Food Chemistry 183 (2015) 58–63

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Novel liquid chromatography–mass spectrometry method for sensitive determination of the mustard allergen Sin a 1 in food

Maria Posada-Ayala [a,1], Gloria Alvarez-Llamas [a,1], Aroa S. Maroto [a], Xavier Maes [b], Esther Muñoz-Garcia [a], Mayte Villalba [c], Rosalía Rodríguez [c], Marina Perez-Gordo [d], Fernando Vivanco [a,c], Carlos Pastor-Vargas [a,*,1], Javier Cuesta-Herranz [e,1]

[a] Department of Immunology, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain
[b] Agilent Technologies GmbH, Waldbronn, Germany
[c] Department of Biochemistry and Molecular Biology I, Faculty of Chemistry, Universidad Complutense de Madrid, Spain
[d] Institute of Molecular Applied Medicine (IMMA), and San Pablo CEU University School of Medicine of Madrid, Spain
[e] Department of Allergy, IIS-Fundación Jiménez Díaz, UAM, Madrid, Spain

ARTICLE INFO

Article history:
Received 8 May 2014
Received in revised form 4 February 2015
Accepted 27 February 2015
Available online 12 March 2015

Keywords:
Allergens
Hidden allergen
LC–MS
Mustard allergy
Quantification
Sin a 1
SRM

ABSTRACT

Mustard is a condiment added to a variety of foodstuffs and a frequent cause of food allergy. A new strategy for the detection of mustard allergen in food products is presented. The methodology is based on liquid chromatography analysis coupled to mass spectrometry. Mustard allergen Sin a 1 was purified from yellow mustard seeds. Sin a 1 was detected with a total of five peptides showing a linear response (lowest LOD was 5 ng). Sin a 1 was detected in mustard sauces and salty biscuit (19 ± 3 mg/kg) where mustard content is not specified. Sin a 1, used as an internal standard, allowed quantification of this mustard allergen in foods. A novel LC/MS/MS SRM-based method has been developed to detect and quantify the presence of mustard. This method could help to detect mustard allergen Sin a 1 in processed foods and protect mustard-allergic consumers.

1. Introduction

Mustard is a condiment made from the seeds of a mustard plant belonging to the Brassicaceae family, which includes other vegetables such as radish, rutabaga, cabbage, broccoli, turnip, watercress, horseradish, castor oil plant, and rapeseed. There are several varieties of mustard, the most important being (1) yellow mustard (*Sinapis alba*), the most commonly used variety in Europe, and (2) oriental mustard (*Brassica juncea*), commonly used in United States and Asia. Mustard seed is added to an assortment of foodstuffs such as pickled products, processed meats, seasoning blends, salad dressings, sauces, and condiments in order to improve flavor. However, mustard is also frequently included as a hidden component in sauces, flavoring powders, or salad dressings.

Mustard has been reported as a frequent cause of food allergy. Symptoms in allergic reactions to mustard range from oral allergy syndrome to immediate skin response and angioedema as well as more severe reactions, such as anaphylactic shock in hypersensitive patients (Caballero et al., 2002). For this reason, mustard was included in the European Union Regulation 1169/2011 as one of the allergens that are required to appear on food labels. The prevalence of mustard allergy and the cross-reactivity between mustard and other plant-derived foods have been reported increasingly in recent years (Sirvent et al., 2012; Vereda et al., 2011); cross-reactivity occurs between mustard and nuts, legumes, and Rosaceae fruits. No treatment has been described at present, and strict avoidance of mustard-containing foods is currently the only method to avoid allergic reactions. In yellow mustard seeds, 4 allergens have been identified and characterized. Sin a 1 and

Abbreviations: LC–MS, liquid chromatography coupled to mass spectrometry; LC–MS/MS, liquid chromatography coupled to mass spectrometry in tandem; LOD, limit of detection; LOQ, limit of quantification; SRM, selected reaction monitoring; MS, mass spectrometry; QQQ, triple quadrupole mass spectrometer; OD, optical density.

