to
240 minimize the computation time.

241 *Analytical solution*. In the case of a spherical geometry, and considering constant
242 diffusivity values, an analytical solution of Equation (4) with the initial and boundary
243 conditions (5) and (6) was used (Crank, 1975):

244 $$\frac{\left(C_t - C_0\right)}{\left(C_\infty - C_0\right)} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\left(\frac{1}{n^2}\exp\left(\frac{-D_{eff}n^2\pi^2 t}{r_{\max}{}^2}\right)\right) \qquad \text{Equation 7}$$

245 Where C_t is the total amount of water entering the sphere at time t.

246 Equation (7) was programmed using MATLAB software (The Mathworks Inc., Natick,
247 MA).

248 *Diffusivity identification.* Moisture diffusivity was identified for both whole kernels and
249 endosperms with the equivalent radius r_e as an input parameter in Equation (7) and by
250 minimizing the root mean squared error (RMSE) between the experimental and predicted
251 moisture content values using the Gauss-Newton algorithm (Gill et al., 1981). A dedicated
252 MATLAB function (nlinfit) was used for this identification. This value of RMSE was also
253 used to estimate the fitting model's quality.

$$RMSE = \sqrt{\frac{\left(C_{pred} - C_{\exp}\right)^2}{N - p}} \quad \text{Equation 8}$$

Where C_{pred} and C_{exp} were, respectively, the predicted and experimental moisture content, N was the number of moisture content measurements and p the number of estimated model parameters (p=1 in this study).

2.6. Statistical analysis

Statistical significance of the experimental results was assessed using a single-factor analysis of variance (ANOVA, routine function of MATLAB). Multiple comparisons were then performed by calculating the least significant difference. All tests were conducted at the 5 % significance level.

3-Results and discussion

3.1. Malting quality and enzyme activities in green malt

Four barley samples displaying contrasting malting quality in industrial conditions were selected: one from a spring barley (*Sebastian*) and three from winter barleys (*Azurel*, *Esterel* and *Cervoise*) as expressed in Table 1 by their values of fine extract, friability and viscosity. The sample from *Sebastian* was shown to display a higher friability (FI=92) in comparison with those from *Azurel* (FI= 86), *Esterel* (FI= 80) and *Cervoise* (FI= 67). The same classification of the four barley samples could be obtained on the basis of fine extract values or malt viscosity. Confirming these results of malt quality, brewers have widely recognized that *Sebastian* and *Azurel* (grown in 2007 in the North of France) had higher malting quality than *Esterel* and *Cervoise* (same year and location of growing).

Table 1

In order to analyse deeper the malting quality of these four barley samples, enzymatic activities involved in malting (β-glucanase and α-amylase) as well as in cell walls' degradation (revealed with β-glucans measurements) were evaluated in a standard steeping diagram (8 h steep, 14 h air-rest and 6 h steep followed by 3 d of germination at 15°C). Figure 1 illustrates results of β-glucanase activities and subsequent changes in β-glucan levels in green malts. *Sebastian* sample was found to display the highest β-glucanase activity while *Cervoise* grains displayed the lowest activity. This resulted in a lower residual amount of β-glucans in *Sebastian* (around 0.3 % dw) after 3 d of germination compared with *Cervoise* (around 1 % dw). It should be pointed out that residual amount of β-glucans was not related to the initial quantity found in the mature kernel. Indeed 12 % of the initial β-glucan amount was remaining in *Sebastian* grains after the end of the steeping diagram whereas 33% was observed to remain in *Cervoise* grains. It appears from these results that the malt quality, especially grain friability, could be more correlated to β-glucanase activity than to the initial content in β-glucans. In a previous study, lowest remaining levels of β-glucan were also observed in barley samples displaying greater production of β-glucanase throughout germination (Ellis et al., 1997).

Figure 1

Concomitantly α-amylase activity was also measured in green malts and also allowed to point out the highest level in *Sebastian* grains compared with *Azurel*, *Esterel* and *Cervoise* samples (Figure 1). α-amylase activity was insignificant in barley grains and reached activity

ranging between 100 to 300 units.g^{-1} in green malt after 3 d of germination as previously observed for other barley samples (Arends et al., 1995; Georg-Kraemer et al., 2001). By contrast, total β-amylase activity which is not determinant for malting quality was found to display more similar enzymatic activity levels between grains and green malt in each of the samples (Figure 1).

