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Rapid assignment of malting barley varieties by matrix-assisted laser desorption–ionisation – Time-of-flight mass spectrometry

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ABSTRACT

A method for discriminating malting barley varieties based on direct matrix-assisted laser desorption–ionisation – time-of-flight mass spectrometry (MALDI-TOF MS) fingerprinting of proteins was developed. Signals corresponding to hordeins were obtained by simple mixing of powdered barley grain with a MALDI matrix solution containing 12.5 mg mL^{-1} of ferulic acid in an acetonitrile:water:formic acid 50:33:17 *v*/*v*/*v* mixture. Compared to previous attempts at MALDI-TOF mass spectrometric analysis of barley proteins, the extraction and fractionation steps were practically omitted, resulting in a significant reduction in analytical time and costs. The discriminatory power was examined on twenty malting barley varieties and the practicability of the method was tested on sixty barley samples acquired from Pilsner Urquell Brewery. The method is proposed as a rapid tool for variety assignment and purity determination of malting barley that may replace gel electrophoresis currently used for this purpose.

1. Introduction

Due to their influence on malting quality, the identity of malting barley varieties and their purity (i. e. the affiliation of the grains within the entire batch to a single variety) is a fundamental step preceding the process of beer production (Newton, Swanston, Guy, & Ellis, 1998). Routinely, the identity and purity of malting barley varieties are determined by means of one-dimensional polyacrylamide gel electrophoresis (1D-PAGE) of the hordein fraction (Almgard & Landegre, 1974). In this approach, which has been used for several decades as a standard method in breweries, hordeins are extracted from crushed barley grains overnight, followed by electrophoretic separation on the second day and visualisation of protein bands on the third day. Variety identification and purity determination are based on a comparison of protein band patterns with those of standard malting barley varieties or comparisons between several grains from the same batch, respectively.

As well as involving time-consuming analytical steps, this standard protocol is complicated by safety issues, due to the use of toxic chemicals (such as acrylamide, *N*,*N*-methylenebisacrylamide or mercaptoethanol). In addition, it has been shown that some barley varieties are not distinguishable by means of 1D-PAGE (Almeida & Cavalli-Molina, 2000).

Matrix-assisted laser desorption–ionisation – time-of-flight mass spectrometry (MALDI-TOF MS) caused a revolution in microbial diagnostics (Bizzini & Greub, 2010; Clark, Kaleta, Arora, & Wolk, 2013; Welker & Moore, 2011; Šedo, Sedláček, & Zdráhal, 2011). Due to its rapidity and high discriminatory power, it has become the method of choice for identification of bacterial pathogens. Aside from bacteria and fungi, other types of materials can also be subjected to MALDI-TOF MS profiling analysis.

The concept of cereal protein profiling by MALDI-TOF MS, with the aim of discriminating different cereal varieties, has already been investigated. Specific gliadin signals obtained by MALDI-TOF MS after a 20 min extraction of milled grains enabled the identification of wheat varieties with the aid of artificial neural networks (Bloch, Kesmir, Petersen, Jacobsen, & Sondergaard, 1999). A similar approach was also demonstrated on rye and barley (Bloch et al., 2001), while data treatment based on multivariate analysis was used for this purpose (Gottlieb et al., 2002).

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Discrimination between malting barley varieties on the basis of MALDI-TOF mass spectral comparison of hordeins was achieved for the first time by Šalplachta and Bobáľová (2009) after optimisation of the extraction procedure. MALDI-TOF MS also enabled brewing process monitoring, as was shown by MALDI-TOF MS profiling of water-soluble proteins (Laštovičková, Mazanec, Benkovská, & Bobáľová, 2010). A fundamentally different approach for identification of malting barley varieties was developed by Pattermore, Rice, Marshall, Waugh, and Henry (2010), who employed MALDI-TOF MS profiling of DNA fragments in a multiplexed single nucleotide polymorphism (SNP) assay.

In the present paper, we introduce a rapid and easy method for discrimination between malting barley varieties based on MALDI-TOF MS analysis of proteins obtained directly from the barley material.

2. Materials and methods

2.1. Barley samples

Certified standards of registered malting barley varieties were obtained from Oseva Pro (Prague, Czech Republic; varieties Akcent, Bojos, Laudis, Malz, Marthe, Tipple, and Wintmalt), from Bio-Rad spol s.r.o. (Prague, Czech Republic; varieties Ebson and Esterel), and Central Institute for Supervising and Testing in Agriculture (Brno, Czech Republic; varieties Advent, Aksamit, Aktiv, Blaník, Delphi, Despina, Kangoo, Radegast, Sebastian, Tolar, and Xanadu). Standards for Malz and Wintmalt varieties were obtained after being grown at two different locations in the Czech Republic (samples labelled "CZ") and Slovakia (samples labelled "SK").

Sixty samples of malting barley selected for evaluation of the method were delivered to Pilsner Urquell Brewery from different producers over a two-year period (2013–2014). The sampling was carried out by acquiring eight small sub-samples from different parts of each batch.