* Corresponding author at: Department of Immunology, Hospital Fundación Jimenez Diaz, Avda. Reyes Católicos 2, 28040 Madrid, Spain. Tel.: +34 915504800x2202; fax: +34 915448246.
E-mail address: cpastor@fjd.es (C. Pastor-Vargas).

[1] Both authors and seniors researchers contributed equally to this work.

http://dx.doi.org/10.1016/j.foodchem.2015.02.139
0308-8146/

Sin a 2 are specific seed-storage proteins and major allergens in mustard. Sin a 1 belongs to the 2S albumin family, a group of proteins that are highly resistant to proteolysis and remain stable under heat treatments (Menendez-Arias, Moneo, Dominguez, & Rodriguez, 1988). Sin a 2 belongs to the 11S globulin family (Palomares, Vereda, Cuesta-Herranz, Villalba, & Rodriguez, 2007), and Sin a 3 and Sin a 4 were identified as a non-specific lipid transfer protein (nsLTP) and a profilin, respectively (Sirvent et al., 2009). In addition, the mustard allergens Sin a 3 and Sin a 4 are involved in IgE cross-reactivity with fruits such as peach and melon, respectively.

Allergens in mustard can be introduced in food products accidentally, by means of a deficient processing (e.g., improper handling, cross-contamination, or incomplete cleaning (Jackson et al., 2008)), or due to labeling errors. Also, in some countries, the presence of mustard in foods is not specifically mentioned on the label and is rather included under the generic term "spices." Therefore, the development of sensitive analytical methods for the determination of mustard content in foods is very important to protect allergic consumers.

Several ELISA methods have been described to detect mustard traces (Koppelman et al., 2007; Lee, Niemann, Lambrecht, Nordlee, & Taylor, 2009; Shim & Wanasundara, 2008). These tests have decreased the limit of protein quantification to 1 ppm, and they are capable of detecting mustard proteins from different species, including yellow and oriental mustard (Cuhra, Gabrovska, Rysova, Hanak, & Stumr, 2011).

Methods based on mass spectrometry (MS) have advanced significantly in terms of sensitivity and specificity, having improved the identification, characterization, and determination of food allergens (Faeste, Ronning, Christians, & Granum, 2011). Since the first study by Shefcheck and Musser (2004), liquid chromatography (LC) coupled to MS analysis has become an important detection tool to identify allergens in food (Abdel Rahman, Kamath, Gagne, Lopata, & Helleur, 2013; Abdel Rahman, Kamath, Lopata, Robinson, & Helleur, 2011; Careri et al., 2007; Heick, Fischer, & Popping, 2011; Mattarozzi, Bignardi, Elviri, & Careri, 2012). LC–MS/MS has superior characteristics, improved reproducibility, recovery, sensitivity, dynamic range, and quantification (Heick, Fischer, Kerbach, Tamm, & Popping, 2011). In this sense, selected reaction monitoring (SRM) is a highly specific quantitative methodology based on the measurement of specific proteotypic peptide masses (peptide precursor and precursor fragments masses) corresponding to the protein of interest in a triple quadrupole (QQQ) mass spectrometer (Picotti, Bodenmiller, & Aebersold, 2013). A proteotypic peptide is defined as a peptide that identifies a protein uniquely; thus, this protein can be specifically quantified by peptide measurement.

The aim of this study was to develop a sensitive and specific method based on LC–MS/MS measurements of allergens for the detection of mustard traces in food products. Such a method would make it possible to reliably and accurately detect mustard allergens. The new method could be applied to other allergens and create an essential tool for the food industry and regulatory agencies in their effort to control food allergens and better serve the food-allergic consumer.

2. Materials and methods

2.1. Yellow mustard seed extract Sin a 1 purification

Mustard powder obtained from seeds of *S. alba* L. was suspended in 0.15 mol/l sodium borate buffer, pH 8, at a 10% (w/v) ratio, gently stirred for 1 h and centrifuged at 4 °C. The pellet was extracted twice with borate buffer and the supernatants were combined and lyophilized. The remaining plant material was then washed three times with 10% (w/v) acetone. The pellets were air-dried before being dissolved in 0.15 mol/l ammonium bicarbonate, pH 8.0. After centrifugation at 8700*g*, at 4 °C, supernatants were lyophilized and stored at −20 °C.