Differences in β-glucanase and α-amylase activities between barley grains and green malt could be related to (i) differences in enzyme production and/or specific activity or to (ii) differences in maximal water uptake and/or water diffusion within grain or endosperm in the first step of steeping (first 24h). Indeed, water uptake and diffusion was suggested to play a major role in hormones and enzyme migration required for cell walls degradation. This second hypothesis was mainly tested in this study.

3.2. Water uptake and diffusivity in whole kernels and corresponding endosperm

Soaking experiments were carried out at 22°C on grain and endosperm samples obtained after grain abrasion. Example of typical curves illustrating the increase in water content with, respectively, grains or corresponding endosperm are given in Figure 2 for *Sebastian* and *Cervoise* samples.

Figure 2

322 The maximal water uptake (or equilibrium water content) obtained for the four barley
323 samples studied are reported in Table 2 and Table 3 for whole grains and endosperm samples,
324 respectively. *Sebastian* retained significantly lower amount of water compared with the three
325 other grain samples. Maximal water uptake was also found lower in the endosperm samples
326 compared with the whole kernels (between 5 and 10% less) suggesting a significant
327 contribution of the outer layers and germ to the overall water uptake. Indeed maximal water
328 uptake carried out on hand-isolated germ or outer-layers from *Sebastian* grains were found
329 1.7 fold higher than values obtained with endosperm samples (data not shown).

330

331 **Table 2**

332

333 Experimental soaking data were adjusted with modelled curves using an analytical solution
334 based on the second Fick's law and considering a sphere geometry (Equation 7) in order to
335 determine water diffusivity. Volumes of the four barley grain samples were measured in order
336 to calculate equivalent radius (Table 2). Data obtained with *Sebastian* and *Cervoise* grain
337 samples were found significantly lower than those measured on *Azurel* and *Esterel* samples.
338 As they are also not completely spherical, equivalent radius of endosperm samples were
339 calculated as the average value between breadth and length (Table 3).

340

341 **Table 3**

342

343 A good fitting of the experimental data was obtained for all of the barley samples as
344 illustrated for *Sebastian* and *Cervoise* grain samples in Figure 2A and corresponding

345 endosperms in Figure 2B. The RMSE values calculated using Eq. 8 were for each sample
346 lower than 0.5 % (fresh weight basis) and were very closed to the experimental error (lying
347 between 0.1 and 0.5 % fw).

348 To validate our diffusion model, simulations were conducted for *Sebastian* samples using
349 either the analytical solution considering a sphere geometry or the numerical model which
350 takes into account a 2D ellipsoidal geometry. Figure 3 shows an example of the two simulated
351 curves obtained for the *Sebastian* sample (diffusivity value of 5.98×10^{-12} m^2/s). For whole
352 grains, differences between the two simulated curves was less than 6% on the entire range of
353 moisture content investigated and was thus considered negligible. This difference was in the
354 same range as already observed for moisture diffusivity in wheat by Gaston et al. (2002).
355 Therefore the simplified model based on Equation (7) could be used to predict the soaking
356 curves of barley grains and identify diffusivity values. For the endosperm samples
357 comparison between the numerical model and predictions from Equation (7) proved that the
358 endosperm could be definitively considered as a sphere for modeling purpose (no significant
359 differences observed).

360 **Figure 3**

361 Water diffusivity in grains varied between 5.28×10^{-12} m^2/s to 7.81×10^{-12} m^2/s depending on
362 the barley samples (Table 2). These water diffusivity values were found in the same order of
363 magnitude than those previously reported for barley by Tagawa et al. (2003): 3.5 to 39×10^{-12}
364 m^2/s as a function of the temperature ranging from 10 to 50°C. They were also in the same
365 range than those measured in wheat (4 to 25×10^{-12} m^2/s at 22°C) by Kang and Delwiche
366 (2000) or in maize (11×10^{-12} m^2/s at 30°c) by Verma and Prasad (1999).

