JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Enhanced Approaches for Identifying Amadori Products: Application to Peanut Allergens

Katina L. Johnson,[†] Jason G. Williams,[†] Soheila J. Maleki,[‡] Barry K. Hurlburt,[‡] Robert E. London,[§] and Geoffrey A. Mueller*,[§]

[†]Epigenetics & Stem Cell Biology Laboratory, and [§]Genome Integrity & Structural Biology Laboratory, National Institute of Environmental Health Sciences (NIEHS), 111 T.W. Alexander Drive, Research Triangle Park, North Carolina 27709, United States

[‡]Agricultural Research Service, United States Department of Agriculture, 1100 Robert E. Lee Boulevard, New Orleans, Louisiana 70124, United States

ABSTRACT: The dry roasting of peanuts is suggested to influence allergic sensitization as a result of the formation of advanced glycation end products (AGEs) on peanut proteins. Identifying AGEs is technically challenging. The AGEs of a peanut allergen were probed with nano-scale liquid chromatography−electrospray ionization−mass spectrometry (nanoLC−ESI−MS) and tandem mass spectrometry (MS/MS) analyses. Amadori product ions matched to expected peptides and yielded fragments that included a loss of three waters and HCHO. As a result of the paucity of b and y ions in the MS/MS spectrum, standard search algorithms do not perform well. Reactions with isotopically labeled sugars confirmed that the peptides contained Amadori products. An algorithm was developed on the basis of information content (Shannon entropy) and the loss of water and HCHO. Results with test data show that the algorithm finds the correct spectra with high precision, reducing the time needed to manually inspect data. Computational and technical improvements allowed for better identification of the chemical differences between modified and unmodified proteins.

KEYWORDS: *peanuts (Arachis hypogaea), allergen, advanced glycation end products, Amadori products, Maillard reactions, Shannon entropy*

INTRODUCTION

Allergies to peanuts are an increasing concern in public health. In the United States, 1−2% of the population, or nearly 3 million people, are allergic to peanuts.[1] The molecular basis for peanut allergy has been the subject of extensive commentary and research.[2] One hypothesis suggests the common method of processing peanuts in the U.S., i.e., dry roasting, increases the allergenic nature of peanuts and tree nuts.[3,4] During dry roasting, the proteins can be extensively modified with advanced glycation end products (AGEs), and the connection to allergy has been supported by several *in vitro* studies, suggesting that AGE-modified proteins skew the immune response toward allergy.[5−7] Recently, these findings were corroborated in a murine study where dry roasting enhanced peanut allergic sensitization across mucosal and cutaneous routes.[8] Because the AGE modifications appear to influence sensitization, knowledge of their chemical structures will be important for understanding the mechanism of action.

Characterizing the AGE modifications on peanut allergens has proven challenging. Early studies used antibodies specific for certain types of AGEs.[9] More recent studies have used mass spectrometry (MS) to specifically identify chemical modifications and the modified residues.[10−12] This provided detailed atomic information, but several technical challenges make this a difficult process. First, the modifications on lysine and arginine residues prevent digestion of the allergens with commonly used trypsin-related proteases. Second, the proteins were difficult to extract and purify from roasted peanuts[13] and required extraction with urea[11] or multiple chromatography steps.[12] Despite problems, some commonly modified peptides have been identified.[10−12]

AGEs are created in a non-enzymatic mechanism known as the Maillard reaction. This process is accelerated by higher temperatures and dry roasting, which leads to an increased number of AGE modifications compared to boiling.[14] The first step in the formation of an AGE occurs when sugars react primarily with free amines, forming a Schiff base.[15] The modifications are most common on lysines and are less frequently observed on arginines, the N terminus, and cysteines.[16] The sugar modification then undergoes a further Amadori rearrangement to create a covalently modified protein. The ketone of the Amadori product, however, can initiate further decomposition, resulting in a variety of different AGEs. The most common AGE created is a carboxymethyllysine, which is produced by additional adduct oxidation.

During manual examination of tandem mass spectrometry (MS/MS) spectra of peanut extract for modifications, it was noticed that many spectra were observed that demonstrated significant neutral loss of water but very little to no b and y ion formation from the peptide backbone.[12] It was previously noted that peptides modified by the Amadori product produce a common fragmentation pattern in MS/MS experiments, in which ions that correspond primarily to neutral loss of three and four waters and an ion that corresponds to neutral loss of

Received: November 18, 2015
Revised: January 14, 2016
Accepted: January 25, 2016

ACS Publications

This article not subject to U.S. Copyright. Published XXXX by the American Chemical Society

DOI: 10.1021/acs.jafc.5b05492
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

