

to biotic and abiotic stress. In contrast with the plant model for dicots and monocots, *i.e.*, *Arabidopsis thaliana* and *Oryza sativa*, respectively, the lack of complete genomic databanks for barley limits the efficiency of proteomics for this application. However, preliminary maps of soluble barley seed proteins, *i.e.*, albumins and globulins, have been obtained either on mature [5, 6] or developing and germinating grains [7–9]. Recently, more specific protein maps have been obtained on the major compartments of the seed, *i.e.*, starchy endosperm, aleurone layer, and embryo [10]. Most of these studies were performed using 2-DE in the pH range of 4–7. A recent study reported the proteomics analysis of barley seed proteins in the basic pH range of 6–11, with an emphasis on the modification of protein pattern from barley to malt [11]. In all these studies, the analyses were concerned with total proteins and the data cannot be used in the context of malting and brewing end-uses of barley. Therefore, in order to highlight all the proteins likely to be involved in the texture of head foams, we have focused proteomics analysis on the heat-stable water-soluble proteins from barley seed and corresponding malt in relation to the protein composition of beer. To our knowledge, this is the first time where a proteomics approach is performed with the objective of identifying proteins involved in a technological process.

2 Materials and methods

2.1 Protein extraction

Barley and malt (*Hordeum vulgare* cultivar Esterel, France) were milled in liquid nitrogen and 1 g of flour was extracted in 10 mL of deionized water for 2 h at room temperature. Extracts were boiled at 100°C for 30 min, filtered and then freeze-dried. Beers were provided by the Institut Français des Boissons de la Brasserie Malterie (IFBM). They were degassed and freeze-dried.

2.2 2-DE

Freeze-dried protein extracts were dissolved in 8 M urea, 2% w/v CHAPS, 2% v/v IPG buffer pH 3–10 (Amersham Biosciences, Uppsala, Sweden), 0.01% bromophenol blue, 20 mM DTT, and were centrifuged at 12 000 rpm for 10 min. IEF was performed using a Pharmacia Biotech IPGphor electrophoresis system (Amersham Biosciences). Prior to second dimension, the IPG strips were equilibrated for 10 min in equilibration buffer (50 mM Tris/HCl pH 8.8, 6 M urea, 30% v/v glycerol, 2% w/v SDS, 0.01% bromophenol blue) containing 10 mg/mL DTT, followed by 10 min in equilibration buffer containing 25 mg/mL iodoacetamide. The second dimension was run on 15% acrylamide gels on a vertical system. Gels were stained with silver nitrate [12] or colloidal CBB [13], or transferred on nitrocellulose membrane for immunoblotting with anti-LTP1 antibodies.

2.3 Enzymatic digestion of proteins in-gel

Gel spots were washed with 100 µL of 25 mM NH_4HCO_3 , followed by dehydration with 100 µL of 50% ACN in 25 mM NH_4HCO_3 . Proteins were reduced by incubation with 10 mM DTT (1 h, 57°C), and alkylated with 100 µL of 55 mM iodoacetamide (45 min at room temperature). Gel spots were further washed with NH_4HCO_3 , dehydrated as described above and then dried in a vacuum centrifuge. The proteins were digested overnight at 37°C by the addition of 10 to 20 µL of trypsin (12.5 ng/µL in 25 mM NH_4HCO_3 ; modified trypsin purchased from Promega, Madison, WI, USA). The resulting peptides were extracted by the addition of 50 mL of 70% ACN/20% H_2O /10% formic acid. Supernatant was collected and dried in a vacuum centrifuge. For LC-MS/MS analysis, peptides were resolubilized in 10 µL of solvent A (2% ACN/0.1% formic acid).

2.4 Mass spectrometric analysis

Nanoscale capillary liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses of the digested proteins were performed using a Famos-Switchos-Ultimate capillary LC system (LC-Packings, Amsterdam, Netherlands) coupled to a hybrid quadrupole orthogonal acceleration TOF mass spectrometer (Q-TOF Global; Waters/Micromass, Manchester, UK). Chromatographic separations were conducted on a reverse-phase capillary column (Pepmap C18, 75 µm id, 15 cm length; LC-Packings) at a flow rate of 200 nL/min. The gradient consisted of a linear increase from 0 to 50% of solvent B (80% ACN/0.08% formic acid) in 60 min, followed by a rapid increase up to 100% of B within 15 min. Mass data acquisitions were piloted by the MassLynx software (Waters) using the so-called “survey” scanning mode: MS data are recorded for 1 s, after which the three most intense ions are selected and fragmented in the collision cell. MS/MS data are recorded for each of these three ions during 8 s. The collision energy profiles were optimized for various mass ranges of precursor ions.

2.5 Protein identification

Mass data collected during a LC-MS/MS analysis were processed with Protein Lynx Global Server software (Waters). Protein identification was performed by searching the peptide masses and MS/MS sequence stretches against the Swiss-Prot and NCBI non-redundant sequence databank, or against The Institute of Genomic Research (TIGR) expressed sequence tag (EST) databank.