367 Water diffusivities in the endosperm samples were found 5 to 7 fold higher, ranging
368 between 35.2×10^{-12} m^2/s and 49.5×10^{-12} m^2/s (Table 3). They were in accordance with those

obtained at the same temperature by Kang & Delwiche (2000) in wheat endosperm (39 to 109×10^{-12} m^2/s depending on the cultivar). Comparison between water diffusivities within grains or endosperm samples showed that *Sebastian* and *Azurel* displayed significantly lower values than those from *Esterel* and *Cervoise* samples.

The lower water diffusivity obtained for the whole kernel has been related to the singular barrier property of the peripheral layer. Similarly, Klamczynski et al. (1998) also pointed out a quicker water uptake after removal of the outer layers from barley grains. Indeed, Hinton (1955) previously reported that the seed coat (testa) may act as a barrier to water flux. The testa contained in the peripheral layer of barley grain may radically contribute to slow down the water flow entering the whole kernel, yielding to a lower apparent diffusivity within whole kernel than within the endosperm. The barrier role of the peripheral layers was also confirmed by Rathjen et al. (2009) using Resonance Micro-Imaging study of wheat grains.

3.3. Biochemical composition and water uptake / diffusivity

Analysis of the biochemical composition of the four barley grain samples was done in order to test its relationship with maximal water uptake and water diffusivity. As shown in Table 1, the barley samples displayed significantly distinct values of protein and starch content. As an example, the *Sebastian* sample shows the lowest protein content and the highest starch content however it was also found to display the lowest maximal water uptake (Table 2). This appears in contradiction with what it is generally stated, *i.e.* a low protein content was usually related with an increase in water uptake through lower grain hardness or vitreousness (Bathgate et al., 1978; Chandra et al., 1999; Molina-Cano et al., 2002). However, others did not find any correlation between protein content and water uptake (Gamlath et al., 2008; Leach et al., 2002; Swanston et al., 2006).

393 The arabinoxylan content of the four samples studied did not differ significantly in contrast
394 with β-D-glucan. But, β-D-glucan content did not appear correlated to the maximal water
395 uptake neither to the water diffusivity, contrary to Gamlath et al. (2008) who pointed out a
396 negative correlation between arabinoxylan and β-D-glucan initial quantity and maximal water
397 uptake. However, correlation coefficients described by Gamlath et al. (2008) were low (R^2
398 ranging between 0.25 and 0.54).

399 The variability of the four samples under study probably did not differ enough to observe
400 clear trends between biochemical composition and water uptake or diffusivity.

401

402 **3.4. Structure and water uptake / diffusivity**

403 Physical characteristics of the four barley samples were determined and reported in Table 4. It
404 is commonly accepted that the particle size index (PSI) measurement could be assimilated to a
405 measure of hardness (Psota et al., 2007; Williams and Sobering, 1986), lower PSI values
406 corresponding to harder endosperm. Grain sample from *Sebastian* cultivar which displayed
407 the harder grain structure (lowest PSI value) was also found the most vitreous, followed by
408 *Azurel*, *Esterel* and then, *Cervoise* sample, which appeared the mealiest. Density of the grain
409 sample from the spring cultivar, *Sebastian*, was also found significantly higher (Table 4) in
410 accordance with vitreousness measurements. Our data revealed a negative linear correlation
411 between PSI and vitreousness measurements (R^2= 0.99), i.e. a positive linear relationship
412 between hardness and vitreousness. This relation between hardness and vitreousness was
413 never reported even for other cereals, but vitreousness and hardness characteristics are
414 generally mixed in the literature. Indeed recently Walker et al. (2011) pointed out a
415 relationship between grain density and endosperm hardness. Differences in grain vitreousness
416 could be related to changes in the grain structure as revealed by porosity measurements. As

417 expected, the hardest and the most vitreous grains displayed the lowest porosity (*Sebastian*
418 and *Azurel* cultivars) whereas grains from *Cervoise* displayed a 2 fold higher porosity. Barley
419 porosities measurements (between 5.3 to 11.5%) appeared to be close to those obtained with
420 soft (between 5.3 to 7.0 %) or hard (between 3.6 and 5.0 %) wheat grains or sorghum (8.9 to
421 10.6 %) but lower than those obtained from maize grains (11.7 to 13.3 %), which displayed a
422 more porous structure (Chang, 1988).

