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The quantification of free Amadori compounds and amino acids allows to model the bound Maillard reaction products formation in soybean products

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*N*ε-(2-furoylmethyl)-L-lysine (furosine, PubChem CID123889)
*N*ε-(carboxymethyl)-L-lysine (CML, PubChem CID123800)
lysine (PubChem CID5962)
N-(1-deoxy-D-fructos-1-yl)-L-phenylalanine (PubChem CID71316982)
N-(1-deoxy-D-fructos-1-yl)-L-lysine (PubChem CID123708)
N-(1-deoxy-D-fructos-1-yl)-L-glycine (PubChem CID3081391)
N-(1-deoxy-D-fructos-1-yl)-L-valine (PubChem CID71777427)
N-(1-deoxy-D-fructos-1-yl)-L-tryptophan (PubChem CID159983)
N-(1-deoxy-D-fructos-1-yl)-L-asparagine (PubChem CID71316980)
N-(1-deoxy-D-fructos-1-yl)-L-glutamic acid (PubChem CID56971968)

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ABSTRACT

The quantification of protein bound Maillard reaction products (MRPs) is still a challenge in food chemistry. Protein hydrolysis is the bottleneck step: it is time consuming and the protein degradation is not always complete. In this study, the quantitation of free amino acids and Amadori products (APs) was compared to the percentage of blocked lysine by using chemometric tools. Eighty thermally treated soybean samples were analyzed by mass spectrometry to measure the concentration of free amino acids, free APs and the protein-bound markers of the Maillard reaction (furosine, *N*ε-(carboxymethyl)-L-lysine, *N*ε-(carboxyethyl)-L-lysine, total lysine). Results demonstrated that Discriminant Analysis (DA) and Correlated Component Regression (CCR) correctly estimated the percent of blocked lysine in a validation and prediction set. These findings indicate that the measure of free markers reflects the extent of protein damage in soybean samples and it suggests the possibility to obtain rapid information on the quality of the industrial processes.

1. Introduction

Maillard reaction (MR) contributes to the final quality of thermally processed and long term stored foods through a network of reactions involving reducing sugars, free amino groups, intermediates, volatiles and Maillard reaction end-products (MRPs) or dietary advanced glycation end-products (d-AGEs) (van Boekel et al., 2010). Amadori products (APs) and their analogous Heyns products (HPs) are the first stable compounds that lead to the formation of desired and undesired molecules following similar pathways. When carbonyls attachment occurs on the nucleophilic side chains of proteins, the ε-amino group of lysine and guanidino side chain of arginine, bound MRPs are formed. In most foods, the large majority of amino acids is present as part of the proteins so a large percentage of MRPs is bound to the protein (Hellwig & Henle, 2014). Their detection needs acidic or enzymatic hydrolysis of the protein peptide bonds to release the free compounds. The most relevant bound MRPs include *N*ε-(carboxymethyl)-L-lysine (CML), *N*ε-(carboxyethyl)-L-lysine (CEL), pyrraline, glucosepan, pentosidine,

Abbreviations: DA, discriminant analysis; PLS, partial least squares; VIPs, variable importance on projections; CCR, correlated component regression; APs, Amadori products; CML, *N*ε-(carboxymethyl)-L-lysine; CEL, *N*ε-(carboxyethyl)-L-lysine; HILIC, hydrophilic interaction liquid chromatography; HRMS, high resolution mass spectrometry; MS/MS, tandem mass spectrometry

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glyoxal-lysine dimer (GOLD), methylglyoxal-lysine dimer (MOLD), 3-deoxyglucosone-derived imidazolium cross-link (DOGDIC), hydroimidazolones and lysino-alanine (LAL) (Thornalley & Rabbani, 2014). LAL is formed upon β-elimination of the carbanion intermediates between lysine and serine or cysteine and, although is not really a MRPs, it is often used as a marker of thermal treatments (Friedman, 1999).