2.6 Western blotting

Proteins separated by 2-D gel were blotted on to nitrocellulose membrane. Immunodetection was carried out using a primary antibody raised in rabbit against barley LTP1 prepared in our laboratory. Goat anti-rabbit horseradish perox-

ydase-conjugated antibody was used as the secondary antibody, and the signal was detected by chemiluminescence with 4-chloro-1-naphthol.

3 Results and discussion

To obtain a detailed characterization of the protein profiles of barley, malt and beer, the high-resolution 2-DE was performed with immobilized pH 3–10 gradients in the first dimension, followed by silver, colloidal CBB staining or Western blotting. The observed changes in the protein patterns displayed in the silver-stained gels can be related to the events occurring in malting and brewing.

3.1 Comparison between total water-soluble proteins and heat-stable water-soluble proteins from barley, malt and beer

Figure 1 shows the 2-D gels of water-soluble proteins of barley, malt and beer. As expected, clear differences could not be extracted from the 2-D maps. Indeed, the high intensity of protein spots in the range of 40–60 kDa on the barley gel (Fig. 1A) prevented comparison with the corresponding protein spots in beer. Moreover, the malt gel displayed a much higher number of spots in the pI 3–6 range (Fig. 1B), while the profile of beer proteins was more simple (Fig. 1C). Considering the heat-stable fraction of water-soluble proteins, a simplified pattern was obtained (Fig. 2) that made the comparison of barley and malt proteins easier. Furthermore, this pattern was more representative of the proteins found in beer after malting and brewing processes. In Fig. 2 we observed a significant decrease in the number of protein spots in the range of 20–35 and 40–70 kDa for barley (Figs. 1A and 2A), and in the pI range of 4–6 for malt (Figs. 1B and 2B).

In the case of heat-stable proteins, the transition from barley to beer was accompanied by changes in the protein patterns of 2-DE, especially in the range of 9–20 and 40–

55 kDa. However, it is worthy to note that the protein profiles of malt and beer are quite similar (Fig. 2B and C). Therefore the malt heat-stable proteins are good indicators of the proteins recovered in beer. Finally, approximately 150 well-defined spots could be detected on each gel in the pI range of 3–10 using the 2-D gel analysis software Melanie (Amersham Biosciences).

3.2 Protein identification by MS

Identification of proteins from barley, malt and beer 2-D gels using MS relies on sequence information in the databanks. The Swiss-Prot databank (release 43.5, June 7, 2004: 153 017 entries) contains only 300 sequences from barley, and the TrEMBL databank (release 26.5, June 7, 2004: 1 062 416 entries) 1178 sequences. The Institute of Genomic Research (TIGR) expressed sequence tag (EST) databank from barley (release 8, January 9, 2004) was also used to identify proteins. Because of this limited number of protein sequences for barley in the databanks, protein identification was not always successful, even if good spectra were obtained.

Using the combination of nanoLC-MS/MS and databank searching, we could identify a certain number of proteins. Some were identified based on the correlation of the MS/MS data for multiple peptides to the protein sequence. These matches were considered to be valid. The databank searches also retrieved proteins for which a single peptide matched. To confirm or rule out the identification, these single peptide matches were examined manually according to the score, the quality of the MS/MS spectrum, and the continuous stretch of the protein sequence covered by either the y- or b-ion series. Yet, for a number of spots (circled in white on Fig. 2), mass data did not allow the identification of the protein, even by using *de novo* sequencing. This suggests that complete sequence data for these proteins are missing in the databanks.

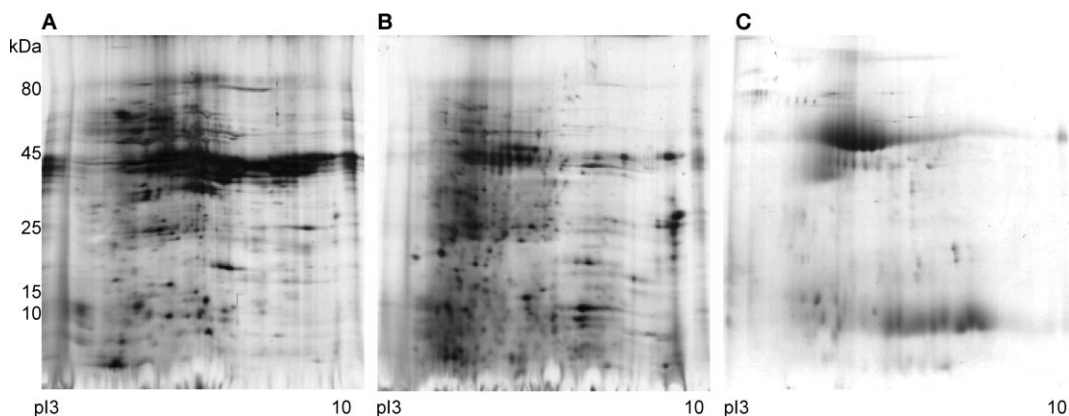


Figure 1. 2-D gel protein patterns of water-soluble proteins of barley (A), malt (B) and beer (C). Proteins were separated in the first dimension on a IPG strip pI 3–10 and in the second dimension on a 15% acrylamide SDS gel. The proteins were silver stained.