423

424 **Table 4**

425

426 High water diffusivity values have already been assigned to an increase of the overall
427 porosity as demonstrated in various porous cereal-based products such as pasta (Waananen
428 and Okos, 1996; Xiong et al., 1991), cakes (Baik and Marcotte, 2003) and sponge cakes
429 (Roca et al., 2008; Roca et al., 2006; Guillard et al., 2003). However it has never been pointed
430 out in grains except by Glenn and Johnson (1994) who pointed out the effect of vitreousness
431 on water diffusivity in wheat endosperm. For the four studied barley samples, water
432 diffusivities in the whole kernel, as well as in the endosperm, appeared to be well correlated
433 with grain porosity (R^2= 0.65 and 0.85, respectively), a lower porosity yielding to a lower
434 water diffusivity value. As expected, moisture diffusivity was the lowest in the hardest and
435 the most vitreous kernel in accordance to Chandra et al. (1999), who noticed a slower
436 penetration of water into steely grains (qualitatively established with a colorimetric method).

437 Our results appear in contradiction with previous studies concluding that mealy endosperm
438 facilitates moisture and thus enzyme movements within the endosperm contributing to higher
439 degradation of cell walls during malting (Swanston et al., 1995; Schildbach and Rath, 1994;
440 Gamlath et al., 2008; Chandra et al., 1999). Indeed, the analyzed barley samples displaying

441 the highest β-glucanase and alpha-amylase activities (*Sebastian* and *Azurel*) were also the less
442 porous and showed the lowest moisture diffusivity values. Therefore an efficient diffusion
443 and distribution of water is surely an important factor for malting quality but does not appear
444 as a limiting factor.

445

Conclusion

Studies of water diffusivity in four distinct barley samples have allowed to point out a 5-7 fold lower water diffusion within grains compared with their corresponding starchy endosperm, confirming the important role of the outer layers as a water barrier. A significant relationship was also found between water diffusivity within the endosperm and structural parameters (i.e. porosity, vitreousness, hardness). These results should, of course, be confirmed on more samples, considering that the values of some components (i.e. proteins) are quite similar in this study. Higher hydrolytic activities of enzymes involved in malting (β-glucanase and α-amylase) were not correlated with maximal water uptake or water diffusivity. Therefore, water diffusivity values within grain or the endosperm do not appear as the limiting factor for enzyme activities involved in malting. Indeed, further studies on enzyme activation will be necessary to pursue in order to better understand the parameters involved in the expression of malt quality.

Moreover, from a practical aspect, it is interesting to point out that the developed soaking assays could be used to optimise the time required to obtain optimal grain hydration in the first step of the steeping diagram. For instance, one could pointed out that it would take less than 10 h for *Cervoise* grains to reach 40 % of water content whereas more than 15 h was required for grains of the *Sebastian* sample. The steeping diagram could thus be adjusted by the brewers according to the experimental soaking data of the analysed sample in order to determine the minimum required steeping time.

Acknowledgements

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Table 1: Biochemical characteristics of whole grain and quality parameters of the corresponding green malt from the four barley samples

	Grain				Green malt		
	Proteins *(% dry weight basis)*	**Starch** *(% dry weight basis)*	**Beta-glucans** *(% dry weight basis)*	**Arabinoxylans** *(% dry weight basis)*	**Fine extract♮** *(% dry weight basis)*	**Friability♮** *(%)*	**Viscosity♮** *(mPa.s)*
Sebastian	10.31 (0.01) [a]	64.76 (0.43) [d]	2.47 (0.06) [b]	6.94 (0.17) [a]	83.5 (0.5) [c]	92.0 (3.0) [c]	1.46 (0.05) [a]
Azurel	11.20 (0.03) [b]	59.60 (0.01) [b]	2.08 (0.01) [a]	7.74 (0.09) [b]	82.1 (0.5) [b]	86.5 (3.0) [c]	1.55 (0.05) [a]
Esterel	11.42 (0.05) [c]	62.80 (0.32) [c]	2.64 (0.02) [c]	7.51 (0.56) [a, b]	79.5 (0.5) [a]	80.0 (3.0) [b]	1.68 (0.05) [b]
Cervoise	10.91 (0.18) [d]	57.40 (0.51) [a]	3.00 (0.02) [d]	7.47 (0.64) [a, b]	79.6 (0.5) [a]	67.3 (3.0) [a]	1.70 (0.05) [b]