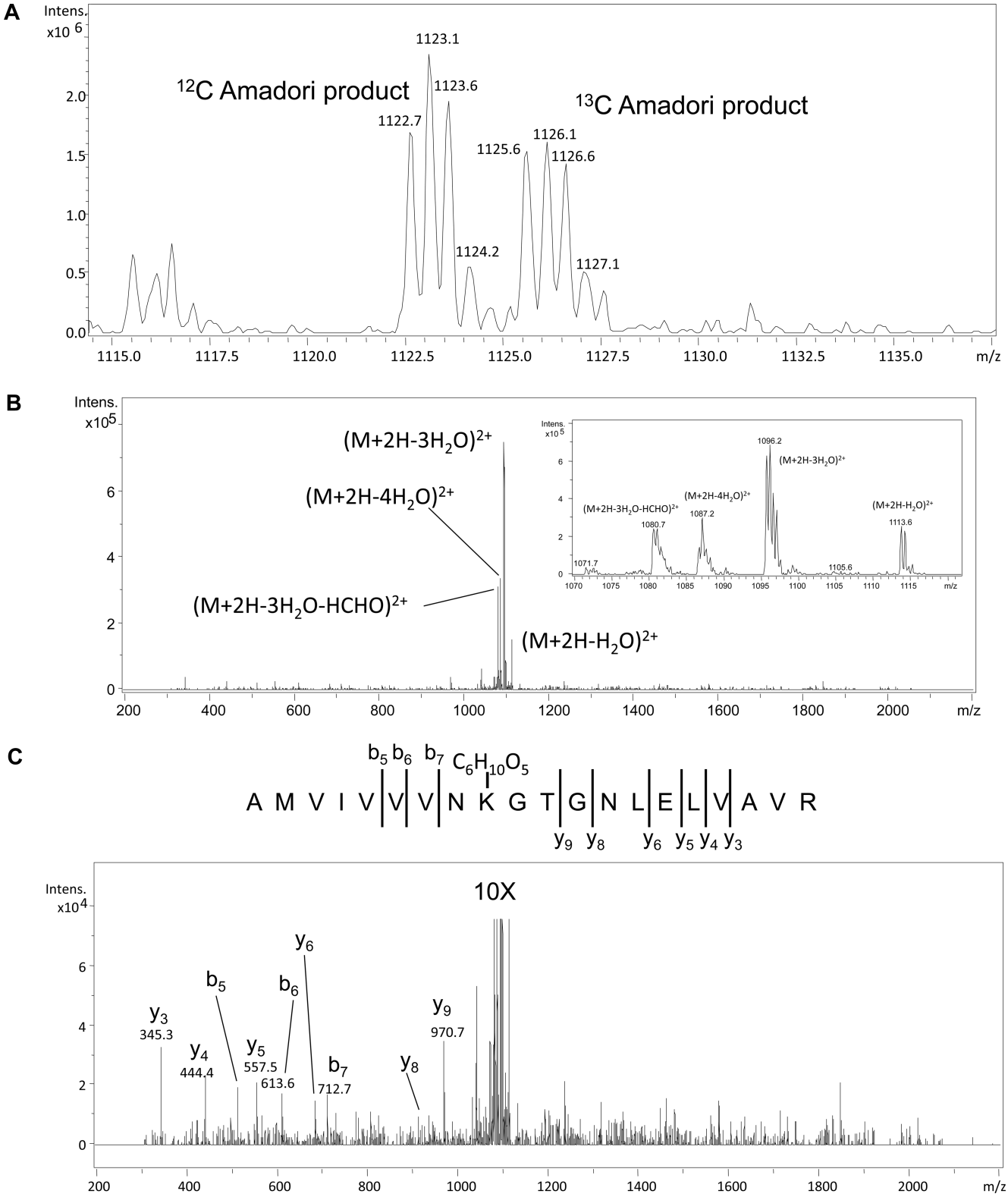

Figure 1. Mass spectra of the tryptic peptide spanning amino acids 452−471 from glycated recombinant Ara h 1. Panel A shows the mass spectrum of the dyad of ions at m/z 1122.7 and 1125.6 that correspond to amino acid residues 452−471, with K460 modified by glycation that arises from the incubation of rAra h 1 with 1:1 ^{12}C glucose/^{13}C glucose. Panel B is the low-information MS/MS spectrum of ion m/z 1122.7, with an inset showing a zoom from m/z 1070 to 1120. The neutral losses of three waters and three waters plus HCHO are characteristic for Amadori-product-modified peptides. Panel C is the 10× zoom of MS/MS of ion m/z 1122.7, which shows very low-abundance b- and y-series ions.

three waters and HCHO are observed.[17] Very few b and y ions are generated from the fragmentation of these peptides, making it difficult to identify the peptide.[17] These spectra are commonly scored poorly by standard software packages because little information on the peptide sequence is obtained, and even when b and y ions are present, dominant fragment

DOI: 10.1021/acs.jafc.5b05492
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

ions (the neutral loss of three and four waters and the neutral loss of three waters and HCHO) are unassigned. However, a careful manual examination can sometimes find b- and y-series ions at low signal-to-noise levels that can then be employed for assignment of the peptide sequence. In this paper, the correspondence between the low-information spectra and the glycated peptides was confirmed with isotopic labeling of the precursor sugars and recombinant peanut protein, an idea suggested by other glycation studies.[18,19] To target these low-information spectra for glycation analysis, a new computational algorithm was designed using a Shannon-entropy-based method.

MATERIALS AND METHODS

Glycation of Recombinant Ara h 1. Purified recombinant Ara h 1 (0.5 mg/mL) (mature sequence, amino acid residues 85−626 of UniProtKB/Swiss-Prot accession P43238.1)[12] was solubilized in phosphate-buffered saline (PBS) and incubated at 55 °C in the presence of 0.25 mol/L glucose, xylose, 1:1 ^{12}C glucose/^{13}C glucose, or 1:1 ^{12}C xylose/^{13}C xylose for 0 or 10 days. Trypsin was added at a 20:1 substrate/enzyme ratio, and digestion was carried out at 37 °C for 14 h. Reactions were stopped by freezing at −80 °C for use in subsequent MS analysis.[12]

Nano-scale Liquid Chromatography−Electrospray Ionization−Tandem Mass Spectrometry (NanoLC−ESI−MS/MS). NanoLC−ESI−MS/MS analyses were performed using an Agilent 1100 nanoLC system online with an Agilent XCT Ultra ion trap mass spectrometer with the chip cube interface. A total of 20 μL of the peptide digest was loaded onto an Agilent C_{18} chip (75 μm × 43 mm). Peptides were eluted by applying a linear gradient from 5% acetonitrile and 0.1% formic acid to 50% acetonitrile and 0.1% formic acid to the column over 45 min. The mass spectrometer was used in the positive-ion mode and standard enhanced mode and included settings of a mass range from m/z 200 to 2200, an ionization potential of 2.1 kV, an ion charge control (ICC) smart target of 200 000 or 200 ms of accumulation, and a 1.0 V fragmentation amplitude. MS/MS data were acquired using a data-dependent acquisition format, with the six most abundant ions from each MS scan further interrogated by MS/MS. The automated switching for MS/MS required a threshold of 10 000 counts.

Generation of Peak Lists and Traditional Database Searching. Peak lists were generated from the data obtained from the nanoLC−ESI−MS/MS analysis using the Data Extractor feature of the Spectrum Mill software from Agilent. The Data Extractor settings included limiting the data search to deconvoluted ions observed between 400 and 6000 Da and a retention time between 10 and 50 min. Moreover, of the remaining MS/MS spectra, only spectra that contained sequence tag information greater than or equal to 1 residue were submitted for database searching. The resulting extracted data were then searched iteratively against the sequence of the recombinant Ara h 1 (gi:347447590) using the MS/MS Search function in the Spectrum Mill software. Search settings included a trypsin specificity with two missed cleavages allowed, a precursor ion mass tolerance of 2 Da, a product ion mass tolerance of 0.7 Da, variable methionine oxidation, and a minimum matched spectral intensity of 70%. Putative matches were manually validated, and the remaining unmatched spectra were then searched again against the rAra h 1 sequence using similar settings but allowing for lysine modification by either carboxymethyl, carboxyethyl, or Amadori products.