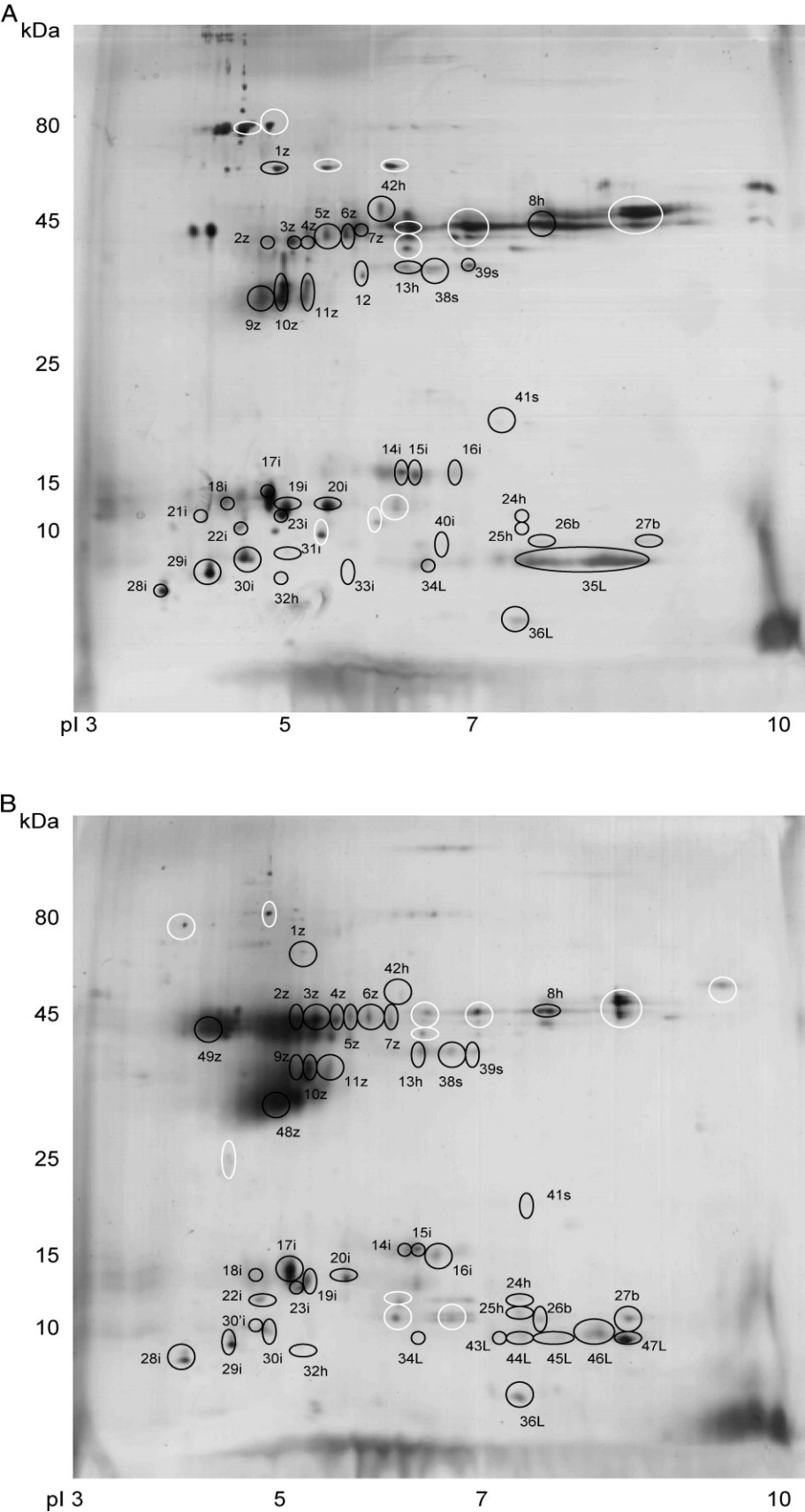


Figure 2.