♮ measured after a standard steeping diagram (8 h steep, 14 h air-rest and 6 h steep followed by 3 d of germination at 15°C) – values between brackets represent uncertainty of the method.
Content of the main grain compounds was expressed as means of at least 3 measurements ± standard deviation
[a, b, c, …] Values with same letters are not significantly different (ANOVA test + Fisher's Least Significant Difference)

Table 2: Dimensional and volume characteristics, initial and maximal water uptake and effective water diffusivity (D_{eff}) at 22°C of the four barley samples (whole grains)

	Volume (v) *(mm^3)*	**Equivalent radius (r_e)** *(mm)*	**Initial water content** *(g/100 g fw)*	**Maximal water uptake*** *(g/100 g fw)*	***D_{eff}*** *(10^{-12} m^2/s)*
Sebastian	32.3 (0.5) [a]	1.97 (0.010) [a]	10.3 (0.1) [a]	45.9 (0.1) [a]	5.98 (0.10) [a]
Azurel	35.4 (0.3) [b]	2.03 (0.006) [b]	11.9 (0.2) [b]	47.1 (0.2) [b]	5.28 (0.09) [a]
Esterel	33.4 (0.4) [c]	2.00 (0.008) [c]	11.6 (0.4) [b]	47.1 (0.5) [b]	7.81 (1.08) [b]
Cervoise	32.4 (0.2) [a]	1.98 (0.004) [a]	11.7 (0.3) [b]	48.3 (0.4) [b]	7.61 (0.26) [b]

* after 50 h of soaking

Means of at least 3 measurements ± standard deviation

[a, b, c, ...] Values with same letters are not significantly different (ANOVA test + Fisher's Least Significant Difference)

Table 3: Equivalent radius, water content after 20 h of soaking at 22°C and effective water diffusivity (D_{eff}) of the four barley endosperm samples

	Equivalent radius (r_e) *(mm)*	**Maximal water content** *(g/100 g fw)*	***D_{eff}*** *(10^{-12} m^2/s)*
Sebastian	1.62 (0.11) [a]	41.80 (0.36) [a]	35.2 (0.25) [a]
Azurel	1.72 (0.10) [a]	44.01 (0.48) [b]	36.8 (0.39) [a]
Esterel	1.78 (0.12) [a]	45.01 (0.35) [b]	38.0 (0.12) [a]
Cervoise	1.84 (0.10) [a]	45.16 (0.19) [b]	49.5 (0.35) [b]

Means of at least 3 measurements ± standard deviation

[a, b, c, …] Values with same letters are not significantly different (ANOVA test + Fisher's Least Significant Difference)

Table 4: Particle size index (PSI), vitreousness, apparent density (D_2) and porosity of the four barley samples

	PSI	Vitreousness	Apparent density	Porosity
	(%)	*(%)*	*(kg/m^3)*	*(%)*
Sebastian	13.06 (0.78) [a]	64.01 (2.14) [d]	1446 (3) [d]	5.32 (0.21) [a]
Azurel	16.81 (0.97) [b]	49.01 (1.41) [c]	1428 (2) [c]	6.48 (0.45) [b]
Esterel	18.81 (0.64) [b, c]	39.00 (4.95) [a, b]	1408 (4) [b]	8.84 (0.58) [c]
Cervoise	20.38 (1.22) [c]	32.25 (3.18) [a]	1388 (6) [a]	11.54 (0.45) [d]

Means of at least 3 measurements ± standard deviation

[a, b, c, …] Values with same letters are not significantly different (ANOVA test + Fisher's Least Significant Difference)

