Food Control 46 (2014) 41–48

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Selected product ion monitoring for quantification of 5-hydroxymethylfurfural in food products by capillary zone electrophoresis-tandem ion trap mass spectrometry

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ARTICLE INFO

Article history:
Received 9 February 2014
Received in revised form
29 April 2014
Accepted 30 April 2014
Available online 14 May 2014

Keywords:
5-Hydroxymethylfurfural (HMF)
Capillary zone electrophoresis
Ion trap mass spectrometry

ABSTRACT

5-hydroxymethylfurfural (HMF) is an important marker of food processing and storage widely recognized as an important parameter of food freshness and quality. Its amount depends on the composition of a food product and on the thermal treatments to which the food is subjected. The aim of this work was to propose for the first time the optimization and validation of an innovative CE-MS^2 method for the quantification of HMF in food products exploiting selected product ion monitoring as acquisition mode. The method was applied to the analysis of different kind of samples: cereal-based baby foods, coffee, soft beverages and vinegars. In order to show the reliability of the proposed method, results were compared with those obtained by RP-HPLC-UV, and a good accordance between the two sets of data was found.

1. Introduction

During heat treatment of foods, complex chemical reactions take place, contributing to desired color, taste and aroma of heated foodstuff. The advanced stage of the Maillard reaction (MR) is characterized by the accumulation of undesirable compounds, such as furfurals, that are useful tools used to evaluate the severity of heat treatment applied and the effect of storage (Rada-Mendoza, Sanz, Olano, & Villamiel, 2004; Ramírez-Jiménez, García-Villanova, & Guerra-Hernández, 2001). In details, two pathways leading to furfural production start from sugar decomposition: the caramelisation, where the reducing carbohydrates suffer 1–2 enolisation, dehydration and cyclisation reactions, and the MR, where the Amadori product is submitted to enolisation and dehydration of the sugar moiety, releasing the intact amino acid (Cardenas Ruiz, Guerra-Hernández, & García-Villanova, 2004; Kroh, 1994; Ramírez-Jiménez, Guerra-Hernández, & García-Villanova, 2000).

5-hydroxymethylfurfural (HMF) is a common product of these two reactions. In addition to temperature, the amount of HMF in foods is dependent on the type of sugar, pH, water activity and cations concentration (Capuano & Fogliano, 2011).

HMF represents a recognized parameter for evaluating food freshness and quality, especially for honey and apple juice (Directive 2001/110/EC, 2001; Khalil, Sulaiman, & Gan, 2010). Several food matrices can form furfurals, and the amount of HMF is directly related to the food processing and storage. A common source of HMF is represented by ingredients used in the formulation such as caramel solutions or honey. In 2011, EFSA revised a scientific opinion about caramel colors (E 150 a, b, c, d), defining that some of its constituents, including 5-HMF and furans, may be present in food products at levels that may be of concern. Therefore it considers that the specifications should include maximum levels for these constituents (EFSA, 2011).

Although the concentrations in some food items are extremely high, bread and coffee are the most important contributors to dietary intake (Murkovic & Pichler, 2006). The estimated exposure is several orders of magnitude higher than the daily intake for other heat-induced food toxicants such as acrylamide and furan (Morehouse, Nyman, McNeal, Dinovi, & Perfetti, 2008; Svensson et al., 2003). Based on data reported in literature it is not clear whether human exposure to HMF represents a potential health risk (Capuano & Fogliano, 2011): the major concern is related to its enzymatic conversion to sulphoxymethylfurfural (SMF) which has been reported to be mutagenic (Lee, Shlyankevich, Jeong, Douglas, & Surh, 1995; Surh, Liem, Miller, & Tannenbaum, 1994), as also reported by EFSA in a report concerning its genotoxic potential (EFSA, 2005). Moreover, SMF in the blood of mice after HMF intravenous administration has been recently detected (Monien, Frank, Seidel, & Glatt, 2009), and associated risk for humans may be higher since human enzymes are more active than in rodents (Capuano & Fogliano, 2011). Although the correlation of adverse health effects

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http://dx.doi.org/10.1016/j.foodcont.2014.04.049
0956-7135/

and exposure to HMF is not conclusive (Abraham et al., 2011; Janzowski, Glaab, Samimi, Schlatter, & Eisenbrand, 2000), analytical measurements of HMF in foods seem opportune for an objective risk assessment, as well as for quality evaluation of food. Therefore, new methods with a simplified protocol and lower cost are still in demand to ensure the high-throughput screening and the high efficiency. The common analytical technique employed is HPLC-UV (Aquino, Rodrigues, Nascimento, & Casimiro, 2006; Gaspar & Lucena, 2009; Pereira, Albuquerque, Ferreira, Cacho, & Marques, 2011; Spano, Ciulu, Floris, Panzanelli, & Piloa, 2009; Zappalá, Fallico, Arena, & Verzera, 2005), reference method of the Association of the Official Analytical Chemists (AOAC, 1996). During the last years several analytical methods have been developed. Liquid chromatography with pulsed amperometric detection (Xu, Templeton, & Reed, 2003), refractive index detection (Xu et al., 2003), or coupled to mass spectrometry (Gökmen & Senyuva, 2006; Teixidó, Moyano, Santos, & Galceran, 2008) have been used. Recently, gas chromatography coupled to mass spectrometry (Teixidó, Santos, Puignou, & Galceran, 2006), and electrochemical biosensors (Lomillo, Campo, & Pascual, 2006) have been proposed for HMF analysis in honey, baby-foods, jam, orange juice and bakery products. However, LC methods proposed often require long analysis time and consume considerable amounts of solvents, while a derivatization procedure is mandatory in GC analysis to increase volatility and overcome adsorption to the column.