Test Data Set. A LC−ESI−MS/MS test data set was generated using the tryptic digest of a 10 day incubation of rAra h 1 with ^{12}C xylose. The 2929 MS/MS spectra were manually evaluated for fragmentation in MS/MS that was low-information and also suggested neutral loss of water(s) being the primary fragment ion(s). The resulting spectra were then manually matched to peptides of rAra h 1 modified by Amadori products or AGEs. The resulting 12 spectra with confirmed Amadori products were then used for evaluation of the Shannon-entropy-based algorithm.

NanoLC−ESI−MS/MS/MS. NanoLC−ESI−MS/MS/MS analyses were performed using an Agilent 1200 nanoLC system online with an Agilent 6340 ion trap mass spectrometer with the chip cube interface using the same LC and MS/MS conditions described above. Ions that demonstrated a neutral loss of m/z 27, 18, or 13.5 from the precursor in the MS/MS spectrum were further analyzed in an automated way by MS/MS/MS experiments.

Computational Algorithm. Shannon entropy is a measure of the information content of a message or data set.

$$H = -\sum_i P(x_i)\log_b(P(x_i)) \tag{1}$$

H is the Shannon entropy, so named because it resembles the Boltzman equation for thermodynamic entropy. P can be any data. In this case, it is the intensity at a m/z ratio of x. The equation sums the product of $P(x)$ times $\log(P(x))$ over all i points in the mass spectrum. The base of logarithm can be any number; in this case, $b = 10$. In data not shown, there was very little discriminatory gain with different bases. For each mass spectrum analyzed here, the ion peak lists were generated using the Data Extractor function of Spectrum Mill MS Proteomics Workbench (Agilent) and binned into vectors of length i, with a cumulative intensity in each bin. The intensities for each vector $P(x)$ were normalized prior to calculating H.

After sorting the values of H for the spectra with the lowest values, the second step of the algorithm was to check for the ion pattern typical of the Amadori products, namely, that the maximum abundance ion had a neutral loss of a water and a loss of HCHO. All of the ions in the top 10% of intensities were checked for two corresponding mass peaks at the 2+, 3+, or 4+ states for $3H_2O$ and HCHO, with a mass tolerance of 2 Da. Both the Shannon-entropy- and ion-pattern-based filtering steps are evaluated below.

RESULTS

Amadori Products Identified from ^{12}C/^{13}C Glucose-Treated Samples. The use of stable isotopes has routinely been used in conjunction with MS for quantitation of metabolites and, more recently, proteins. In fact, a stable isotope dilution strategy has recently been employed to quantitate Amadori compounds in foods.[20] In addition to aiding with quantitation, mixtures of isotopes can also be used to create mass signatures, so that post-translational or chemical modifications can be more readily detected, as was performed by Priego-Capote et al. in efforts to identify AGEs.[18] In efforts to identify previously uncharacterized Amadori products or AGE modifications, recombinant Ara h 1 was treated with a 1:1 mixture of ^{12}C/^{13}C glucose and subjected to digestion, followed by nanoLC−MS and MS/MS. Modification by glucose and subsequent rearrangement to the Amadori product and/or decay to an AGE results in peptides that are readily observed as a dyad of ions, with one ion arising from the ^{12}C glucose and a sister ion arising from the ^{13}C glucose. These dyads are easily recognized when manually interrogating individual MS spectra. The mass difference between the pair of ions should be 1 Da for each carbon incorporated, as demonstrated by Stefanowicz et al. and Priego-Capote et al.[18,19] Figure 1A shows a MS spectrum of the dyad (m/z 1122.7 and 1125.6), where a peptide is modified by a six-carbon product. The ion at m/z 1122.7 corresponds to the species that contains all ^{12}C, and the ion at m/z 1125.6 corresponds to the species that contains all ^{13}C. Both species are doubly charged, hence the m/z difference of 3 for a six-carbon modification. In this example, the dyad corresponds to the tryptic peptide containing residues 452−471 of our construct of Ara h 1 modified by an Amadori product at lysine 460. Figure 1B shows example data characteristic of a low-information MS/MS spectrum similar to those previously

DOI: 10.1021/acs.jafc.5b05492
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

