Asparagine Analysis in Food Products

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The current concern over acrylamide levels in food has unexpectedly brought amino acid analysis to the forefront. Acrylamide (2-propenamide) is a known mutagen in rats and also a neurotoxin and probable cancinogen in humans. Its levels are strictly regulated in drinking water. Swedish researchers have discovered surprisingly high levels of this toxic compound in common food products such as chips and french fries [1]. This unexpected finding was even more puzzling as raw food ingredients showed no or trace levels of acrylamide. It was found that acrylamide is being generated in significant quantities during high temperature processing of raw food materials (primarily by frying and baking).

The source of acrylamide in processed foods is believed to be linked to the Maillard reaction involving amino acids and sugars [2]. At high temperatures, the amino acid asparagine (ASN; and possibly others as well) in reaction with carbohydrates generates acrylamide. The yield of formation of acrylamide in model systems made of asparagine and glucose heated to over 110°C is very low. Nevertheless, foods like potato, rice, and corn, rich in both amino acids and carbohydrates, show alarming levels of acrylamide when fried or baked at high temperatures [3]. The current understanding is that all food-borne acrylamide originates with free asparagine, along with minor contributions by other free amino acids like methionine, glutamine, aspartic acid and cysteine [4].

The current research efforts are focused on four different aspects:

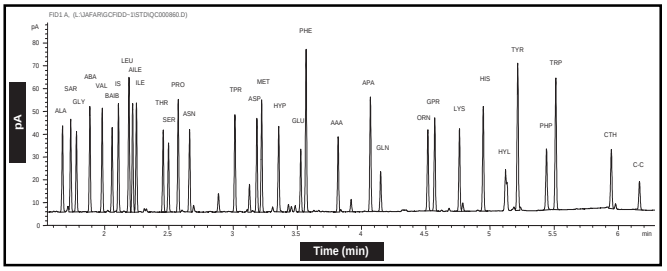
1. A comprehensive evaluation of acrylamide levels in different food products *(regulatory)*
2. A better assessment of the suspected harmful effects of acrylamide in food on human health *(toxicologic)*
3. A better understanding of the chemistry behind acrylamide generation during food processing *(biochemical research)*
4. An investigation into safer food processing practices which would insure reduced acrylamide levels in food products *(remediatory)*

These last two efforts involve the monitoring of precursors like asparagine in raw food ingredients, and also the selection of low amino acid containing food varieties.

Most of the current methods used for the analysis of amino acids in biological matrixes are laborious, time consuming, and expensive. Several of them require the use of dedicated (and expensive) instrumentation like amino acid analyzers, or of specialized detectors (amperometric). Most require a time consuming sample preparation step meant to remove proteins and other matrix components. Next, a derivatization procedure is applied, but some derivatives are less stable than others. The one method which is fast, requires only common instrumentation readily available in most laboratories, and is cost effective is based on the EZ:faast® Amino Acid Analysis Kit (Phenomenex, Inc., Torrance, CA). The total cycle time for the analysis of ASN in food with this kit is 15 minutes. The EZ:faast® procedure involves a simple solid phase extraction (SPE) step, followed by a rapid derivatization reaction conducted in aqueous phase at room temperature. This reaction is concomitant to a liquid-liquid extraction step resulting in pure samples amenable for GC (FID/NPD) analysis. Alternative kits are available for either GC/MS, or for LC/MS analysis, respectively. The total cycle time for sample preparation followed by gas-chromatographic analysis is 15 minutes. The sample preparation for LC/MS analysis is similar to the procedure used for gas-chromatographic analysis. The LC/MS analysis takes 12 min, and it's followed by a 4 min column re-equilibration time.