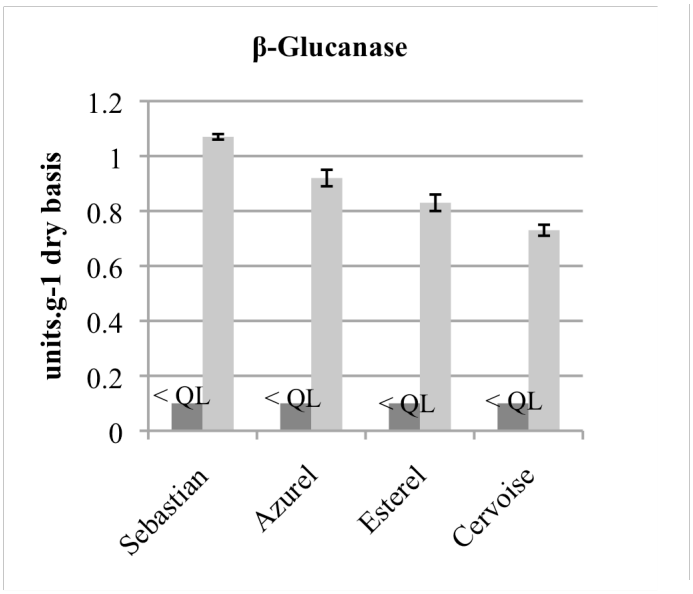
Figures captions

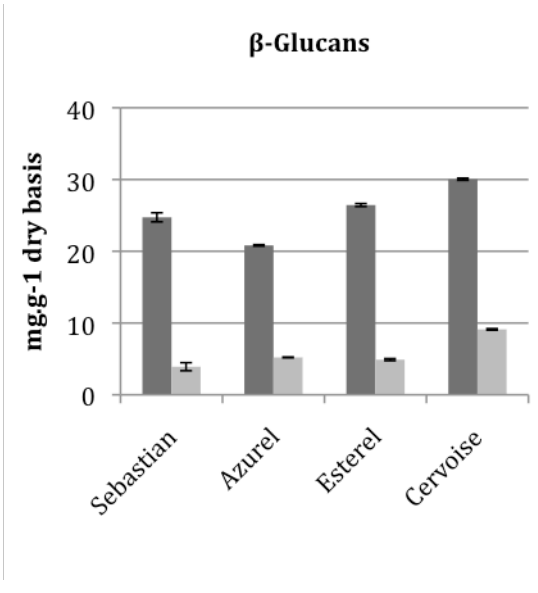
Figure 1: Evolution of total β-glucanase, α-amylase and β-amylase activities compared to those of the β-glucan content measured in non-malted grains (first column) and in grains after malting (second column) for the four barley samples under study (means of 3 measurements ± standard deviation ; < QL means lower than the quantification level)

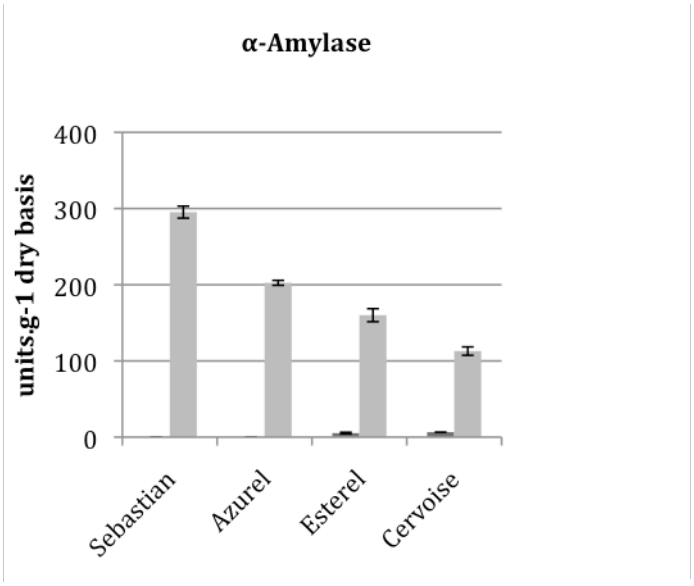
Figure 2: Experimental values (symbols) and model predicted (solid line; calculated with Equation 8) soaking curve at 22°C for *Sebastian* (□) and *Cervoise* (○) barley samples (A- whole grains and B- corresponding endosperm).