Free MRPs can be categorized as all the MRPs that are not bound to proteins and therefore do not require an hydrolysis step to be quantified by chromatographic techniques. A brief overview of such category includes small aldehydes, ketones, pyrazines, pyrroles, furan, thiols, dicarbonyls, hydroxycarbonyls, free APs (i.e. the APs formed on amino acids which are not part of polypeptide chains), 5-hydroxymethylfurfural, acrylamide and 4-methylimidazole.

Mass spectrometry techniques are the golden standard for both unbound and bound MRPs quantification. The simultaneous detection of free amino acids and unbound APs was recently proposed by our group and a preliminary overview of multivariate data analysis was presented by using the "FancyTiles" schema (Troise, Ferracane, Palermo, & Fogliano, 2014; Troise, Fiore, Roviello, Monti, & Fogliano, 2015). Three examples of systematic analysis of free markers were recently reported: Schwarzenbolz and co-workers showed that the analysis of free MRPs is a suitable tool to distinguish between organic and conventional production methods of milk. They monitored free CML, pyrraline, methylhydroimodazolinone (MG-H), and *N*-(1-deoxy-D-fructos-1-yl)-L-lysine as markers of the intake of glycated proteins in cows feed (Schwarzenbolz, Hofmann, Sparmann, & Henle, 2016). Our group monitored free APs to evaluate the deglycating activity of fructose-amine oxidase I in low lactose UHT milk (Troise, Buonanno, Fiore, Monti, & Fogliano, 2016) and the side proteolytic activity of lactase in dairy products (Troise et al., 2016).

Bound markers such as CML and CEL were quantified in many study (Thornalley & Rabbani, 2014) while a HPLC-UV method was developed to indirectly quantify bound APs of lysine by measuring furosine after acidic hydrolysis (Resmini, Pellegrino, & Battelli, 1990). Mass spectrometry coupled to stable isotope dilution assay and solid phase extraction allows the robust and simultaneous detection of many products including CML, CEL and furosine along with the quantification of non-reacted lysine (Troise, Fiore, Wiltafsky, & Fogliano, 2015). However this quantification procedure has a severe bottleneck which is the acidic hydrolysis of the proteins. It is time consuming, the strong acid treatment destroys some MRPs and the protein degradation is not always complete. Moreover, this step is influenced by the ratio proteins/acids, by the concentration of the acids, by the matrix, the time, temperature and by the effective reduction of fructose-lysine into hexitol-lysine by using sodium borohydride.

Several authors have used the MR as a source of chemical and statistical inputs to model food quality. Stanimirova and coworkers (2011) performed a detailed study on the relationship between amino acids, sugars mixtures, products formed and aroma sensory profiles by using GC-MS. These "multiblock" data were analyzed by comparing three different methods: the consensus principal component analysis (CPCA), SUM-PCA and multiple factor analysis (MFA). Another approach involved the Classification and Regression Trees (CART) and Multivariate Regression Trees (MRT) to perform supervised feature selection by modeling one response variable by some explanatory variables. These techniques were successfully applied to the detection of cluster structure in data of the sensorial evaluations of MRPs mixtures (Questier, Put, Coomans, Walczak, & Heyden, 2005). The formation of acrylamide in biscuits with different recipes and baking conditions was evaluated by combining the potential of high-throughput direct analysis in real time–high resolution mass spectrometry (DART–HRMS) and multivariate regression analysis via PCA and partial least square regression (PLSR) analysis of the data matrix issued from positive and negative ionization mode fingerprints (Vaclavik, Capuano, Gokmen, & Hajslova, 2015).

In this study, the possibility to avoid protein hydrolysis and the successive samples purification by solid phase extraction, was explored. The hypothesis was that free APs and free amino acids concentrations could be correlated to the bound markers of MR and used in a chemometric model to assess the extent of the protein glycation in an homogenous sampling of industrially treated soybean products.