Sin a 1 was purified as previously described (Menendez-Arias et al., 1988). Briefly, lyophilized seed extract was dissolved in 0.15 mol/l ammonium bicarbonate buffer (pH 8.0) and applied to a Sephadex G-50 *Fine* column. Fractions containing 2S albumin protein (identified by a specific rabbit polyclonal antibody against Sin a 1) were pooled and resolved by ion-exchange chromatography on an SP-Sephadex C-25 column equilibrated with 3 mmol/l pyrophosphate buffer (pH 9.0). Proteins were eluted with a gradient from 3 to 100 mmol/l pyrophosphate buffer (pH 9.0). Fractions containing protein were pooled and resolved by ion-exchange chromatography using a CM-cellulose (NaCl gradient from 0.15 to 0.3 mol/l). Fractions containing Sin a 1 were pooled.

2.2. Protein extraction of commercial samples

Commercial sauces (mustard sauce, garlic mayonnaise, barbecue sauce, honey-mustard sauce, ketchup, and mayonnaise) and salty biscuits were purchased from a local market (Madrid, Spain) (Supplementary Table 1). 0.5 g of each sample was suspended in 50 mmol/l ammonium bicarbonate buffer pH 8.0, at a 10% (w/v) ratio, gently stirred for 48 h at 4 °C and centrifuged at 20,000×*g*, at 4 °C. After centrifugation, the supernatants were lyophilized, resuspended in 0.5 ml PBS and loaded onto a Superdex 25 column (GE Healthcare, Uppsala, Sweden) equilibrated with 50 mmol/l ammonium bicarbonate (pH 8.0). Fractions containing proteins (identified by OD at 280 nm) were lyophilized and resuspended in 1 ml 50 mmol/l ammonium bicarbonate (pH 8.0). Total protein content was determined by Coomasie Plus Bradford Assay (Pierce, Rockford, IL, USA) and 50 μg of total protein was taken for digestion.

2.3. Liquid chromatography–mass spectrometry

For LC–MS analysis, protein samples (purified protein or extracts obtained from commercial samples) were reduced with dithiothreitol (DTT), Bio-Rad (Hercules, CA, USA) and alkylated with iodoacetamide (IAA), Bio-Rad (Hercules, CA, USA). Digestion was performed with sequencing-grade modified bovine trypsin, Roche (Branford, CT, USA), at a final concentration of 1:50 (trypsin:protein) for commercial extracts (50 μg total protein digested) or by adding 0.2 μg trypsin to all standard solutions of purified Sin a 1 used in the calibration curve. Tryptic peptide solutions were cleaned with C18 spin columns, Protea Biosciences (Morgantown, WV, USA) according to manufacturer instructions, and diluted 1:1 with mobile phase A (0.1% formic acid, Sigma–Aldrich (St. Louis, MO, USA) containing 5% acetonitrile, Merck (Darmstadt, Germany)). Samples were analyzed using a 6460 Triple Quadrupole on-line connected to a HPLC-Chip Cube interface, Agilent Technologies (Palo Alto, CA, USA) and 1200 Series LC Modules, Agilent Technologies (Palo Alto, CA, USA) provided with a pre-cooled nano LC autosampler. Peptide separation was carried out on a ProtID Zorbax 300B-C18-5 μm chip with a 43 × 0.075 mm analytical column and a 40 nl enrichment column, Agilent Technologies (Palo Alto, CA, USA). One microliter of sample was injected at 3 μl/min and separation took place at 0.4 μl/min in a continuous acetonitrile gradient as follows: 5% for 1 min, 5–30% B for 1 min, 30–60% B for 8 min, up to 95% B in 1 min, and 95% B for 2 min. The system was controlled by MassHunter Workstation software v4.01 Agilent Technologies (Palo Alto, CA, USA). The mass spectrometer was operated in positive ion mode with capillary voltage of 1950 V, 325 °C source gas temperature and 5 L/min source gas flow. Fragmentor potential was set to