JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2018, 66, 212−217

pubs.acs.org/JAFC

Measurement of Fructose−Asparagine Concentrations in Human and Animal Foods

Jikang Wu,[†] Anice Sabag-Daigle,[‡] Thomas O. Metz,[§] Brooke L. Deatherage Kaiser,[∥] Venkat Gopalan,[†] Edward J. Behrman,[†] Vicki H. Wysocki,*[,†] and Brian M. M. Ahmer*[,‡]

[†]Department of Chemistry and Biochemistry, The Ohio State University, Columbus, Ohio 43210, United States

[‡]Department of Microbial Infection and Immunity, The Ohio State University, Columbus, Ohio 43210, United States

[§]Biological Sciences Division, Pacific Northwest National Laboratory, Richland 99352, Washington, United States

[∥]Signature Sciences and Technology Division, Pacific Northwest National Laboratory, Richland 99352, Washington, United States

Supporting Information

ABSTRACT: The food-borne bacterial pathogen, *Salmonella enterica*, can utilize fructose−asparagine (F−Asn) as its sole carbon and nitrogen source. F−Asn is the product of an Amadori rearrangement following the nonenzymatic condensation of glucose and asparagine. Heating converts F−Asn via complex Maillard reactions to a variety of molecules that contribute to the color, taste, and aroma of heated foods. Among these end derivatives is acrylamide, which is present in some foods, especially in fried potatoes. The F−Asn utilization pathway in *Salmonella*, specifically FraB, is a potential drug target because inhibition of this enzyme would lead to intoxication of *Salmonella* in the presence of F−Asn. However, F−Asn would need to be packaged with the FraB inhibitor or available in human foods. To determine if there are foods that have sufficient F−Asn, we measured F−Asn concentrations in a variety of human and animal foods. The 400 pmol/mg F−Asn found in mouse chow is sufficient to intoxicate a *Salmonella fraB* mutant in mouse models of salmonellosis, and several human foods were found to have F−Asn at this level or higher (fresh apricots, lettuce, asparagus, and canned peaches). Much higher concentrations (11 000−35 000 pmol/mg dry weight) were found in heat-dried apricots, apples, and asparagus. This report reveals possible origins of F−Asn as a nutrient source for *Salmonella* and identifies foods that could be used together with a FraB inhibitor as a therapeutic agent for *Salmonella*.

KEYWORDS: *Salmonella, fructosamines, fructose−asparagine, Amadori products, Maillard reaction, acrylamide, foods, browning*

INTRODUCTION

Fructose−asparagine (F−Asn, Figure 1) is an Amadori product that can be utilized as a carbon and nitrogen source by *Salmonella enterica*.[1] Amadori products are formed via the nonenzymatic condensation of the carbonyl group of reducing sugars and the nucleophilic amino group of amino acids and proceed through the formation of a Schiff base that spontaneously rearranges to form a stable ketoamine.[2−6] In the case of F−Asn, the reactants are glucose and asparagine.[3] The Amadori product is the first relatively stable compound in the sequence of reactions collectively referred to as the Maillard reaction.[2,7] The reactions are controlled by many factors, including pH, temperature, solvent, and pressure. Water is lost during the reaction, so the use of water as solvent favors the return to starting materials. Harsh reaction conditions convert F−Asn to advanced glycation end products (AGEs). Kinetically, this is an example of a series reaction in which the formation of an intermediate is followed by its subsequent decay.[8,9]

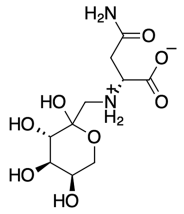

Figure 1. Structure of fructose−asparagine.

Maillard chemistry is significant in vivo; the current gold standard marker for long-term blood glucose control, hemoglobin A_{1c}, is an Amadori product.[10−13] Subsequent steps in the reaction lead to formation of AGEs that are associated with the development of diabetic complications,[14,15] among other disease pathologies.[16,17] In addition, this reaction occurs in virtually all high temperature prepared and stored foods.[18] Many Maillard reaction products are involved in food browning and are responsible for some of the distinct aromas and flavors of cooked foods. F−Asn is best known as the precursor of acrylamide, a neurotoxic compound found in some human foods.[19−27] Acrylamide is found in several cereals and potato products, and its concentration correlates with asparagine concentration, an important nitrogen storage compound in these plants.[28] Because heat accelerates, and water is inhibitory, for formation of acrylamide, acrylamide is found in concentrations in fried potatoes higher than those in

Received: September 12, 2017
Revised: December 8, 2017
Accepted: December 12, 2017
Published: December 12, 2017