2. Material and methods

2.1. Chemicals and reagents

Acetonitrile, methanol and water for solid phase extraction (SPE), liquid chromatography tandem mass spectrometry and (LC-MS/MS) and liquid chromatography high resolution mass spectrometry (LC-HMRS) analysis were obtained from Merck (Darmstadt, Germany). The ion pairing agent perfluoropentanoic acid (nonafluoropentanoic acid, NFPA), formic acid, hydrochloric acid (37%), ammonium formate, the analytical standards [4,4,5,5-d_4]-L-lysine hydrochloride (d_4-Lys) and the 20 L-amino acids analytical standards were purchased from Sigma-Aldrich (St. Louis, MO). Analytical standards *N*ε-(2-furoylmethyl)-L-lysine (furosine) and *N*ε-(2-furoyl[$^2H_4$]methyl)-L-lysine (d_4-furosine) were obtained from Polypeptide laboratories (Strasbourg, France). *N*ε-(carboxymethyl)-L-lysine (CML), *N*ε-(carboxy[$^2H_4$]methyl)-L-lysine (d_4-CML), *N*ε-(carboxyethyl)-L-lysine (CEL), *N*ε-(carboxy[$^2H_4$]ethyl)-L-lysine (d_4-CEL), *N*-(1-deoxy-D-fructos-1-yl)-L-isoleucine and *N*-(1-deoxy-D-fructos-1-yl)-L-phenylalanine were purchased from TRC-Chemicals (North York, Canada). The other APs standards *N*-(1-deoxy-D-fructos-1-yl)-L-lysine (Fru-Lys), *N*-(1-deoxy-D-fructos-1-yl)-L-asparagine (Fru-Asn), *N*-(1-deoxy-D-fructos-1-yl)-L-aspartic acid (Fru-Asp), *N*-(1-deoxy-D-fructos-1-yl)-L-histidine (Fru-His), and *N*-(1-deoxy-D-fructos-1-yl)-L-glycine (Fru-Gly) were synthesized according to the procedure previously reported (Troise et al., 2015).

2.2. Soybean samples

Soybean samples (80) were provided by Evonik Nutrition & Care GmbH (Germany). Samples were collected from different regions: Brazil (7), Australia (4), Austria (3), China (10) and Germany (56). Two different soy products were used: soybean meal and soybean full-fat.

2.3. Free amino acids and APs

Free amino acids and APs were analyzed according to Troise et al. (2015) with some modifications. Samples were weighed (0.1 mg) and 4 mL of a ternary mixture of acetonitrile/water/formic acid (85: 14.9: 0.1, v/v/v) was added. The samples were centrifuged at 4 °C, for 15 min at 21,100*g* then filtered by using PVDF filters (0.22 μm Millipore, Billerica, MA). The chromatographic separation of amino acids and their respective APs was achieved by three mobile phases consisting in 0.1% formic acid in acetonitrile (solvent A), 0.1% formic acid in water (solvent B) and 50 mM ammonium formate (solvent C). The following linear gradient of solvent B (min/%B): (0/2), (1.20/2), (4/40), (6/40) was used, while mobile phase C was constant at 10% in order to keep unaltered the ionic strength. The flow rate was set to 400 μL/min and the injection volume was 5 μL. Chromatographic separation of amino acids and APs was achieved through a thermostated (35 °C) Kinetex 2.6 μm (75 × 2.1 mm) core shell silica HILIC column and a guard column with the same stationary phase (Phenomenex, Torrance, CA). The Accela 1250 U-HPLC system (Thermo Fisher Scientific, Bremen, Germany) was directly interfaced to an Exactive Orbitrap HRMS (Thermo Fisher Scientific, Bremen, Germany) and the analytes were detected through a heated electrospray interface (HESI-II) operating in the positive mode. The current ion associated to each compound listed in Table 1 was scanned in the *m/z* range of 60–400. The resolving power was set to 75,000 full width at half maximum (FWHM, *m/z* 200) resulting in a scan time of 1 s. The automatic gain control was used in high dynamic range mode (3×10^6 ions); maximum injection time was