Figure 3: Example of simulated soaking curves obtained for Sebastian sample by using the analytical model (Eq. 7) (black curve) and the numerical model for a 2D ellipsoidal geometry (grey curve)

Figure 1


β-Glucanase
units.g-1 dry basis
1.2
1
0.8
0.6
0.4
0.2
0
< QL
< QL
< QL
< QL
Sebastian
Azurel
Esterel
Cervoise


β-Glucans
mg.g-1 dry basis
40
30
20
10
0
Sebastian
Azurel
Esterel
Cervoise


α-Amylase
units.g-1 dry basis
400
300
200
100
0
Sebastian
Azurel
Esterel
Cervoise

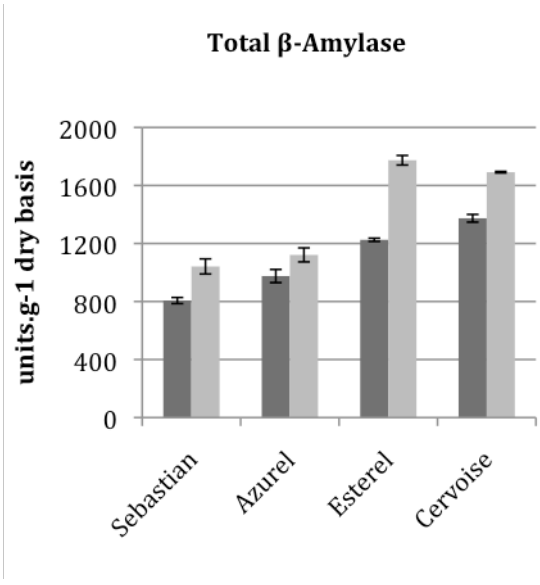
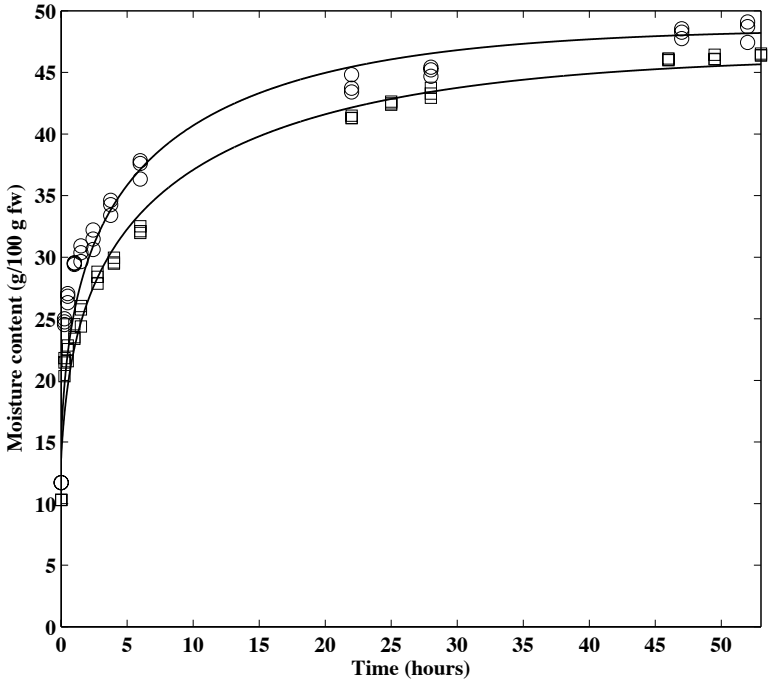
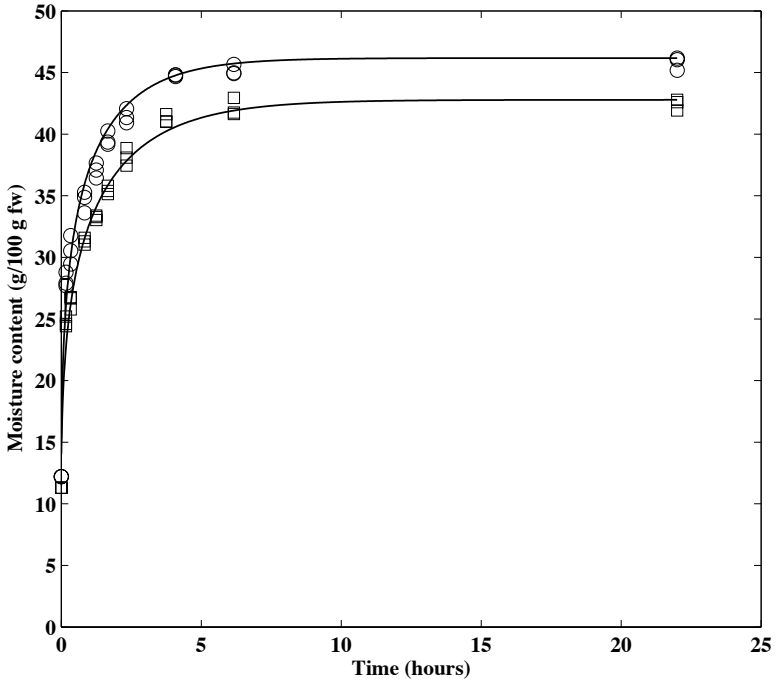