DOI: 10.1021/acs.jafc.7b04237
J. Agric. Food Chem. 2018, 66, 212−217

raw or boiled potatoes. While many studies measured acrylamide in foods, few measured F−Asn, so it is unclear whether F−Asn is present in the potatoes before frying, or whether frying increases the formation of F−Asn during the process of forming acrylamide. There is evidence that F−Asn formation does not require heating, so it may be present in the potatoes before cooking.[29] The concentration of F−Asn and related products has been measured in apricots and peaches.[5] Interestingly, F−Asn was not detectable in fresh fruits, but was abundant after months of storage.[5] F−Asn was also abundant in dried vegetables, with dried asparagus containing the highest concentration of 48 000 pmol/mg dry weight.[4] Dried tomato, red pepper, and bell pepper also had high concentrations, while dried cauliflower, carrot, and celery were the lowest at 3 400 pmol/mg dry weight.[4]

While F−Asn is known as a precursor of acrylamide, it was not known to be a nutrient for any organism until the recent discovery that *Salmonella* can utilize this compound as a carbon and nitrogen source.[1] The genes required for F−Asn utilization (the *fra* genes) appear to have been acquired horizontally shortly after divergence from *E. coli*.[1] The divergence of *E. coli* and *Salmonella* is estimated to have occurred roughly 140 million years ago,[30] which places the acquisition of the *fra* locus by *Salmonella* much earlier than the evolution of the modern form of humans. We therefore postulated that F−Asn is present in raw foods and served as a nutrient source for *Salmonella*. Here, we tested this hypothesis and found that F−Asn is found in some raw foods, although heat drying or frying greatly increases its abundance. Additionally, we previously reported that F−Asn is toxic to a *fraB* mutant of *Salmonella*.[31] This suggests that a combination of F−Asn and a FraB enzyme inhibitor would be toxic to wild-type *Salmonella* and could represent a novel therapeutic. In this study, we addressed the question of whether the F−Asn content in foods is sufficiently high to inhibit a *fraB* mutant of *Salmonella*.

Liquid chromatography coupled with tandem mass spectrometry has been used to quantify Amadori products such as fructose−leucine, fructose−phenylalanine, fructose−methionine, fructose−valine and fructose−histidine in pepper and tomato.[32,33] Heavy labeled standards facilitated the absolute quantification of Amadori products in food with correction for the losses during sample preparation and degradation of analytes[32] and we follow a similar procedure here.

MATERIALS AND METHODS

Food Preparation. The following food items were purchased from local grocery stores: Ohio sweet corn, basmati rice, Russet potato, Yukon Gold potato, red potato, fresh apricot, fresh peach (Hirsch fruit farm), canned apricot in heavy syrup (Libby's), dried apricot (Sunmaid Mediterranean), white bread (the crust and the crumb were measured separately), canned peach in heavy syrup (Libby's), green lettuce (Little Gem), and asparagus. Food items were either raw or cooked using two methods, frying or boiling. Raw items were chopped and placed in preweighed microcentrifuge tubes. Boiled items were obtained by placing the food of interest in boiling water for 7 min prior to chopping the boiled item and placing in preweighed microcentrifuge tubes. Fried items were obtained by slicing (as in the case of potatoes) and frying in olive oil at 216 °C for 5 min per side of the food item before chopping and placing in a preweighed microcentrifuge tube. Additionally, F−Asn was measured in the following items without further manipulation: Lays Classic potato chips, Wendy's French fries, Horizon Grains hog feed, and Purina Flock Raiser poultry feed.

Synthesis of Amadori Products. ^{13}C-labeled fructose−asparagine was synthesized as described,[3] but using glucose uniformly labeled with ^{13}C (Cambridge Isotope Laboratories). The resulting product is ∼95% pure and has been characterized by ^{1}H and ^{13}C NMR, exact mass, and specific rotation.[3] The fructose−asparagine exists as a mixture of the zwitterion and the ammonium salt; the main impurity is ethylene glycol, whose content we know from NMR data.

