Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1163 (2007) 80–85
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

# Proteomic identification of technologically modified proteins in malt by combination of protein fractionation using convective interaction media and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Janette Bobalova, Josef Chmelik *

*Research Centre for Study of Extract Compounds of Barley and Hop, Institute of Analytical Chemistry, v.v.i., Academy of Sciences of the Czech Republic, Veveří 97, CZ-602 00 Brno, Czech Republic*

Received 4 April 2007; received in revised form 31 May 2007; accepted 5 June 2007
Available online 9 June 2007

**Abstract**

A method for the fast separation of proteins and identification of their modifications based on the use of monolithic chromatographic media and mass spectrometric techniques is described. This method has been developed and applied to the analysis of malt proteins and its posttranslational modifications (glycation). Glycation, one of the most common forms of posttranslational modifications (PTM), can be detected in both biological and industrial samples. Our attention was focused on the investigations of possible chemical modifications of water-soluble barley proteins during malting process by combination of anion-exchange chromatography with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The malt extract was directly fractioned by anion-exchange chromatography using short monolithic columns and a linear gradient from 0 to 700 mM NaCl. Sufficient fractionation was obtained for malt sample, which demonstrates the potential of anion-exchange chromatography on this type of column. Proteins in separated fractions were identified by MALDI-TOF/TOF MS. Our proteomic analysis provided the identification of the major proteins present in the malt that were found to be heterogeneously glycated after malting. One of these proteins: nonspecific lipid transfer protein 1 (LTP1) can be used as a marker for characterization of glycation during malting. This protein and its modifications can be easily determined by the developed method.


*Keywords:* Post-translation modification; Glycation; Marker; Protein identification; MALDI-TOF/TOF MS; Anion exchange chromatography; Monolithic columns

## 1. Introduction

Malting as an important process in production of beer is a procedure applied to cereal grains, in which the grains are made to germinate and then are quickly dried before the plant develops [1]. The importance of barley grain protein profile for malting and brewing is remarkable [2]. Proteins contained in barley grains are determinants of beer quality and are also modified during malting [3]. Only a relatively small number of proteins from barley grains survive malting. Some of them are relevant components of beer (e.g. for formation and stabilization of beer foam) and their identification is an important task [4]. Proteins associated with beer foam formation and stability are protein Z, lipid transfer proteins, hordein and glutelin fragments [5–7]. Of those, protein Z (Mr ∼43 kDa) and the 9.6 kDa lipid transfer protein 1 (LTP1), both originating from barley, are the predominant proteins in beer [8]. These proteins display high stability to heating and proteolysis. Their resistance to extreme pH, high temperatures and their protease-inhibiting properties may explain the survival of these proteins during the malting and brewing processes [9]. Further, it was found that glycated proteins of beer exist in three groups: those responsible for causing haze, those responsible for providing foam stability and a third group which appeared to have no role in physical or foam stability [10].

Protein Z is glycated during the malting process through Maillard reactions [11]. The latter study showed that glycated protein Z has improved foam stability [12] and glycation might prevent protein from precipitation on unfolding during the wort

* Corresponding author. Tel.: +420 532 290 145; fax: +420 541 212 113.
*E-mail address:* chmelik@iach.cz (J. Chmelik).

0021-9673/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2007.06.006

boiling step. It has been reported that barley LTP1 is modified during the brewing process and these forms contributed to foam formation, while foam stability depended on protein Z [13].

Post-translational modifications result in an increase or decrease in the molecular weight of proteins as compared with their unmodified forms. These mass shifts provide information regarding to the identity of the modifications [14]. Mass spectrometry is well suited to the task of determining the mass shifts and identity of the post–translational modifications. Protein identification is obtained after data base search using the measured protein molecular mass and protein sequence tags determined by MALDI-TOF/TOF MS.