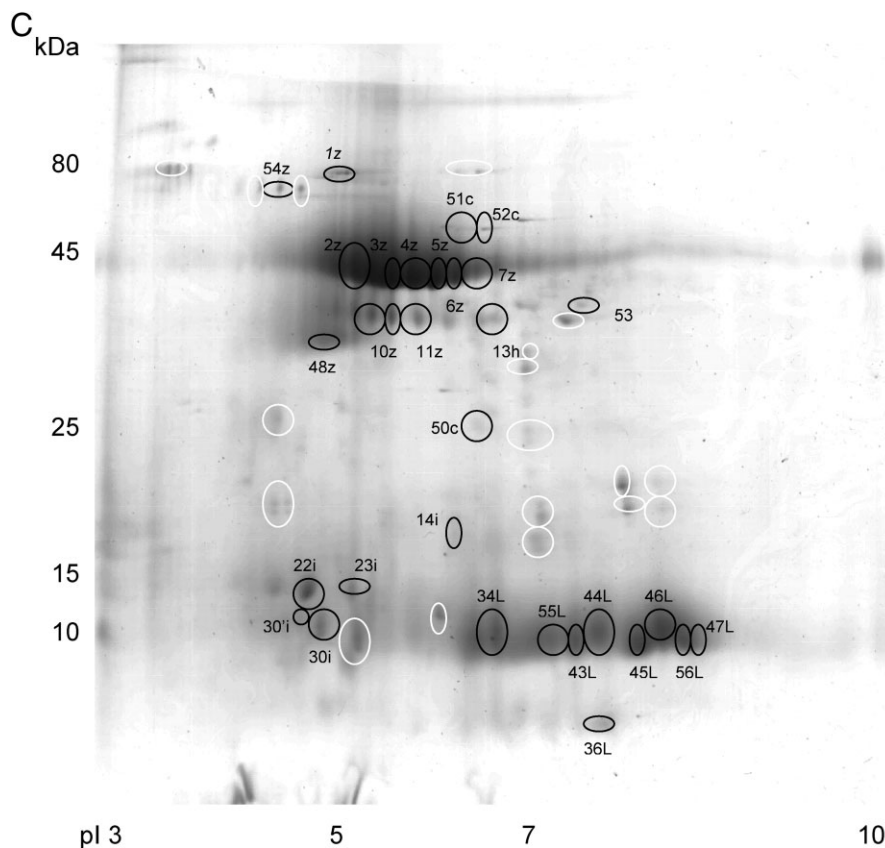


Figure 2. 2-D gel protein patterns of water-soluble heat-stable proteins of barley (A), malt (B) and beer (C). Proteins were separated in the first dimension on a IPG strip pI 3–10 and in the second dimension on a 15% acrylamide SDS gel. The proteins were silver stained. Identified proteins are listed in Table 1. The spots circled in white were submitted to MS analysis but could not identified.

In the current work, identification of proteins in major spots from 2-D gels concerns 40 proteins from barley gel, 41 proteins from malt gel, and 30 proteins from beer gel (Table 1, Fig. 2). Enzyme inhibitors constituted the largest group of identified proteins from barley, malt and beer gels, including various α -amylase/trypsin inhibitors, a subtilisin-chymotrypsin inhibitor, and protein Z. Other significantly represented proteins include non-specific lipid transfer proteins (LTP1 and LTP2), hordeins, barwin (barley wound induced protein), a fragment of cold-regulated protein 1, and proteins from *Saccharomyces cerevisiae* (enolase 2 and triose phosphate isomerase) (Table 1). Moreover, as observed for LTP1 and protein Z, two foaming proteins, a protein can be found in several spots.

3.3 Protein Z spots

Barley and especially malt and beer gels displayed a remarkably high number of abundant proteins in the pI range 5–6 and molecular mass range 40–50 kDa. These spots were identified as protein Z (Table 1, Fig. 2). Protein Z belongs to the serpin family and can be subdivided into protein Z4 and protein Z7, which are expressed from two separate (although highly related) small gene families [16]. The biochemical role of plant serpins remains unclear, with no target protease yet identified in plants. Serpins could provide protection against

insect predators [17]. These serpins, which are major components of the albumins of barley endosperm, could also have a role as storage proteins in plants, contributing for a substantial part to the lysine content of the grain due to their high content in this cationic amino acid [7]. In barley and malt, protein Z4 is the dominant form, accounting for approximately 80% of all protein Z [2]. As such, it is assumed that most of the investigations conducted so far on protein Z are actually referring primarily to protein Z4.

Protein Z is composed of several proteins with an acidic pI that is glycosylated during the malting process through Maillard reactions [16, 18]. It has also been reported that about 16% of the lysine content of protein Z is glycosylated during the brewing process through Maillard reactions [18]. Partial modification of lysine residues with sugars may explain the migration of protein Z as a diffuse zone. It is one of the few barley proteins recognized in beer, and contributes to foam stability and/or haze formation [16, 19]. The latter study showed that glycosylated protein Z has improved foam stability [19].

When the malt gel was stained with silver, two dark stained areas were first resolved into well-defined spots. However the resolution was lost while continuing the staining for the visualization of other proteins (Fig. 2B). Similar intense dark areas were observed for the 2-D gel from beer (Fig. 2C), and it is likely that the proteins in these spots have

Table 1. Barley, malt and beer proteins identified by LC-MS/MS from the 2-D gels (Fig. 2). The accession number refers to Swiss-Prot or NCBI database. Molecular mass (M_r) and isoelectric point (pI) values listed in the table were calculated using ExPASy tools (<http://www.expasy.ch>).