Liquid Chromatography−Mass Spectrometry (LC−MS). To measure the concentration of F−Asn in food samples, the samples were lyophilized and ground on dry ice with a pestle in a preweighed 1.5 mL microcentrifuge tube. Approximately 10 mg of dry material was then transferred to a new 1.5 mL centrifuge tube and weighed, followed by the addition of 500 μL of chilled methanol and 500 μL of H_2O spiked with 0.16 nmol [^{13}C]-F−Asn. After vortexing and centrifugation at 14 800*g* for 1 h, the supernatant was transferred into a new 1.5 mL centrifuge tube, frozen, and lyophilized. Before mass spectrometric analysis, these dried pellets were resuspended in 500 μL of acetonitrile/water, 80:20% with 0.1% (v/v) formic acid (LC−MS grade, Thermo Scientific) and filtered using a 0.2 μm PTFE filter (Thermo Scientific). A recovery test of sample preparation was performed, and results are shown in Supplementary Table 1. The supernatant of the flow-through fraction was injected for LC−MS analysis. A nanoACQUITY Ultra Performance LC system (Waters, Milford, MA, United States) with a UPLC M-class BEH 130 amide column (Waters, 75 μm × 100 mm, 1.7 μm) was coupled to a triple quadrupole mass spectrometer (Waters Xevo TQ-S) for F−Asn quantification. Buffer A (0.1% FA in water with 10% acetonitrile) and buffer B (0.1% FA in acetonitrile) were used as mobile phases for gradient separation, which started with 80% B for 6 min at a flow rate of 0.5 μL/min and then followed by gradient: 6−20 min, 80−50% B; 20−26 min, 50% B; 26−28 min, 50−80% B; 28−35 min, 80% B. The chromatograms of synthesized F−Asn, [^{13}C]-F−Asn, and F−Asn detected from food samples are shown in Supplementary Figure 1. The mass spectrometer was operated in positive ion nanoelectrospray ionization mode (nano-ESI+) with capillary voltage 3.5 kV, source temperature 70 °C, cone voltage 2 V, and source offset 2 V. The gas flow rate for the collision cell was 0.15 mL/min. The transition m/z 295 → 211 of F−Asn with collision energy 13 eV was selected for quantitation, and the corresponding transition m/z 301 → 216 of [^{13}C]-F−Asn with collision energy 13 eV was used for normalization. While m/z 295 → 211 was used as the quantifier, transitions m/z 295 → 277 and m/z 295 → 259 were used as qualifiers to verify the presence of F−Asn. The tandem mass spectra of synthesized F−Asn, [^{13}C]-F−Asn, and F−Asn detected from food samples are shown in Supplementary Figure 2. Skyline-daily (v 3.5, MacCoss Lab, Department of Genome Sciences, University of Washington, Seattle, WA, United States) was used for calculating the peak area of transitions. The reproducibility of F−Asn measurements was tested using heat dried apple, and the results are shown in Supplementary Figure 3.

RESULTS

Fructose−Asparagine Concentrations in a Sampling of Human and Animal Foods. Acrylamide is a neurotoxin that has been found in fried potato products, including French fries and potato chips.[4,20−25,29,34] Concerns over this finding led to the measurement of acrylamide in a large number of human foods.[27,42] While acrylamide itself has been measured in numerous foods, the concentration of its precursor, fructose−asparagine (F−Asn), has been measured in very few.[4,5,35,36] The F−Asn content of foods has become more significant with the finding that F−Asn is utilized by the food-borne pathogen *Salmonella* as a carbon and nitrogen source.[1] In this study, we measured F−Asn in a variety of foods, including raw, boiled, and fried potatoes (French fries and potato chips); raw, boiled, and fried rice; bread; bread crust; raw and boiled asparagus and corn; canned peaches; and fresh, dried, and canned apricots.

DOI: 10.1021/acs.jafc.7b04237
J. Agric. Food Chem. 2018, 66, 212−217

We used three different types of potato and then fried or boiled them ourselves so that the results could be directly compared to the raw starting material (see Materials and Methods). In each of the three types of potatoes, we found that frying increased the F−Asn content by between 7- and 21-fold (Table 1, Figure 2). After frying, the concentrations in the three types of potatoes ranged between 560 and 1900 pmol/mg. Boiling had no effect. We also tested commercial potato chips and French fries obtained from a local restaurant. In both cases, the F−Asn concentration was 80 pmol/mg, which is similar to what we observed in raw potatoes (between 70 and 130 pmol/mg) and far lower than when we fried the potatoes ourselves. This is presumably because the food industry has developed pretreatment procedures to decrease acrylamide formation.[37,38]

The browning of foods results in numerous Maillard reaction products.[39−43] Because F−Asn is a precursor to Maillard reaction products, we hypothesized that it may be present in bread or bread crust. Therefore, we measured the F−Asn content of bread crust and compared this to the F−Asn content of a sample from the middle of the same slice of bread. The F−Asn concentration in the crust was below our detection limit of 1.4 pmol/mg. In the middle of the slice of bread, the F−Asn was 13 pmol/mg (Table 1, Figure 2).

We purchased raw rice and then fried it or boiled it ourselves so that the results could be directly compared (see Materials and Methods). Raw rice had a fairly low concentration of F−Asn at 10 pmol/mg. Frying and boiling reduced the F−Asn concentration in rice to below our detection limit of 1.4 pmol/mg (Table 1, Figure 2).

Fresh asparagus, lettuce, and apricot had significant concentrations of F−Asn (500, 500, and 800 pmol/mg, respectively). Boiling asparagus reduced the F−Asn concentration from 500 to 50 pmol/mg. The concentration of F−Asn in apricots was 800 pmol/mg in fresh and 500 pmol/mg in commercially canned. However, F−Asn was extremely high in commercially dried apricots at 8000 pmol/mg (Table 1, Figure 2). Fresh peaches had 66 pmol/mg F−Asn, while the concentration in commercially canned peaches was much higher at 500 pmol/mg.