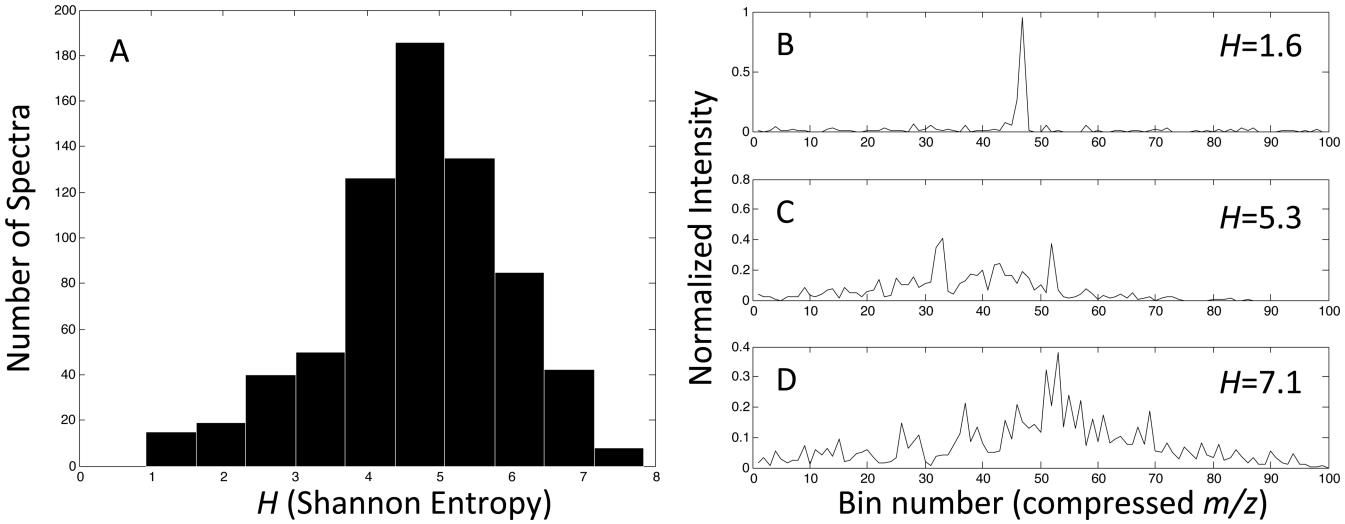

Figure 2. Shannon entropy of binned MS spectra. The Shannon entropy H was calculated for the test set of spectra according to eq 1. (A) Histogram of H for the test spectra. (B−D) Examples of binned MS spectra (a compressed m/z scale) for visual comparison of calculated H values.

observed in studies of *in vitro* glycated human serum and model peptides.[17,21,22] A few characteristics to note are the scarcity of fragment ions, with those few that are observed being of high abundance and corresponding to the neutral loss of waters and the neutral loss of three waters and HCHO from the precursor ion. This behavior matches well with pervious observations[21] and the scheme suggested by Frovol et al.[22] However, looking on an intensity scale 10 times larger, Figure 1C demonstrates that the MS/MS spectrum does contain low-abundance b and y ions that can be manually interpreted. This suggested that further targeted manual analyses of these characteristic spectra may be useful in identifying the modified peptides and the sites of Amadori product formation.

Design of the Shannon Entropy Algorithm. A single nanoLC−MS/MS analysis of *in vitro* glycated recombinant Ara h 1 yielded 2929 individual MS/MS spectra. These spectra were triaged using the Data Extractor routine from the Agilent Spectrum Mill MS Proteomics Workbench, resulting in 706 spectra meeting the criterion outlined in the Materials and Methods. Among this set of 706 MS/MS spectra, 12 spectra with data similar to that shown in Figure 1A were manually confirmed as containing Amadori products. Figure 2 shows a histogram of the calculated Shannon entropy (H), for the test set, using a bin size of 100. Panels B, C, and D show three examples of the normalized and binned MS data for which the H values are 1.6, 5.3, and 7.1, respectively. Qualitatively, this confirmed that a low value of the Shannon entropy would be able to identify the type of spectra containing the typical Amadori product fragmentation pattern seen in Figure 1A.

The goal of the search algorithm was to significantly reduce the number of MS/MS or MS^3 spectra that needed to be manually interrogated in efforts to identify b and y ions that may lead to peptide identification and localization of the site of the Amadori product modification but, at the same time, not miss any of these rare modifications. Hence, the parameters were optimized to include a high false-positive rate. For example, the optimal size of the number of bins is a trade-off between m/z resolution and increased noise. The Shannon entropy was calculated for different bin sizes, and the data were assessed for whether or not the known correct 12 spectra landed in the bottom percent of the H values. Figure 3 shows that, at small binning values, the resolution of the data is

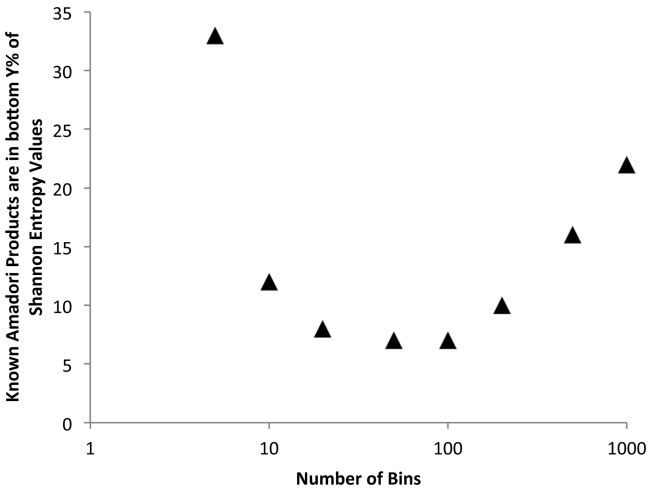

Figure 3. Optimizing search strategy. The number of bins (compressed m/z) is plotted against the bottom percent of H values of the known Amadori product spectra that occur. The optimum number of bins (between 50 and 100) puts the known spectra among the fewest of false positives, about 7%.

inadequate to use H to spot the 12 spectra, while at binning values >100, noise begins to dominate the calculation and H is again ineffective at identifying the correct spectra. A bin size of 50 or 100 was equivalent in maximally reducing the number of spectra that needed to be searched, by 93%. To be conservative in future studies, the top 90% of H values was eliminated from further examination using a bin size of 50.

Next, the peak lists of the bottom 10% of H values (n = 71) were examined for spectra that contained the characteristic fragmentation pattern in Figure 1A. These characteristics were the maximum abundance ion had a neutral loss of three water molecules from a 2+, 3+, or 4+ precursor ion and a neutral loss of HCHO. Empirically, the neutral loss of three waters was the most abundant fragment ion. This eliminated 34 more spectra from consideration and retained 37 spectra that still contained the correct 12 spectra. Hence, the false-positive rate was 68%, but the combination of the two filtering procedures reduced the number of data sets that needed to be examined by 95%. As an aside, if the Shannon entropy filter is not used, a filter based

DOI: 10.1021/acs.jafc.5b05492
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