Protein name	Accession number	M_r (kDa)	pI	Spot number
Protein Z				
Protein Z-type serpin	CAA66232.1	43.19	5.85	Barley 1z, 2z, 3z, 4z, 5z, 6z, 7z, 9z, 10z, 11z Malt 1z, 2z, 3z, 4z, 5z, 6z, 7z, 9z, 10z, 11z, 48z, 49z Beer 1z, 2z, 3z, 4z, 5z, 6z, 7z, 9z, 10z, 11z, 48z
Protein Z4	P06293	43.27	5.72	Beer 54z
Lipid transfer protein				
LTP1	P07597	9.69	8.19	Barley 34L, 35L Malt 34L, 43L, 44L, 45L, 46L, 47L Beer 34L, 43L, 44L, 45L, 46L, 47L, 55L, 56L
LTP2	P20145	6.98	6.98	Barley 36L Malt 36L Beer 36L
Trypsin/α-amylase inhibitor				
CMa	P28041	13.11	5.51	Barley 18i, 19i, 20i Malt 20i
CMb	P32936	14.19	5.78	Barley 17i, 18i, 19i, 20i, 22i, 23i Malt 19i, 23i Beer 23i
CMd	CAA31585.1	16.1	5.24	Barley 17i Malt 17i
CMd3	AAB63440	18.47	8.01	Barley 17i, 23i Malt 17i, 19i
CMe	CAA35188.1	13.25	6.95	Barley 16i Malt 16i
BTI-CMe2.1	CAA11026.1	13.71	6.31	Barley 14i, 15i, 17i Malt 14i, 15i Beer 14i
BMAI-1	P16968	14.44	6.16	Barley 17i, 18i, 19i, 23i, 31i Malt 18i, 19i
BDAI-1	P13691	13.10	5.06	Barley 29i, 30i, 31i Malt 29i, 30i, 30'i Beer 30i, 30'i
BTICMc	Q9LEI6	15.17	6.68	Barley 40i
pUP13	1208404A	14.7	5.44	Barley 21i, 22i Malt 22i Beer 22i
PUP38	1208404B	12.02	4.94	Barley 33i
Hordeins				
Hordein B3	P06471	30.19	7.74	Barley 8h, 42h Malt 8h

Table 1. Continued

Protein name	Accession number	M_r (kDa)	pI	Spot number
Hordein γ 3	T05718	32.84	6.60	Barley 13h Malt 13h Beer 13h
Hordein B3 (fragment)	P06471	30.19	7.74	Barley 24h, 25h Malt 24h, 25h
Hordein D (fragment)	CAA59104.1	50.78	7.60	Barley 32h Malt 32h
Proteins similar to proteins of other organisms				
12S seed storage protein (<i>Arabidopsis thaliana</i>)	NP_849464.1	43.72	6.08	Barley 38s, 39s Malt 38s, 39s
12S seed storage protein (<i>Arabidopsis thaliana</i>)	NP_171884.1	50.55	6.53	Barley 41s Malt 41s
P0529E05.3 (<i>Oryza sativa</i>)	BAB84388	55.70	10.47	Beer 53
Proteins from <i>Saccharomyces cerevisiae</i>				
Enolase 2	P00925	46.63	6.04	Beer 51c, 52c
Triosephosphate isomerase	P00942	26.79	5.74	Beer 50c
Other proteins				
Barwin	P28814	13.72	7.3	Barley 26b, 27b Malt 26b, 27b
Subtilisin-chymotrypsin inhibitor CI-1A	P16062	8.88	5.24	Barley 28i Malt 28i
Cold regulated protein 1 (fragment)	A45512	72.25	6.8	Barley 12

the same origin. Other studies observed a similar dark area on the 2-D gel separating the proteins from malt [8]. Another characteristic feature of the 2-D gel from beer is a series of well-defined spots at slightly higher pI and molecular mass than the dark areas. Protein Z was a prominent protein identified in several spots from these areas: 10 resolved spots for barley, 12 spots for malt, and 11 spots for beer (Table 1, Fig. 2).

3.4 Lipid transfer protein spots

Another prominent protein in barley and especially in malt and beer is lipid transfer protein 1 (LTP1). The *in vivo* function of barley LTP1 remains unknown. Initially, barley LTP1 was thought to be an inhibitor of α -amylase or proteases and was named PAPI (probable α -amylase/protease inhibitor) [20]. This inhibitory activity has been a matter of controversy [21]. Today, many data have been obtained for different LTPs that suggest a role of these proteins in the defense of plants against their microbial pathogens and in the formation of hydrophobic cuticle layer covering plant organs [22, 23]. In

brewing, barley LTP1 concentrates in beer foam. It contributes to foam formation while protein Z confers foam stability [16]. In fact, the native barley seed LTP1 displays poor foaming properties and becomes a foam promoting agent only after unfolding on wort boiling [24–27]. LTP1 is glycosylated due to Maillard reactions that occur on malting [24] and an acylated form is also observed in aqueous barley and malt extracts [28, 29]. All these modifications could improve the surface activity of LTP1.