Because the fresh, canned, and dried apricots were all from different sources, the effects of canning and drying are not directly comparable. Therefore, we bought fresh apricots and dried them ourselves, either with or without additional heat. The fresh apricots had an average F−Asn concentration of 600 pmol/mg. Drying at room temperature without additional heat (fan only) required 4 days and yielded an average F−Asn concentration of 3400 pmol/mg. In contrast, drying with heat required only 1 day and the average F−Asn concentration was 35 000 pmol/mg (Table 1, Figure 2). We then performed the same drying experiment with apples, blueberries, bananas, and asparagus. In all cases except the blueberries, drying with heat yielded high concentrations of F−Asn (4000 to 18 000 pmol/mg) (Table 1, Figure 2).

We measured the F−Asn content in commercial hog feed, chicken feed, and laboratory mouse chow and obtained 7, 70, and 400 pmol/mg, respectively (Table 1, Figure 2).

Table 1. Fructose−Asparagine Concentrations in Human and Animal Foods[a]

food sample	average [F−Asn] (pmol/mg dry weight)	standard deviation
raw rice	10	10
fried rice	0.6	0.5
boiled rice	0.9	0.1
bread crust	0.54	0.08
bread inside	13	8
raw corn	18	9
boiled corn	7	2
raw asparagus (batch 1)	500	200
boiled asparagus (batch 1)	50	10
raw lettuce	500	200
raw apricot (batch 1)	800	200
commercial dried apricot	8000	1000
canned apricot	500	100
raw peach	66	1
canned peach	500	300
raw russett potato	130	40
fried russett potato	1900	400
boiled russett potato	75	7
raw Yukon Gold potato	76	7
fried Yukon Gold potato	560	70
boiled Yukon Gold potato	120	30
raw red potato	70	10
fried red potato	1400	50
boiled red potato	150	50
French fries	80	10
potato chips	80	10
raw apple	34	5
fan dried apple	350	70
heat dried apple	11 000	3000
raw blueberries	1.5	0.5
fan dried blueberries	6	1
heat dried blueberries	39	7
raw apricot (batch 2)	600	300
fan dried apricot (batch 2)	3400	500
heat dried apricot (batch 2)	35 000	4000
raw asparagus (batch 2)	70	20
fan dried asparagus (batch 2)	2300	100
heat dried asparagus (batch 2)	18 000	5000
raw banana	19	5
fan dried banana	400	100
heat dried banana	4000	1000
hog feed	7	2
chicken feed	70	20
mouse chow	400	10

[a]The average of three measurements and the standard deviation are shown for each food type. The limit of detection was 1.4 pmol/mg. The standard deviation of pooled quality control runs was 7.4%. These results are graphed in Figure 2.

DISCUSSION

The various steps of the Maillard reaction occur during the preparation and processing of foods characteristic of the Western diet and are promoted by high temperatures (>140 °C). These reactions are responsible for the browning or darkening of foods, such as the browning of bread into toast, the color of beer from malted barley, and the browning of various meats when seared or grilled. They contribute to the aroma, flavor, color, and texture of foods but also result in decreased nutritional value through a variety of mechanisms.[18]