Total β-Amylase
units.g-1 dry basis
2000
1600
1200
800
400
0
Sebastian
Azurel
Esterel
Cervoise

Figure 2

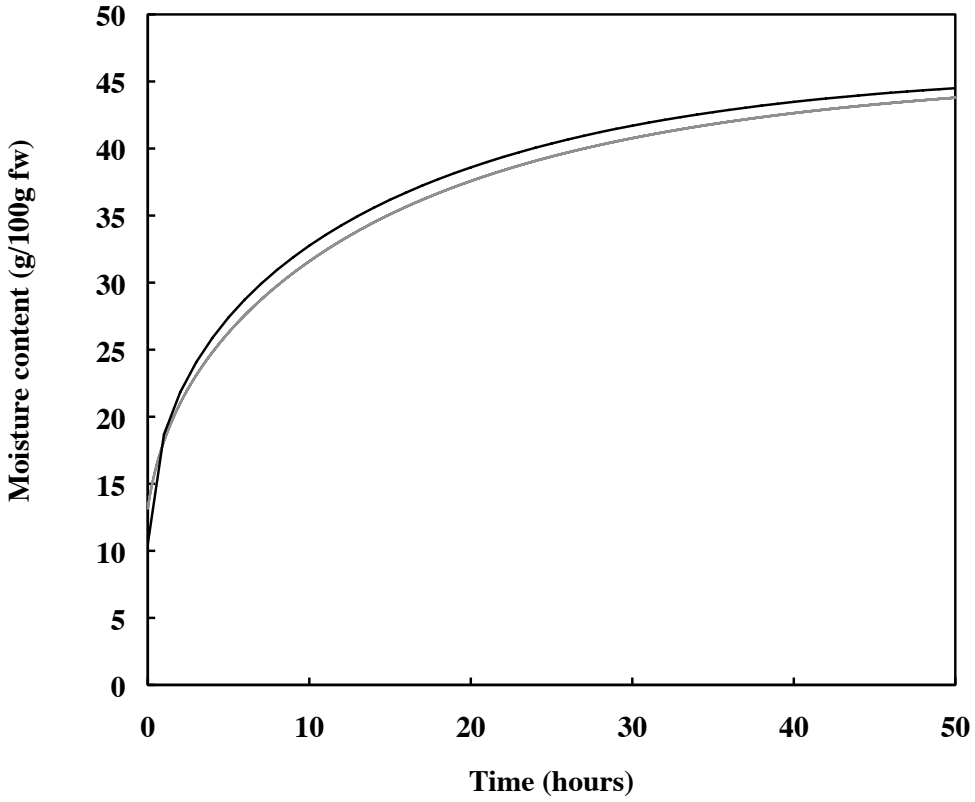

Moisture content (g/100 g fw)
Time (hours)
0
5
10
15
20
25
30
35
40
45
50

- A -


Moisture content (g/100 g fw)
Time (hours)
0
5
10
15
20
25
30
35
40
45
50

- B -

Figure 3


Moisture content (g/100g fw)
50
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
Time (hours)