In 2-DE of boiled aqueous barley extracts in the basic pH range of 8–9, LTP1 was mainly identified as two spots (Fig. 2A: spot 35). In malt and beer, LTP1 was detected as several spots scattered over a large pI range, *i.e.*, from 6 to 9 (Fig. 2). Immunoblots using polyclonal antibodies raised against LTP1 confirmed these identifications (data not shown). However, as for protein Z, we could not detect by MS any clear modification of LTP1 that could explain the observed scattered pattern. In barley, one of the two major LTP1 spots was more acidic than the other; it could correspond to LTP1 with a covalently attached oxygenated fatty acid adduct [28, 29]. However, this modification could not be

highlighted in the present work because trypsin hydrolysis cleaves the ester bond between the acyl adduct and the protein (Marion D., unpublished data). Concerning the most acidic spots of LTP1 observed in malt and beer, they might originate from acylated and/or glycosylated products. Actually, glycation through Maillard reactions modifies the amine group of lysine side chain and of the *N*-terminal amino acid, and produces a decrease of the protein pI. It was estimated that LTP1 was glycosylated with glucose to an extent of about 5% probably through Maillard reactions [25]. The attempt to detect glycation for LTP1 on 2-DE transferred on nitrocellulose membrane was unsuccessful.

Another LTP was identified on barley, malt and beer 2-D gels: LTP2 (Fig. 2: spot 36L). This protein, with a molecular mass of about 7 kDa, is also glycosylated in beer [25], but the glycosylated form could not be evidenced in the neighboring region of malt and beer LTP2 spot in 2-DE. This was probably due to the low amount of LTP2 in the malt extract and in beer. Cross-linked polypeptides through reduced cystine group were previously observed between LTP1 and LTP2 [25]. In the present work these disulfide-bridges forms could not be detected since samples were reduced before electrophoresis.

Concerning the location of LTP1 and LTP2, it is worthy to note that both proteins are located in the aleurone layers. Glycation of these proteins on malting and especially the kilning step means that the overall cell compartmentalization of the grain was probably lost during these processes since glucose and maltose involved in the Maillard reactions are provided by hydrolysis of the starch located in the endosperm. Detailed analysis of the LTP1 spots in barley and malt shows that 2-DE could be a powerful tool to evaluate the impact of kilning conditions in the malting process.

3.5 α -Amylase/trypsin inhibitors

In 2-D gels, numerous spots were identified as being proteins of the α -amylase/trypsin inhibitor family, also called chloroform/methanol (CM) soluble proteins due to their extractability by these mixtures of organic solvents. Noticeably, the inhibitory action of these proteins is probably not directed against barley enzymes, but against enzymes from insects and other pathogens [30]. Some of the CM proteins were known to inhibit either the α -amylase activity from various insects that attack barley or bovine trypsin [31]. None inhibited both α -amylase and trypsin. But the endogenous barley α -amylases, which are *de novo* synthesized during germination, are not inhibited by these α -amylase/trypsin inhibitors. Therefore, it is suggested that their role is to protect starch from insect and mammal predators [7]. These inhibitors are cysteine-rich proteins, containing four or five disulfide bonds, which are essential for their enzyme inhibitory activity.

These inhibitory proteins were found in the pI 4–7 range and molecular mass range of 12–16 kDa of barley and malt gels. Fourteen spots were found on barley gel and 12 spots on

malt gel (Table 1, Fig. 2A and B). However, some spots were less intense in malt gel (Fig. 2B: spots 20i, 23i, 29i and 30i), and disappeared on the beer gel (only five spots were present in this region of the beer gel; Table 1, Fig. 2C). These five spots were assigned as trypsin inhibitor BTI-CMe2.1 (Fig. 2C: spot 14i), α -amylase/trypsin inhibitor pUP13 (Fig. 2C: spot 22i), α -amylase/trypsin inhibitor CMB (Fig. 2C: spot 23i) and barley dimeric α -amylase inhibitor BDAI-1 (Fig. 2C: spots 30i and 30'i). On the other hand, several inhibitors have been annotated to one spot on the gels as spots 17i, 18i, 19i, 20i, 22i, 23i and 31i. For instance, spot 17i could be five different inhibitors (Table 1). The α -amylase inhibitors have close molecular masses and isoelectric points, and they could be post-translationally modified. Therefore, many proteins could be identified in these spots. Otherwise, α -amylase/trypsin inhibitors CMA, CMB and CMD form a heterotetramer with a stoichiometry of one CMA, one CMB and two CMD chains. However, in the reduced conditions of electrophoresis, these oligomeric structures dissociate in their monomeric units. A recent study using barley thioredoxin in order to target proteins on 2-D gels showed that the amount of α -amylase/trypsin inhibitors decreases in germinating seeds compared to mature seeds [9]. This agrees with our present observation. In contrast with LTP1, these enzyme inhibitors did not scatter in many spots when progressing from barley to malt. This suggests that they are not extensively affected by Maillard reactions on kilning in spite of their lysine content. Thus, we might wonder if these lysine residues are accessible in the oligomeric structure. Yet, it has been reported that the inhibitory form of barley monomeric amylase inhibitor (BMAI-1) was post-translational glycosylated, and that CMB existed both in a non-glycosylated and in a glycosylated form, a potentially allergen [32]. Low and even absence of glycation of these proteins on malting could explain, in contrast with LTPs, why these proteins had almost totally disappeared in beer. Actually, glycation could maintain the solubility of unfolded proteins throughout the brewing process. This unfolding was probably promoted by the combination of reducing and heating conditions of the brewing process.