2.2. Chemicals

Ferulic acid (FerA) and 6-aza-2-thiothymine (ATT) were obtained from Sigma–Aldrich (Steinheim, Germany). Trifluoroacetic acid (TFA) and acetonitrile (ACN) were from Merck (Darmstadt, Germany). Formic acid (FA) was from Riedel de Haën (Seelse, Germany). α-Cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxybenzoic acid (DHB), and sinapinic acid (SA) were obtained from Bruker Daltonik (Leipzig, Germany). All chemicals were of analytical grade purity. Water was prepared using a Milli-Q plus 185 apparatus (Millipore, Billerica, MA).

2.3. Sample preparation

A single barley grain per sample was crushed using a mortar and pestle. The certified malting varieties were prepared in

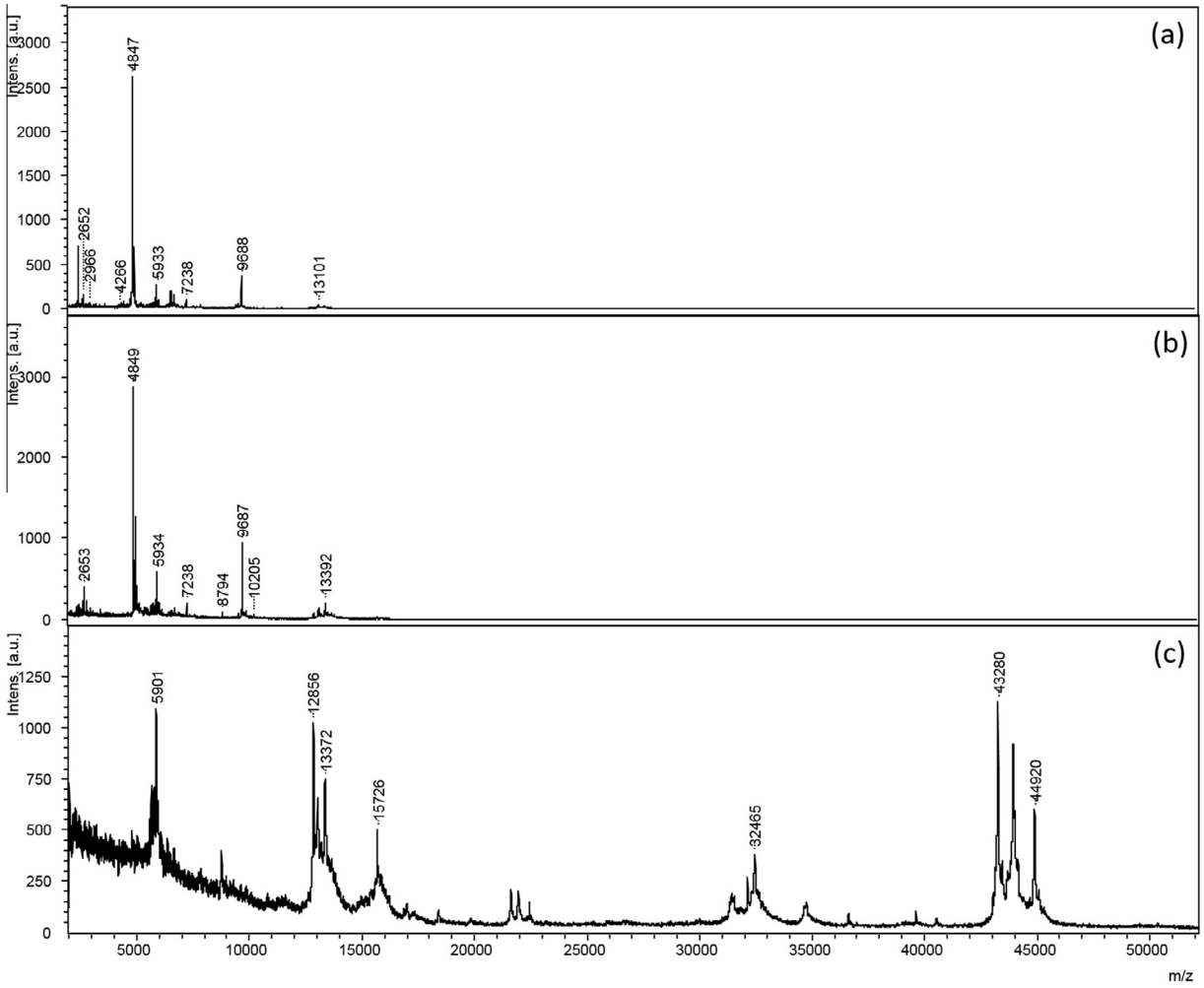

Fig. 1. MALDI-TOF mass spectra of Bojos standard by using (a) CHCA, (b) ferulic acid with 0.1% TFA, and (c) strongly acidified ferulic acid (17% FA).