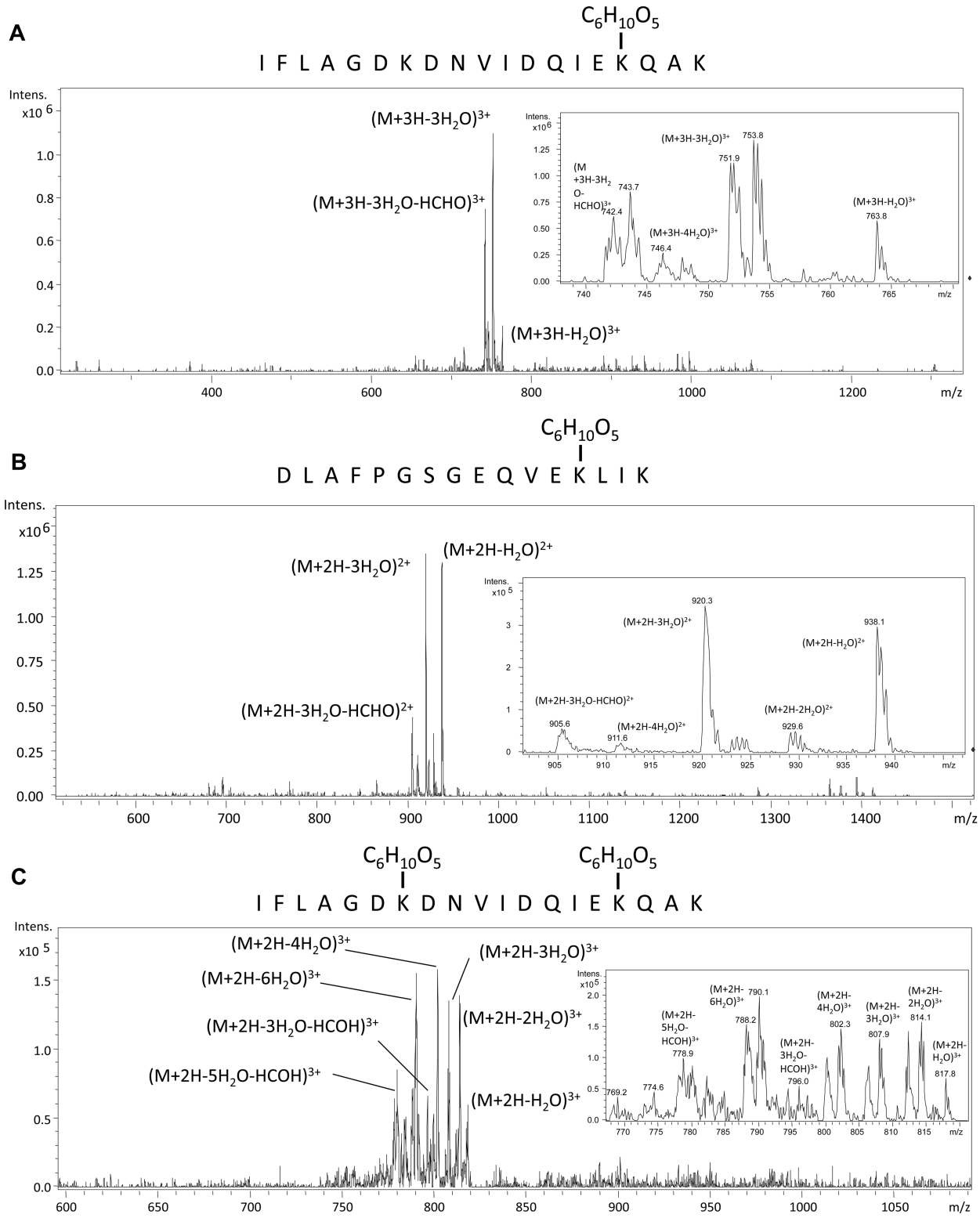

Figure 4. Example mass spectra of several Amadori-product-modified tryptic peptides from rAra h 1. Panel A is the MS/MS spectrum of the peptide containing amino acids 541−559 with K556 modified by an Amadori product. The inset is a zoom from m/z 740 to 770, with ions corresponding to the losses of one, three, four, and three H_2O and HCHO labeled. The sister ions at m/z 743.7, 747.9, and 753.8 correspond to the ^{13}C-labeled Amadori product (m/z 771.8) that co-isolated during MS/MS of the ^{12}C ion, m/z 769.9. Panel B is the MS/MS spectrum of the peptide spanning amino acids 560−575 with K572 modified by an Amadori product. The inset is a zoom from m/z 900 to 945, with ions corresponding to the losses of one, two, three, four, and three H_2O and HCHO labeled. Panel C is the MS/MS spectrum of the peptide containing amino acids 541−559 with both K547 and K556 modified by Amadori products. The inset is a zoom from m/z 775 to 820, with ions corresponding to multiple water losses and water and HCHO losses labeled. This spectrum is extremely complicated because the fragmentation of two Amadori products and the fact that all of the neutral losses exist as ion dyads as a result of the co-isolation of the ^{13}C-labeled Amadori product containing peptide with the natural abundance (99% ^{12}C) Amadori-product-labeled peptide when performing MS/MS.

DOI: 10.1021/acs.jafc.5b05492
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