DOI: 10.1021/acs.jafc.7b04237
J. Agric. Food Chem. 2018, 66, 212−217

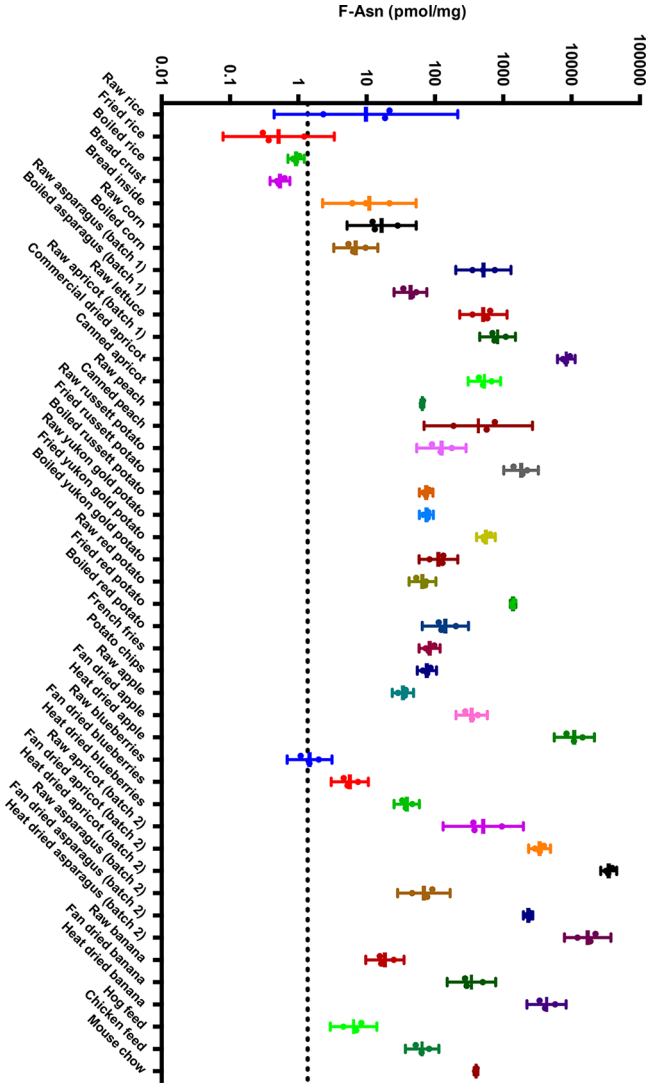

Figure 2. Fructose−asparagine in human and animal foods with each food type indicated along the *x*-axis. Each food type was measured in triplicate with the geometric mean and 95% confidence interval indicated. The limit of detection (1.4 pmol/mg) is indicated by the dotted line. The data in this graph come from Table 1.

In contrast, they have been shown to increase the antioxidant activity in foods and may exert organism-specific influences on the gut microbiota, such as inhibiting growth of *E. coli* and *Enterobacter cloacae*.[18] In a diet that consists largely of foods that have been either prepared at high temperatures (e.g., baking, frying, dehydrating) or stored for long periods of time, ingestion of Maillard reaction products can be significant. For example, the intake of Amadori rearrangement products has been estimated at 0.5−1.2 g/day, and consumption of AGEs may reach 25−75 mg/day.[44] Apart from microoxic fringes, the human gut is an anoxic environment;[45] consequently, the vast majority of the human gut microbiome must employ anaerobic metabolism. The gut community ultimately relies on fermentation for its energy, with the two main fermentative substrates being nondigestible carbohydrates and protein.[44] Except for specific fermentation products (e.g., acetate, H_2) consumed by organisms capable of anaerobic respiration (e.g., sulfate reduction), terminal fermentation products cannot be further metabolized anaerobically. However, some Maillard reaction products are known to be metabolized by colonic microbiota, and the microbial catabolism of these products may modulate the composition of the gut microbiota.[44,46,47]

Salmonella is the first organism identified that can specifically consume F−Asn,[1] although we have recently confirmed that some members of the class Clostridia can also utilize F−Asn.[48] It is not known if F−Asn in the diet could affect a *Salmonella* infection. However, a mutation in the gene *fraB*, which encodes a deglycase essential for the last step of the F−Asn utilization pathway, causes *Salmonella* to be inhibited by F−Asn.[31] This finding suggests that inhibitors of FraB activity might make potent anti-*Salmonella* therapeutics. If small molecule inhibitors of FraB were to be developed, F−Asn would need to be present with the inhibitor for it to function.

One question we addressed in this study is whether there is sufficient F−Asn in human foods or if supplementary F−Asn needs to be administered with a FraB inhibitor. F−Asn has been detected previously in tomato powder, bell pepper, red pepper, asparagus, cauliflower, carrot, celery, apricots, and peaches, especially when these samples were stored for long periods of time or dried.[4,5] We confirmed that asparagus, peaches, and apricots do indeed have high F−Asn concentrations. We found that raw asparagus had 70 pmol/mg of F−Asn and that this batch contained 2300 pmol/mg when fan dried at room temperature for 96 h and 18 000 pmol/mg when heat dried for just 24 h (Table 1, Figure 2). Thus, fan drying and heat drying increased F−Asn content by 33- and 257-fold, respectively. A previous study reported 1.4 g of F−Asn per 100 g of dry weight in dried asparagus,[5] equivalent to 48 000 pmol/mg, approximately 2.7-fold more than what we measured. It is possible that this difference is attributable to methodology, or variance between asparagus samples (see Table 1 for a comparison of different asparagus batches). With apricots, we observed 600 pmol/mg F−Asn in raw apricot that increased to 3400 pmol/mg after fan drying at room temperature for 96 h and 35 000 pmol/mg after heat drying for just 24 h (Table 1, Figure 2). This finding again demonstrates that heat drying leads to the highest concentrations of F−Asn. We also observed very high concentrations of F−Asn in heat-dried bananas and apples (Table 1, Figure 2). For animal foods, we looked at commercial swine food (7 pmol/mg) and poultry food (70 pmol/mg) (Table 1, Figure 2). Both contain F−Asn, but neither was as high as laboratory mouse chow (400 pmol/mg).