3.6 Hordeins

B, γ 3 and D hordeins were also identified on barley and malt 2-D gels (Fig. 2A and B: spots 8h, 13h, 13h, 24h, 25h, 32h and 42h). Hordeins are prolamins, a major family of cereal seed storage proteins. These major proteins of barley endosperm generally display poor solubility in water. They consist of a complex polymorphic mixture, with major groups B, C, D and γ designated on the basis of their apparent molecular mass by electrophoresis. They are characterized by a high proline and glutamine content, and a fraction of the hordein polypeptide fragments found in beer promote haze formation [33]. The B hordeins, which are relatively sulfur-rich, are present as polymers stabilized by interchain disulfide bonds, and are the major hordein fraction present in barley grain,

accounting for about 80% of the total hordeins. The D hordeins, which are also relatively sulfur-rich, only account for a few percent of the fraction, and the γ hordeins are minor components [16].

Most of the hordeins disappeared in beer except hordein $\gamma 3$ (Fig. 2C: spot 13h). The other spots on the right and the left sides of spot 8h on barley and malt gels are thought to be hordeins, too. These spots are less intense on malt gel and disappeared almost completely on beer gel. In contrast with protein Z, LTPs and enzyme inhibitors, hordeins have a very low lysine content and therefore are not susceptible to Maillard reactions. As we suggested previously for enzyme inhibitors, absence of glycation might have prevented the maintenance of these unfolded prolamins in a soluble form throughout the brewing process.

3.7 Other identified spots

Other proteins that could be identified were present only on barley 2-D gel, or on barley and malt 2-D gels but not on beer 2-D gel. This is the case for a fragment of the cold regulated protein 1, barwin and the subtilisin-chymotrypsin inhibitor CI-1A. The fragment of the cold regulated protein 1 is detected only on the barley gel (Fig. 2A: spot 12). Barwin is detected as two spots on barley and malt gels (Fig. 2A and B: spots 26b and 27b) but not on the beer gel. It is probably a plant lectin and it may be involved in defense mechanism. Subtilisin-chymotrypsin inhibitor CI-1A is detected as one spot on barley and malt gels (Fig. 2A and B: spot 28i). This protein inhibits both subtilisin and chymotrypsin, and belongs to the potato chymotrypsin inhibitor I family of serine protease inhibitor. Therefore, most of these proteins did not resist the reducing and heating conditions of the brewing process.

Other spots were present only on the beer gel. Some corresponded to yeast proteins such as enolase 2 (Fig. 2C: spots 51c and 52c) or triosephosphate isomerase (Fig. 2C: spot 50c), which play an important role in several metabolic pathways. If the presence of yeast proteins was expected, to our knowledge, this is the first time that such proteins are evidenced in beer. Their influence on the quality of beer, and especially on the formation and stability of head foams, should be explored in the future.

4 Concluding remarks

Proteomics analysis of heat-stable proteins from barley and malt provided a clear view of the proteins that are recovered in beer. Although no direct investigation was performed concerning the modification that can occur during malting and brewing (glycation by Maillard reactions, proteolysis, protein unfolding), the scattered protein pattern observed for the major heat-stable proteins on the transition from barley to malt and to beer is well related to the physico-chemical modifications that occur on malting and brewing processes.

Especially for LTP1, it was possible to relate the two main spots of the barley gel to the presence of covalent oxygenated fatty acid adducts observed in our laboratory (Marion D., unpublished data) and the numerous spots of the malt gel to the glycation occurring on kilning.

Finally, this study revealed that the heat-stable proteins from barley recovered in beer are mainly composed of proteins involved in the defense of plants against their bio-aggressors. This is in agreement with the fact that most of these proteins are cysteine-rich proteins, *i.e.*, proteins with a fold stabilized by many disulfide bonds, which generally confers high thermal stability to the proteins and sometimes protease resistance.

We gratefully thank N. Ouarnier for providing micromalted samples, and M. Malanda for preparation of beer at IFBM. This work was granted by Malteurs de France, Brasseurs de France and the French Ministries of Research and of Agricultural and Fishing (CIFRE contract).