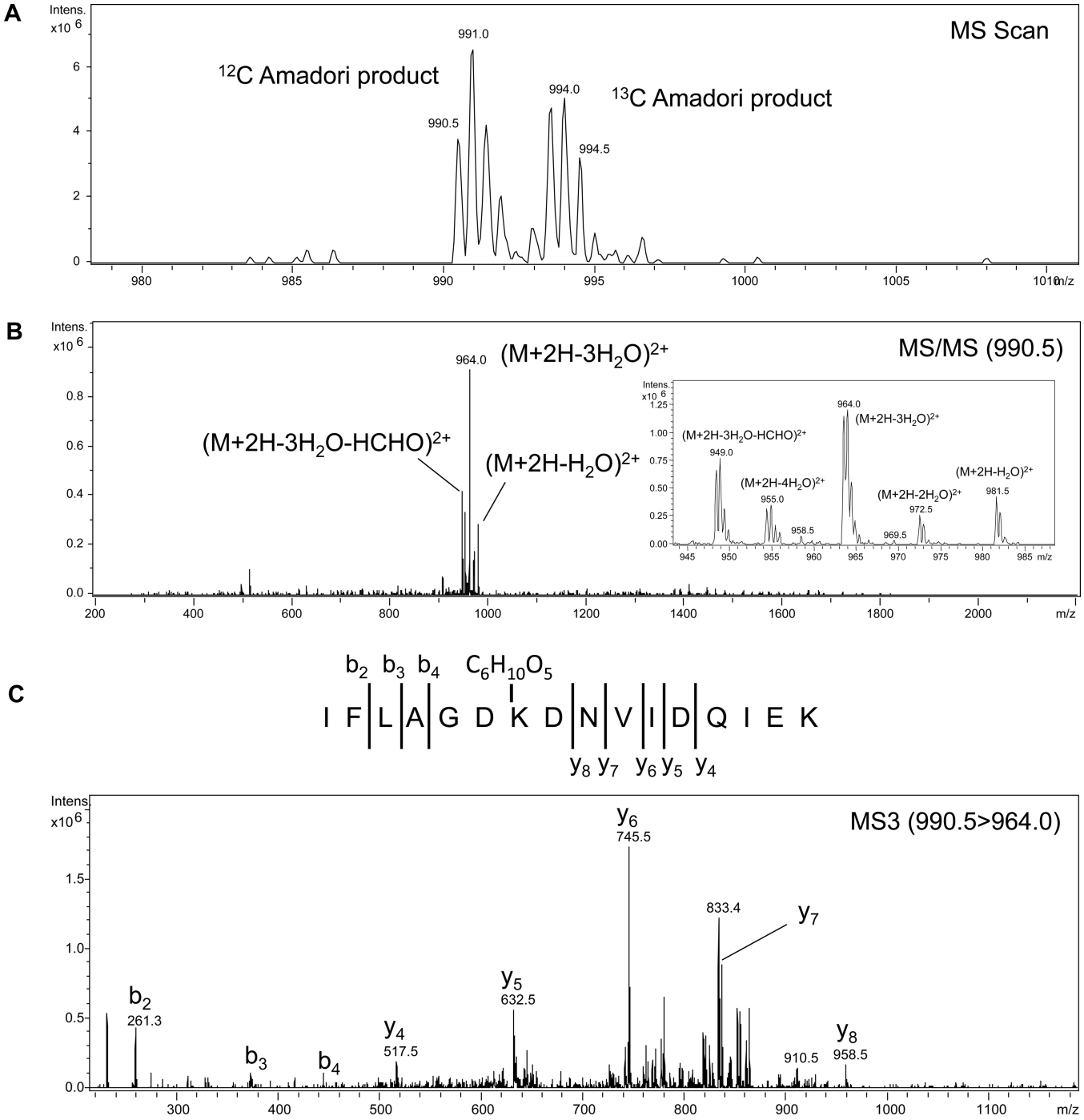

Figure 5. Example of the MS, MS/MS, and MS^3 data that result from a neutral-loss-triggered MS^3 experiment performed on rAra h1. The data in panel A again show a mass spectrum that demonstrates a dyad of ions corresponding to a tryptic peptide that is glycated as a result of the incubation of rAra h 1 with 1:1 ^{12}C glucose/^{13}C glucose. Panel B is the MS/MS spectrum of ion *m/z* 990.5, showing the loss of multiple waters and HCHO, with an inset showing a zoom from *m/z* 945 to 990. Panel C shows the MS^3 of the ion *m/z* 990.5 > 964.0, with the resulting b and y ions annotated that allow for the unambiguous assignment of the Amadori-modified tryptic peptide covering amino acid residues 541−559, with K547 as the site of modification.

only on the loss of water and HCHO correctly retains the 12 spectra of interest but eliminates only 37% of the total spectra for manual interpretation.

Applying the Shannon Entropy and Ion Filter. The algorithm described above was applied to two additional nanoLC−ESI−MS/MS experiments containing 2948 and 3029 MS/MS spectra, and candidate spectra were identified. Panels A (amino acids 541−559 K556−Amadori), B (amino acids 560−575 K572−Amadori), and C (amino acids 541−559 K547−Amadori and K556−Amadori) of Figure 4 show example MS/MS data of several ions found to correspond to an Amadori product modification of recombinant, *in vitro* glycated peanut allergen, Ara h 1. These data further suggested that MS^3 of ions that arose from neutral loss of water in the MS/MS would be useful for the unambiguous assignment of the candidate ions to tryptic peptides from Ara h 1. Hence, we

DOI: 10.1021/acs.jafc.5b05492
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Table 1

sequence number	peptide sequence[a]	predicted MW	m/z	charge state	observed MW	delta mass	corresponding modification[b]
214−228	IVQIEAK*PNTLVLPK	1662	912.7	2	1823.4	161.4	Amadori product
285−307	VAK*ISMPVNTPGQFEDFFPASSR	2524.2	896.4	3	2686.2	162	Amadori product
386−401	K*GSEEEGDITNPINLR	1770.9	967.7	2	1933.4	162.5	Amadori product
402−421	EGEPDLSNNFGK*LFEVKPDK	2262.1	808.4	3	2422.2	160.1	Amadori product
414−421	LFEVK*PDK	974.5	569.5	2	1137	162.5	Amadori product
452−471	AMVIVVVNK*GTGNLELVAVR	2081.2	748.6	3	2242.8	161.6	Amadori product
461−477	GTGNLELVAVRK*EQQQR	1925	661.7	3	1982.1	57.1	CML
461−477	GTGNLELVAVRK*EQQQR	1925	666.5	3	1996.5	71.5	CEL
461−477	GTGNLELVAVRK*EQQQR	1925	696.2	3	2085.6	160.6	Amadori product
541−556	IFLAGDK*DNVIDQIEK	1817	990	2	1978	161	Amadori product
541−559	IFLAGDKDNVIDQIEK*QAK	2144.2	769.9	3	2306.7	162.5	Amadori product
541−559	IFLAGDK*DNVIDQIEK*QAK	2144.2	823.4	3	2467.2	323	2× Amadori product
557−575	QAK*DLAFPGSGEQVEKLIK	2057.1	706.2	3	2115.6	58.5	CML
560−575	DLAFPGSGEQVEK*LIK	1729.9	947	2	1892	162.1	Amadori product

[a]An asterisk indicates the site of peptide modification. [b]CML, carboxymethyllysine; CEL, carboxyethyllysine; and Amadori product, $C_6H_{10}O_5$.

employed a strategy similar to the strategy employed by Zhang et al., where neutral-loss-triggered MS^3 was used.[17] Figure 5 shows the MS, MS/MS, and MS^3 data that result from such an experimental approach. The data in Figure 5A showing a dyad of ions confirm that an isotopic mixture of the two sugars was used in creating the glycation. Figure 5B shows the characteristic low-information MS/MS and ion fragmentation pattern observed when performing MS/MS on many glycated peptides. Finally, Figure 5C shows MS^3 with the annotated b and y ions that were used to identify the modified peptide and glycation product. The sum of these data allowed us to unambiguously identify a tryptic peptide spanning the amino acid residues 541−556 with an Amadori product modification of K547. Table 1 lists the new Ara h 1 modifications that were identified using some or all of this combined procedure. A total of 13 new modifications were identified that had not been previously characterized.