In addition to the fruits and vegetables listed above, we hypothesized that F−Asn may be present in foods that are known to have high acrylamide concentrations.[26] Among foods known to contain acrylamide, we compared russet, Yukon Gold, and red potatoes that were either raw, boiled, or fried. In each case, raw and boiled potatoes had relatively low concentrations of F−Asn (70−150 pmol/mg) (we might expect boiling to remove the soluble precursors) while fried potatoes had higher concentrations (560−1900 pmol/mg). This trend is similar to patterns observed with acrylamide.[26,27,42] Among the potato types, fried russet potatoes had the highest F−Asn content, and these are also known to have high acrylamide content.[49] The FDA has recommended against the use of russet potatoes because of their high reducing sugar content, which leads to high levels of acrylamide in cooked products.[27]

This study elucidated the source of a nutrient, F−Asn, which is utilized by the food-borne pathogen *Salmonella*. While Amadori products are often associated with browning of foods, we found very low concentrations of F−Asn in bread crust. In

DOI: 10.1021/acs.jafc.7b04237
J. Agric. Food Chem. 2018, 66, 212−217

this case, we speculate that this may be due to movement of dissolved glucose and asparagine toward the interior during baking. Instead, we found that heat-dried fruits and vegetables, especially asparagus and apricots, have the highest concentrations of F−Asn in those foods measured in this study. High levels of F−Asn in these dried foods are likely due to the high concentration of the precursors (glucose and asparagine) and the ability of heating as well as removal of water to accelerate Amadori product formation. *Salmonella* acquired the genes required for F−Asn utilization long before humans began heat-drying foods, so we presume that the concentrations available in raw fruits and vegetables are sufficient to benefit *Salmonella* and other organisms that can utilize F−Asn. With regard to drugs targeting *Salmonella* FraB, F−Asn must be available during the inhibition of FraB to intoxicate the bacterium. Therefore, a reliable therapeutic modality would presumably be to package chemically synthesized F−Asn with the inhibitor. However, our study offers an alternative. Because the 400 pmol/mg F−Asn present in mouse chow is sufficient to intoxicate a *Salmonella fraB* mutant in mouse models of inflammation,[31] a similar threshold should suffice as a supplement for a FraB-based therapeutic. We have now demonstrated that F−Asn exists in similar concentrations in human foods such as fresh apricots, lettuce, and asparagus, and in canned peaches. In fact, F−Asn exists in far higher concentrations in dried apricots (35 000 pmol/mg or 35 mM). Clearly, there are several foods that could be eaten with the inhibitor that would provide sufficient F−Asn to intoxicate *Salmonella*.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b04237.

Table of recovery values, liquid chromatograms, tandem mass spectra, and graph of F−Asn measurements (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: wysocki.11@osu.edu; Phone: 614-292-8687.
*E-mail: ahmer.1@osu.edu; Phone: 614-292-1919.

ORCID

Edward J. Behrman: 0000-0002-8797-6625
Brian M. M. Ahmer: 0000-0002-4267-7322

Funding

This work was funded by Grant 1R01AI116119 from the National Institutes of Health.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

F−Asn, fructose−asparagine; F−Asp, fructose−aspartate; 6-P-F−Asp, 6-phosphofructose−aspartate; *fra*, fructose−asparagine utilization gene; *fraB*, fructose−asparagine utilization gene B; FraB, fructose−asparagine utilization protein B

■ REFERENCES

(1) Ali, M. M.; Newsom, D. L.; Gonzalez, J. F.; Sabag-Daigle, A.; Stahl, C.; Steidley, B.; Dubena, J.; Dyszel, J. L.; Smith, J. N.; Dieye, Y.; Arsenescu, R.; Boyaka, P. N.; Krakowka, S.; Romeo, T.; Behrman, E. J.; White, P.; Ahmer, B. M. M. Fructose−asparagine is a primary nutrient during growth of *Salmonella* in the inflamed intestine. *PLoS Pathog.* **2014**, *10*, e1004209.

(2) Hodge, J. E. Dehydrated foods, chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, *1*, 928−943.

(3) Hansen, A. L.; Behrman, E. J. Synthesis of 6-phosphofructose aspartic acid and some related Amadori compounds. *Carbohydr. Res.* **2016**, *431*, 1−5.

(4) Eichner, K.; Reutter, M.; Wittmann, R. In Thermally Generated Flavors. *Detection of Amadori compounds in heated foods* **1994**, *543*, 42−54.

(5) Anet, E. F. L. J.; Reynolds, T. M. Chemistry of non-enzymic browning. II. Reactions between amino acids, organic acids, and sugars in freeze-dried apricots and peaches. *Aust. J. Chem.* **1957**, *10*, 182−191.

(6) Mossine, V. V.; Mawhinney, T. P. 1-Amino-1-deoxy-D-fructose ("fructosamine") and its derivatives. *Adv. Carbohydr. Chem. Biochem.* **2010**, *64*, 291−402.

(7) Maillard, L.-C. Action des Acides Aminés sur les Sucres. *C. R. Hebd. Seances Acad. Sci.* **1912**, *154*, 66−68.

(8) Yaylayan, V. A.; Despointes, A. H.; Feather, D. M. S. Chemistry of Amadori rearrangement products: Analysis, synthesis, kinetics, reactions, and spectroscopic properties. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 321−369.

(9) Frost, A. A.; Pearson, R. G. *Kinetics and mechanism*, 2nd ed.; John Wiley & Sons: New York, 1961; pp 166−169.