In a recent paper, a detailed study of the Direct Analysis in Real Time mass spectra of carbohydrates and HMF using a single quadrupole mass spectrometer has been reported. However, the accurate DART-MS quantitation of HMF in carbohydrates-rich matrices was possible only with a high resolution-mass spectrometer and/or tandem MS (Chernetsova & Morlock, 2012).

Capillary electrophoresis (CE) with UV detection has been selected as an alternative technique to LC for the quantification of HMF in breakfast cereals, toast, honey, orange and apple juice, jam, coffee, chocolate, by employing the micellar electrokinetic capillary chromatography (MECK) mode (Chen & Yan, 2009; Corradini & Corradini, 1992, 1994; Morales & Jimenez-Perez, 2001; Rizelio et al., 2012; Teixidó, Núñez, Santos, & Galceran, 2011; Wong et al., 2012). To the best authors' knowledge, no applications were carried out by capillary-electrophoresis tandem mass spectrometry (CE-MS^2) for quantitation of HMF in food. This technique represents an interesting challenge as allows the combination of low costs in terms of solvent, short time of analysis and good results regarding selectivity and sensibility, as shown by recent papers (Bignardi, Cavazza, & Corradini, 2012; Bonvin, Schappler, & Rudaz, 2012; Huhn, Ramautar, Wuhrer, & Somsen, 2010).

In this work, a new analytical method employing CE-MS^2 for determination of HMF in different foodstuffs was developed and validated. Selected MS/MS ion monitoring (SMIM) was chosen as acquisition mode, programming the detector to perform continuous MS/MS scans on a selected precursor. This acquisition mode has been shown to be convenient when dealing with complex matrices, because it provides the MS/MS spectrum of the analyte to confirm its nature (Jorge et al., 2007).

Analytical conditions and sample preparation (without any pretreatment as SPE purification, often employed) were optimized and applied to different kind of food products. The results were also compared to those obtained with a HPLC-UV method.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical reagent grade. Formic acid, trichloroacetic acid, ammonium hydroxide (25%, w/w), hydrochloric acid (37%, w/w), acetic acid (100%) and sodium hydroxide (50%, w/w) were obtained from J. T. Baker (Deventer, The Netherlands). Methanol (Chromasolv) was purchased from Sigma Aldrich (Milan, Italy). HPLC water was obtained with a MilliQ element A10 System (S. Francisco, CA, USA). 2-Furylmethylketone (FMK) used as internal standard (IS), and 5-hydroxymethylfurfural (99% purity) were obtained from Fluka, Sigma Aldrich (Milan, Italy).

2.2. Sample preparation

Standard stock solutions of HMF and FMK were prepared by dissolving the pure standard in HPLC water at the final concentration of 10 $mgmL^{-1}$. All solutions were kept at 4 °C until needed and used within 2 weeks. Samples of baby food (cereal-based), barley, balsamic vinegar, coffee, and soft beverages were purchased from a local store. Sample treatment was carried out according to a previous method (Chen & Yan, 2009) slightly modified as follows: 300 mg of each sample were dissolved with 6 mL of 1% TCA solution, vortexed for 2 min, ultrasonicated for 10 min and vortexed for 2 min. The resulting mixture was centrifuged at 9000 rpm for 10 min at 4 °C.

Supernatant was collected in a centrifuge tube and another extraction was performed using 3 mL of 1% TCA solution. The supernatants were mixed and the volume was made up to 10 mL with 1% TCA solution. The extract was then filtrated through 0.20 μm nylon filter and 6 μL of 500 mgL^{-1} FMK solution were added to an aliquot of the sample to obtain a concentration of 5 mgL^{-1} in a final volume of 600 μL.

Liquid samples, such as red wine vinegars and soft beverages, were simply diluted (4 and 10 fold, respectively) in 1% TCA solution.

2.3. Capillary electrophoresis

The analysis was carried out on an Agilent Capillary Electrophoresis system (Agilent Technologies, Santa Clara, California) coupled to an Agilent LC/MSD Trap XCT Ultra (Agilent Technologies) with electrospray interface (ESI). Electrophoresis was performed employing untreated fused-silica capillaries (Agilent Technologies, Italy) of 50 μm i.d. and effective length of 60 cm.

New capillaries were conditioned before use by washing sequentially with 0.1 M hydrochloric acid for 5 min, ultrapure water for 5 min, 1 M sodium hydroxide for 5 min, ultrapure water for 5 min. Those rinses were performed offline in order to avoid the ESI contamination. Before each sessions conditioning rinse with the background electrolyte (BGE), formic acid 50 mM adjusted to pH 3.0 with 0.5 M ammonium hydroxide, was performed for 30 min. Between runs the capillary was rinsed with BGE for 2 min by applying a pressure of 900 mbar. Sample introduction was performed by applying a pressure of 50 mbar for 10 s. Optimized conditions employed an operating voltage of 25 kV and temperature of 30 °C. When real samples were injected, an additional rinse was necessary each 9 runs to avoid a strong noise in the baseline, often followed by current drop. The rinse consisted in ultrapure water for 2 min, 1 M ammonium hydroxide for 2 min, water for 2 min and buffer electrolyte for 5 min. Data acquisition and processing were carried out with ChemStation software (Agilent Technologies, Italy).

2.4. Mass spectrometry

Mass spectrometer device was an Agilent LC/MSD Trap XCT Ultra equipped with a pneumatically assisted ESI interface. CE-MS coupling was realized by a co-axial sheath liquid interface with 50% MeOH and 0.1% formic acid as a sheath liquid at a flow rate of 3 $\mu Lmin^{-1}$. ESI-MS^2 parameters were optimized through direct