DISCUSSION

Using isotopic labeling, MS^3, and computational strategies, we were able to formulate a workflow that enabled the rapid identification of Amadori-product-modified peptides. In a test sample containing 2929 total MS/MS spectra, using only computational approaches, we were able to reduce the number of spectra to 37 for manual interpretation. These 37 spectra contained all 12 spectra of the Amadori-product-modified peptides. Two of the modified peptides were observed as both 2+ and 3+ ions, but there was no significant difference in the fragmentation patterns based on which the charge state was selected. It should be pointed out, however, that we cannot make any generalizations with regard to the charge state dependence of the fragmentation of the Amadori products as a result of this extremely small sampling. Nevertheless, this combination of approaches facilitated the identification of 13 additional ions corresponding to peptides containing modifications on the peanut allergen, Ara h 1, that we previously had not been able to characterize.

The peptides in Table 1 show that some of the same peptides are modified with different AGEs. This is theoretically possible because AGEs can be heterogeneous. It is interesting to speculate that the local protein structure and chemistry may have some influence on the type of AGE modifications, but not enough data is available to fully assess this. Some of the peptides in Table 1 were identified in other studies as AGE-modified peptides.[10−12] Whether or not these are "hot spots" for AGE modifications is unknown. Because the AGEs are still problematic to identify, it is difficult to conclude that other solvent-accessible sites are not modified. It is important to not overinterpret the currently small data set of modifications.

If glycation is suspected before mass spectrometric analyses, additional techniques, such as neutral-loss-directed MS^3, multistage activation, and electron-transfer dissociation, would be warranted and could lead to more robust characterization of sites of glycation. The Shannon entropy algorithm detailed here occupies a niche where it is likely best applied as a rescue technique to reanalyze data-dependent acquisition data sets that have already been collected. In other words, if no modified peptides were found with standard software packages, the data can be parsed again with this algorithm to reduce the data set to a manageable size (nearly a 99% reduction in our test case) for manual MS/MS spectra interpretation.

This computational technique is simple and easy to generalize. The utility of the isotopic labeling was only to confirm the initial suspicion that these low-information spectra were indeed the spectra that contained the modified peptides. This is not necessary in future applications. Other applications may include the characterization of glycosylated peptides, where the MS/MS collisional energy is primarily absorbed by the carbohydrate, which also reduces the number of b and y ions for peptide identification. Additional health applications include the identification of glycation markers in patient sera of type 2 diabetes, aging, and cardiovascular disease.[23,24]

In the future, the technique can be applied to peanut extract and other foods.[25] In a preliminary examination of peanut extracts, the method appears applicable; however, the variety and mass degeneracy of AGEs is currently confounding further progress. Further purification of the protein of interest may be required. Nevertheless, knowledge of the common fragmentation patterns and exact masses may be useful for MS detection of trace amounts of peanut allergen in prepared food. This could improve the accuracy and safety of food labeling, as recently proposed.[11] Additionally, identifying the sites of modification and the chemical modifications may explain why dry roasting skews the immune response toward allergy. In a study directly applicable to Ara h 1 studied here, AGE-modified Ara h 1 was demonstrated to influence the proliferation of Caco-2 cells, which are a model for intestinal epithelia. This influence depended upon the incubation time and temperature

DOI: 10.1021/acs.jafc.5b05492
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

of Ara h 1 with the sugars, indicating the possibility that specific AGE modifications may be important for influencing the pro-inflammatory network.[26] The technique developed here will be applicable in characterizing the modifications that lead to the differential effect.

AUTHOR INFORMATION

Corresponding Author

*Telephone: 919-541-3872. Fax: 919-541-5707. E-mail: geoffrey.mueller@nih.gov.

Funding

This research was supported in part by Research Projects Z01-ES102885-01 to Robert E. London and Z01-ES102488-05 to Jason G. Williams in the Intramural Research Program of the National Institute of Environmental Health Sciences, National Institutes of Health.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Andrea Adams for technical assistance and Drs. Peter Thompson and Jeffrey Kuhn for a critical reading of the manuscript.

ABBREVIATIONS USED

AGE, advanced glycation end product; CML, carboxymethyllysine; CEL, carboxyethyllysine; LC, liquid chromatography; ESI, electrospray ionization; MS, mass spectrometry

REFERENCES

(1) Liu, A. H.; Jaramillo, R.; Sicherer, S. H.; Wood, R. A.; Bock, S. A.; Burks, A. W.; Massing, M.; Cohn, R. D.; Zeldin, D. C. National prevalence and risk factors for food allergy and relationship to asthma: Results from the National Health and Nutrition Examination Survey 2005−2006. *J. Allergy Clin. Immunol.* **2010**, *126*, 798−806.

(2) Mueller, G. A.; Maleki, S. J.; Pedersen, L. C. The Molecular Basis of Peanut Allergy. *Curr. Allergy Asthma Rep.* **2014**, *14*, 429.

(3) Beyer, K. B.; Morrowa, E.; Li, X. M.; Bardina, L.; Bannon, G. A.; Burks, A. W.; Sampson, H. A. Effects of cooking methods on peanut allergenicity. *J. Allergy Clin. Immunol.* **2001**, *107*, 1077−1081.

(4) Iwan, M.; Vissers, Y. M.; Fiedorowicz, E.; Kostyra, H.; Kostyra, E.; Savelkoul, H. F. J.; Wichers, H. J. Impact of Maillard Reaction on Immunoreactivity and Allergenicity of the Hazelnut Allergen Cor a 11. *J. Agric. Food Chem.* **2011**, *59*, 7163−7171.

(5) Ilchmann, A.; Burgdorf, S.; Scheurer, S.; Waibler, Z.; Nagai, R.; Wellner, A.; Yamamoto, Y.; Yamamoto, H.; Henle, T.; Kurts, C.; Kalinke, U.; Vieths, S.; Toda, M. Glycation of a food allergen by the Maillard reaction enhances its T-cell immunogenicity: Role of macrophage scavenger receptor class A type I and II. *J. Allergy Clin. Immunol.* **2010**, *125*, 175−183.