(10) Koenig, R. J.; Peterson, C. M.; Jones, R. L.; Saudek, C.; Lehrman, M.; Cerami, A. Correlation of glucose regulation and hemoglobin A_{Ic} in diabetes mellitus. *N. Engl. J. Med.* **1976**, *295*, 417−420.

(11) Diabetes Control and Complications Trial Research Group; Nathan, D. M.; Genuth, S.; Lachin, J.; Cleary, P.; Crofford, O.; Davis, M.; Rand, L.; Siebert, C. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J. Med.* **1993**, *329*, 977−986.

(12) Nathan, D. M.; Cleary, P. A.; Backlund, J.-Y. C.; Genuth, S. M.; Lachin, J. M.; Orchard, T. J.; Raskin, P.; Zinman, B.; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J. Med.* **2005**, *353*, 2643−2653.

(13) Rahbar, S.; Blumenfeld, O.; Ranney, H. M. Studies of an unusual hemoglobin in patients with diabetes mellitus. *Biochem. Biophys. Res. Commun.* **1969**, *36*, 838−843.

(14) Genuth, S.; Sun, W.; Cleary, P.; Gao, X.; Sell, D. R.; Lachin, J.; Monnier, V. M. Skin advanced glycation end products glucosepane and methylglyoxal hydroimidazolone are independently associated with long-term microvascular complication progression of type 1 diabetes. *Diabetes* **2015**, *64*, 266−278.

(15) Genuth, S.; Sun, W.; Cleary, P.; Sell, D. R.; Dahms, W.; Malone, J.; Sivitz, W.; Monnier, V. M. Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. *Diabetes* **2005**, *54*, 3103−3111.

(16) de Groot, L.; Hinkema, H.; Westra, J.; Smit, A. J.; Kallenberg, C. G. M.; Bijl, M.; Posthumus, M. D. Advanced glycation endproducts are increased in rheumatoid arthritis patients with controlled disease. *Arthritis Res. Ther.* **2011**, *13*, R205.

(17) Wong, A.; Lüth, H. J.; Deuther-Conrad, W.; Dukic-Stefanovic, S.; Gasic-Milenkovic, J.; Arendt, T.; Münch, G. Advanced glycation endproducts co-localize with inducible nitric oxide synthase in Alzheimer's disease. *Brain Res.* **2001**, *920*, 32−40.

(18) Bastos, D. M.; Monaro, É.; Siguemoto, É. *Maillard reaction products in processed food: pros and cons, in Food Industrial Processes: Methods and Equipment*; Humana Press: Totowa, NJ, 2012; pp 281−300.