In recent years, the application of polyacrylamide gel electrophoresis and mass spectrometry has become popular in proteomic methods and these methods are powerful tools for determination of changes in protein composition in various biological and technological processes [15]. Although gel electrophoresis is currently the most widely accepted technique for separation of complex protein mixtures, modern downstream processing requires fast and highly effective methods to obtain large quantities of highly pure substances. Among the methods applied for this purpose is chromatography with short monolithic columns which are suitable for very fast separation of proteins in different modes, e.g. in ion-exchange, hydrophobic interaction or affinity mode [16–19]. One of the first useful monolithic stationary phases for the rapid separation of proteins was designed in a disc format [20–21]. It is the basis of the short bed Convective Interaction Media (CIM®) disks monolithic columns, which are specific among the chromatographic columns, because of their monolithic structure and extremely short column length. They have very fast mass transfer between the mobile and stationary phase, which provides high speed and high efficiency of the separation [22]. It is also possible to stack two or more monolithic disks with different ligands into one cartridge. Monoliths have a large potential for application in proteomics technology and their can open new possibilities for mapping of proteins and glycoproteins [23].

The aim of this study is to develop a rapid method for determination of post-translational modifications of barley proteins during malting process. To fulfill this task a combination of anion exchange chromatography with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was chosen.

## 2. Experimental

### 2.1. Chemicals and materials

All chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany), trypsin and chymotrypsin were obtained from Roche Diagnostics (Mannheim, Germany), ZipTip $C_{18}$ pipete tips were purchased from Millipore (Billerica, MA, USA). 4-Hydroxy-3,5-dimethoxycinnamic acid (sinapinic acid, SA) and α-cyano-4-hydroxycinnamic acid (CHCA) were purchased from LaserBio Labs (Sophia-Antipolis Cedex, France).

### 2.2. HPLC conditions

Anion exchange chromatography was performed with a liquid chromatograph Hewlett-Packard 1100 Series. CIM® DEAE disk (BIA Separations, Ljubljana, Slovenia) with diameter 16 mm was used for separation of proteins. A model 1040 HPLC detection system was used for diode-array detection (DAD). 20 μl of aqueous extract of malt sample (8.5 mg/1 ml) was analyzed. Fractionation of proteins from malt extract was performed with a gradient of sodium chloride on a disk monolithic column using a mobile phase consisting of buffer A: 20 mM Tris–HCl, pH 7.4 and buffer B: buffer A containing 1 M NaCl, pH adjusted to pH 7.4. The gradient program for the fractionation at a flow rate of 1 ml/min was as follows: linear gradient 100% to 30% A (2 min). All separations were monitored at 280 nm. We manually collected three fractions at different retention times.

### 2.3. Mass spectrometry

MALDI-TOF/TOF measurements were performed with Applied Biosystems 4700 Proteomics Analyzer (Applied Biosystems, Framingham, MA). This instrument is equipped with linear detector and with Nd:YAG laser (355 nm) of <500-ps pulse. In both MS and MS/MS modes the TOF/TOF operates at 200 Hz repetition rate. Linear mode was used for intact proteins and reflectron mode was used for peptides. All MALDI mass spectra were calibrated externally. For linear TOF mode was used insulin B chain, oxidized (3494.65 Da) and cytochrome C (12361.20 Da); for reflectron TOF/TOF mode peptide mixture (des-arg 1-bradykinin—904.486 Da; angiotensin I—1296.685 Da; glu1-fibrinopeptid—1570.677 Da; ACTH(1–17)—2093.087 Da; ACTH(18–39)—2465.199 Da; ACTH(7–38)—3657.929 Da). MS data were further processed using DataExplorer 4.5 software (Applied Biosystems, Framingham, MA, U.S.A.). Database searching was done using the Mascot program and NCBInr sequence database (ver. 2006) was used for the database searching with the species restriction to green plants.

The dry droplet method was used for measurement of intact proteins, 0.4 μl of each fraction (desalted using ZipTip $C_{18}$ pipete tips) was mixed with a matrix (solution of sinapinic acid (20 mg/ml) in acetonitril/0.1% trifluoroacetic acid (1:1, v/v)). For measurement of protein digests, a solution of α-cyano-4-hydroxycinnamic acid (10 mg/ml) in acetonitril/0.1% trifluoroacetic acid (1:1, v/v) was used. Digests desalted previously with ZipTip were deposited on spot by thin layer method.

### 2.4. In-solution digestion

In-solution digestion was carried out according to Šalplachta et al. [24]. The dried chromatographic fractions were dissolved in 100 μl of 100 mM $NH_4HCO_3$ and the solution was mixed with 10 μl of 50 mM DTT and kept at 56 °C for 45 min in order to reduce disulfide bonds. After cooling, 5 μl of the digestion buffer containing 100 ng/μl of trypsin was added. The mixture was digested at 37 °C overnight. Digestion was also performed with