References

- [1] Slack, P. T., Bamforth, C. W., *J. Inst. Brewing* 1983, **89**, 397–401.
- [2] Evans, D. E., Hejgaard, J., *J. Inst. Brewing* 1999, **105**, 159–169.
- [3] Kaersgaard, P., Hejgaard, J., *J. Inst. Brewing* 1979, **85**, 103–111.
- [4] Sorensen, S. B., Bech, L. M., Muldbjerg, M., Beenfeldt, T., Breddam, K., *Technical Quarterly Master Brewers Association of the Americas* 1993, **30**, 136–145.
- [5] Flengsrud, R., *Electrophoresis* 1993, **14**, 1060–1066.
- [6] Kristoffersen, H. E., Flengsrud, R., *Electrophoresis* 2000, **21**, 3693–3700.
- [7] Finnie, C., Melchior, S., Roepstorff, P., Svensson, B., *Plant Physiol.* 2002, **129**, 1308–1319.
- [8] Ostergaard, O., Melchior, S., Roepstorff, P., Svensson, B., *Proteomics* 2002, **2**, 733–739.
- [9] Maeda, K., Finnie, C., Svensson, B., *Biochem. J.* 2003, **378**, 497–507.
- [10] Finnie, C., Svensson, B., *J. Cereal Sci.* 2003, **38**, 217–227.
- [11] Bak-Jensen, K. S., Laugesen, S., Roepstorff, P., Svensson, B., *Proteomics* 2004, **4**, 728–742.
- [12] Mortz, E., Krogh, T. N., Vorum, H., Görg, A., *Proteomics* 2001, **1**, 1359–1363.
- [13] Neuhaus, V., *Electrophoresis* 1988, **9**, 255–262.
- [14] Liang, F., Holt, I., Karamycheva, S., Salzberg, S. L., Quackenbush, J., *Nucleic Acids Res.* 2000, **28**, 3657–3665.
- [15] Quackenbush, J., Liang, F., Holt, I., Pertea, G., Upton, J., *Nucleic Acids Res.* 2000, **18**, 141–145.
- [16] Evans, D. E., Sheedan, M. C., *J. Am. Soc. Brewing Chemists* 2002, **60**, 47–57.
- [17] Ostergaard, H., Rasmussen, S. K., Roberts, T. H., Hejgaard, J., *J. Biol. Chem.* 2000, **275**, 33272–33279.

- [18] Hejgaard, J., Kaersgaard, P., *J. Inst. Brewing* 1983, 89, 402–410.
- [19] Curioni, A., Pressi, G., Furegon, L., Peruffo, A. D. B., *J. Agric. Food Chem.* 1995, 43, 2620–2626.
- [20] Jones, B. L., *J. Am. Soc. Brewing Chemists* 1997, 55, 58–64.
- [21] Davy, A., Svendsen, I., Bech, L., Simpson, D. J., Cameron-Mills, V., *J. Cereal Sci.* 1999, 30, 237–244.
- [22] Douliez, J. P., Michon, T., Elmorjani, K., Marion, D., *J. Cereal Sci.* 2000, 32, 1–20.
- [23] Blein, J.-P., Coutos-Thévenot, P., Marion, D., Ponchet, M., *Trends Plant Sci.* 2002, 7, 293–296.
- [24] Jégou, S., Douliez, J.-P., Mollé, D., Boivin, P., Marion, D., *J. Agric. Food Chem.* 2001, 49, 4942–4949.
- [25] Jégou, S., Douliez, J.-P., Mollé, D., Boivin, P., Marion, D., *J. Agric. Food Chem.* 2000, 48, 5023–5029.
- [26] Bech, L. M., Vaag, P., Heinemann, B., Breddam, K., *EBC Congress* 1995, 561–568.
- [27] Marion, D., Jégou, S., Douliez, J.-P., Gaborit, T., Boivin, P., 28th EBC Congress 2001, 1–8.
- [28] Douliez, J.-P., Jégou, S., Pato, C., Laré, C., Mollé, D., Marion, D., *J. Agric. Food Chem.* 2001, 49, 1805–1808.
- [29] Lindorff-Larsen, K., Lerche, M. H., Poulsen, F. M., Roepstorff, P., Winther, J. R., *J. Biol. Chem.* 2001, 276, 33547–33553.
- [30] Franco, O. L., Rigden, D. J., Melo, F. R., Grossi-de-Sá, M. F., *Eur. J. Biochem.* 2002, 269, 397–412.
- [31] Jones, B. L., Fontanini, D., *J. Agric. Food Chem.* 2003, 51, 5803–5814.
- [32] Barber, D., Sanchez-Monge, R., Mende, E., Lazaro, A., Garcia-Olmedo, F., Salcedo, G., *Biochim. Biophys. Acta* 1986, 869, 115–118.
- [33] Weiss, W., Postel, W., Görg, A., *Electrophoresis* 1992, 13, 770–773.