DOI: 10.1021/acs.jafc.7b04237
J. Agric. Food Chem. 2018, 66, 212−217

(19) Stadler, R. H.; Robert, F.; Riediker, S.; Varga, N.; Davidek, T.; Devaud, S.; Goldmann, T.; Hau, J.; Blank, I. In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *J. Agric. Food Chem.* **2004**, *52*, 5550−5558.
(20) Surdyk, N.; Rosén, J.; Andersson, R.; Aman, P. Effects of asparagine, fructose, and baking conditions on acrylamide content in yeast-leavened wheat bread. *J. Agric. Food Chem.* **2004**, *52*, 2047−2051.
(21) Zyzak, D. V.; Sanders, R. A.; Stojanovic, M.; Tallmadge, D. H.; Eberhart, B. L.; Ewald, D. K.; Gruber, D. C.; Morsch, T. R.; Strothers, M. A.; Rizzi, G. P.; Villagran, M. D. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.* **2003**, *51*, 4782−4787.
(22) Becalski, A.; Lau, B. P.-Y.; Lewis, D.; Seaman, S. W. Acrylamide in foods: occurrence, sources, and modeling. *J. Agric. Food Chem.* **2003**, *51*, 802−808.
(23) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Food chemistry: Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448−449.
(24) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998−5006.
(25) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Acrylamide: A cooking carcinogen? *Chem. Res. Toxicol.* **2000**, *13*, 517−522.
(26) Lineback, D. R.; Coughlin, J. R.; Stadler, R. H. Acrylamide in foods: A review of the science and future considerations. *Annu. Rev. Food Sci. Technol.* **2012**, *3*, 15−35.
(27) U.S. Food and Drug Administration. *Draft Guidance for Industry: Acrylamide in Foods*; U.S. Food and Drug Administration: Silver Spring, MD, 2013.
(28) Lea, P. J.; Sodek, L.; Parry, M. A. J.; Shewry, P. R.; Halford, N. G. Asparagine in plants. *Ann. Appl. Biol.* **2007**, *150*, 1−26.
(29) Yaylayan, V. A.; Wnorowski, A.; Perez Locas, C. Why asparagine needs carbohydrates to generate acrylamide. *J. Agric. Food Chem.* **2003**, *51*, 1753−1757.
(30) Ochman, H.; Wilson, A. C. Evolution in bacteria: evidence for a universal substitution rate in cellular genomes. *J. Mol. Evol.* **1987**, *26*, 74−86.
(31) Sabag-Daigle, A.; Blunk, H. M.; Sengupta, A.; Wu, J.; Bogard, A. J.; Ali, M. M.; Stahl, C.; Wysocki, V. H.; Gopalan, V.; Behrman, E. J.; Ahmer, B. M. M. A metabolic intermediate of the fructose−asparagine utilization pathway inhibits growth of a *Salmonella fraB* mutant. *Sci. Rep.* **2016**, *6*, 28117.
(32) 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.
(33) Yu, J.; Zhang, S.; Zhang, L. Evaluation of the extent of initial Maillard reaction during cooking some vegetables by direct measurement of the Amadori compounds. *J. Sci. Food Agric.* **2018**, *98*, 190−197.
(34) Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P. A.; Robert, M.-C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002**, *419*, 449−450.
(35) Sanz, M. L.; del Castillo, M. D.; Corzo, N.; Olano, A. Presence of 2-furoylmethyl derivatives in hydrolysates of processed tomato products. *J. Agric. Food Chem.* **2000**, *48*, 468−471.
(36) del Castillo, M. D.; Corzo, N.; Olano, A. Early stages of Maillard reaction in dehydrated orange juice. *J. Agric. Food Chem.* **1999**, *47*, 4388−4390.
(37) Low, M. Y.; Koutsidis, G.; Parker, J. K.; Elmore, J. S.; Dodson, A. T.; Mottram, D. S. Effect of Citric Acid and Glycine Addition on Acrylamide and Flavor in a Potato Model System. *J. Agric. Food Chem.* **2006**, *54*, 5976−5983.
(38) Mestdagh, F.; De Wilde, T.; Fraselle, S.; Govaert, Y.; Ooghe, W.; Degroodt, J.-M.; Verhé, R.; Van Peteghem, C.; De Meulenaer, B. Optimization of the blanching process to reduce acrylamide in fried potatoes. *LWT - Food Science and Technology* **2008**, *41*, 1648−1654.
(39) Hellwig, M.; Henle, T. Baking, ageing, diabetes: a short history of the Maillard reaction. *Angew. Chem., Int. Ed.* **2014**, *53*, 10316−10329.
(40) Tessier, F. J. The Maillard reaction in the human body. The main discoveries and factors that affect glycation. *Pathol. Biol.* **2010**, *58*, 214−219.
(41) Zhang, Q.; Ames, J. M.; Smith, R. D.; Baynes, J. W.; Metz, T. O. A perspective on the Maillard reaction and the analysis of protein glycation by mass spectrometry: Probing the pathogenesis of chronic disease. *J. Proteome Res.* **2009**, *8*, 754−769.
(42) Mottram, D. S.*The Maillard reaction: Source of flavour in thermally processed foods, in Flavours and Fragrances*; Springer Berlin Heidelberg: Berlin, Heidelberg, 2007; pp 269−283.
(43) Brownlee, M. Advanced protein glycosylation in diabetes and aging. *Annu. Rev. Med.* **1995**, *46*, 223−234.
(44) Tuohy, K. M.; Hinton, D. J. S.; Davies, S. J.; Crabbe, M. J. C.; Gibson, G. R.; Ames, J. M. Metabolism of Maillard reaction products by the human gut microbiota–implications for health. *Mol. Nutr. Food Res.* **2006**, *50*, 847−857.
(45) Espey, M. G. Role of oxygen gradients in shaping redox relationships between the human intestine and its microbiota. *Free Radical Biol. Med.* **2013**, *55*, 130−140.
(46) Seiquer, I.; Rubio, L. A.; Peinado, M. J.; Delgado-Andrade, C.; Navarro, M. P. Maillard reaction products modulate gut microbiota composition in adolescents. *Mol. Nutr. Food Res.* **2014**, *58*, 1552−1560.
(47) Reichardt, N.; Gniechwitz, D.; Steinhart, H.; Bunzel, M.; Blaut, M.; Lee, A. H.; Binns, C. W. Characterization of high molecular weight coffee fractions and their fermentation by human intestinal microbiota. *Mol. Nutr. Food Res.* **2009**, *53*, 287−299.
(48) Sabag-Daigle, A.; Wu, J.; Borton, M. A.; Sengupta, A.; Gopalan, V.; Wrighton, K. C.; Wysocki, V. H.; Ahmer, B. M. M. Identification of bacterial species that can utilize fructose-asparagine. *Appl Environ Microbiol. 84*, DOI: 10.1128/AEM.01957-17, in press.
(49) Center for Food Safety and Nutrition. *Chemical Contaminants: Survey Data on Acrylamide in Food: Individual Food Products*; U.S. Food and Drug Administration: Silver Spring, MD, 2002.

DOI: 10.1021/acs.jafc.7b04237
J. Agric. Food Chem. 2018, 66, 212−217