(6) Buttari, B.; Profumo, E.; Capozzi, A.; Facchiano, F.; Saso, L.; Sorice, M.; Rigano, R. Advanced glycation end products of human beta(2) glycoprotein I modulate the maturation and function of DCs. *Blood* **2011**, *117*, 6152−6161.

(7) Hilmenyuk, T.; Bellinghausen, I.; Heydenreich, B.; Ilchmann, A.; Toda, M.; Grabbe, S.; Saloga, J. Effects of glycation of the model food allergen ovalbumin on antigen uptake and presentation by human dendritic cells. *Immunology* **2010**, *129*, 437−45.

(8) Moghaddam, A. E.; Hillson, W. R.; Noti, M.; Gartlan, K. H.; Johnson, S.; Thomas, B.; Artis, D.; Sattentau, Q. J. Dry roasting enhances peanut-induced allergic sensitization across mucosal and cutaneous routes in mice. *J. Allergy Clin. Immunol.* **2014**, *134*, 1453−1456.

(9) Chung, S. Y.; Champagne, E. T. Association of end-product adducts with increased IgE binding of roasted peanuts. *J. Agric. Food Chem.* **2001**, *49*, 3911−3916.

(10) Chassaigne, H.; Norgaard, J. V.; van Hengel, A. J. Proteomics-based approach to detect and identify major allergens in processed peanuts by capillary LC-Q-TOF (MS/MS). *J. Agric. Food Chem.* **2007**, *55*, 4461−4473.

(11) Hebling, C. M.; McFarland, M. A.; Callahan, J. H.; Ross, M. M. Global Proteomic Screening of Protein Allergens and Advanced Glycation Endproducts in Thermally Processed Peanuts. *J. Agric. Food Chem.* **2013**, *61*, 5638−5648.

(12) Mueller, G. A.; Maleki, S. J.; Johnson, K.; Hurlburt, B. K.; Cheng, H.; Ruan, S.; Nesbit, J. B.; Pomes, A.; Edwards, L. L.; Schorzman, A.; Deterding, L. J.; Park, H.; Tomer, K. B.; London, R. E.; Williams, J. G. Indentification of Maillard reaction products on panut allergens that influence binding to the receptor for advanced glycation end products. *Allergy* **2013**, *68*, 1546−1554.

(13) Schmitt, D. A.; Nesbit, J. B.; Hurlburt, B. K.; Cheng, H. P.; Maleki, S. J. Processing Can Alter the Properties of Peanut Extract Preparations. *J. Agric. Food Chem.* **2010**, *58*, 1138−1143.

(14) Uribarri, J.; Woodruff, S.; Goodman, S.; Cai, W. J.; Chen, X.; Pyzik, R.; Yong, A.; Striker, G. E.; Vlassara, H. Advanced Glycation End Products in Foods and a Practical Guide to Their Reduction in the Diet. *J. Am. Diet. Assoc.* **2010**, *110*, 911−916.

(15) Hodge, J. E. The Amadori Rearrangement. *Adv. Carbohyd Chem.* **1955**, *10*, 169−205.

(16) Rabbani, N.; Thornalley, P. J. Glycation research in amino acids: A place to call home. *Amino Acids* **2012**, *42*, 1087−96.

(17) Zhang, Q.; Petyuk, V. A.; Schepmoes, A. A.; Orton, D. J.; Monroe, M. E.; Yang, F.; Smith, R. D.; Metz, T. O. Analysis of non-enzymatically glycated peptides: Neutral-loss-triggered MS3 versus multi-stage activation tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3027−3034.

(18) Priego-Capote, F.; Scherl, A.; Muller, M.; Waridel, P.; Lisacek, F.; Sanchez, J. C. Glycation Isotopic Labeling with C-13-Reducing Sugars for Quantitative Analysis of Glycated Proteins in Human Plasma. *Mol. Cell. Proteomics* **2010**, *9*, 579−592.

(19) Stefanowicz, P.; Kijewska, M.; Kluczyk, A.; Szewczuk, Z. Detection of glycation sites in proteins by high-resolution mass spectrometry combined with isotopic labeling. *Anal. Biochem.* **2010**, *400*, 237−243.

(20) Meitinger, M.; Hartmann, S.; Schieberle, P. Development of Stable Isotope Dilution Assays for the Quantitation of Amadori Compounds in Foods. *J. Agric. Food Chem.* **2014**, *62*, 5020−5027.

(21) Lapolla, A.; Fedele, D.; Reitano, R.; Arico, N. C.; Seraglia, R.; Traldi, P.; Marotta, E.; Tonani, R. Enzymatic digestion and mass spectrometry in the study of advanced glycation end products/peptides. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 496−509.

(22) Frolov, A.; Hoffmann, P.; Hoffmann, R. Fragmentation behavior of glycated peptides derived from D-glucose, D-fructose and D-ribose in tandem mass spectrometry. *J. Mass Spectrom.* **2006**, *41*, 1459−1469.

(23) Beisswenger, P. J. Methylglyoxal in diabetes: Link to treatment, glycaemic control and biomarkers of complications. *Biochem. Soc. Trans.* **2014**, *42*, 450−456.

(24) Simm, A. Protein glycation during aging and in cardiovascular disease. *J. Proteomics* **2013**, *92*, 248−259.

(25) Yu, T. Y.; Morton, J. D.; Clerens, S.; Dyer, J. M. Proteomic Investigation of Protein Profile Changes and Amino Acid Residue Level Modification in Cooked Lamb Meat: The Effect of Boiling. *J. Agric. Food Chem.* **2015**, *63*, 9112−9123.

(26) Teodorowicz, M.; Fiedorowicz, E.; Kostyra, H.; Wichers, H.; Kostyra, E. Effect of Maillard reaction on biochemical properties of peanut 7S globulin (Ara h 1) and its interaction with a human colon cancer cell line (Caco-2). *Eur. J. Nutr.* **2013**, *52*, 1927−1938.

DOI: 10.1021/acs.jafc.5b05492
J. Agric. Food Chem. XXXX, XXX, XXX−XXX