Potato extract samples and amino acid standard mixtures were prepared (7 minutes) and analyzed (8 minutes) in the same way. Standard mixtures were used for both instrument calibration, and for linearity measurements. Potato extracts were analyzed as such, and also after spiking with 100µL of a 200nmol/mL ASN solution. Recovery was calculated at three different concentrations of ASN in potato extracts (prepared at the three different solid/liquid extraction ratios as indicated above). The amount of spike was identical at each level of ASN investigated (20nmols). All materials required for sample preparation and for chromatographic analysis are provided with the kit, except for common laboratory consumables and utilities. One kit provides for the analysis of 384 samples and standard mixtures used for instrument calibration. An Agilent 6890 gas-chromatograph equipped with an FID was used for sample analysis. The chromatographic column with the dimensions 10mx0.25mm ID of a proprietary phase is provided with the kit. The temperature program starts at 110°C, includes a linear ramp of 32°C/min up to 320°C, and a hold time of 1 min. The injector temperature is 150°, while the detector is kept at 320°C. A typical chromatogram showing the separation of 33 amino acids in a standard mixture is shown in *Figure 1.*

Figure 1. Amino Acid Standards by EZ:faast FID



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A typical chromatogram showing the separation of 33 amino acids in a standard mixture prepared by EZ:faast, and analyzed by GC/FID. Instrumental conditions: inlet 250ºC; injecton 2ul with split 15:1; column 10m x 0.25 mm ID ZB AAA; flow 1.5 ml/min He; oven 110-320ºC, 32ºC/min; detector 320ºC.

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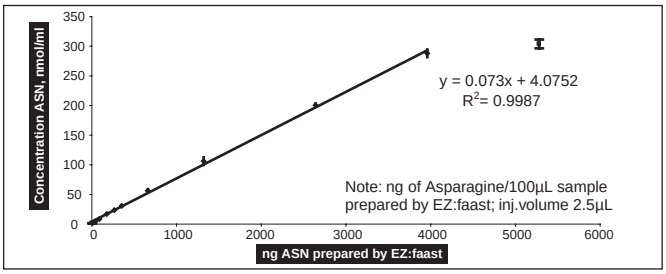
Results

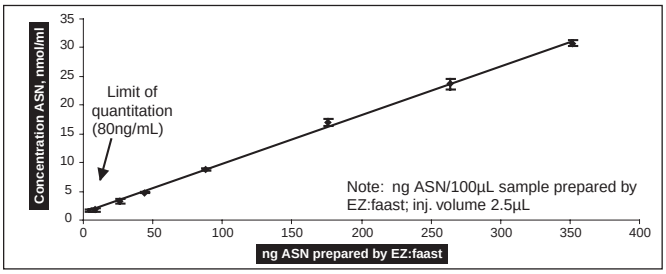
The ASN analysis method based on the EZ:faast® procedure proved linear in the 80-4000ng/mL (0.6-30µM) concentration range, with a correlation coefficient of 0.999 (Figure 2). Amino acid standard mixtures provided with the kit were used for linearity measurements. At the low end of the linearity curve the split ratio was decreased from 15:1 used at all other concentrations to 5:1. The linearity range can be further extended by either adjusting the solid-liquid extraction ratio prior to SPE, or by processing a smaller/larger volume of extract depending on ASN level. A simple dilution of a highly concentrated extract will practically bring any sample high in ASN within the linearity range.

Figure 2. Linear Dynamic Range for Quantitation of Asparagine



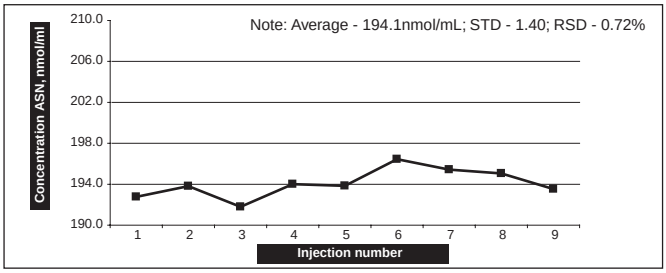
The limit of quantification of 80ng/mL (90pg on column) was determined as the lowest point of the linearity curve, three times analyzed, for which the relative standard deviation is less than 20%, and the accuracy is between 80 and 120% *(Figure 3)*. As the current application concerns only high levels of ASN present in raw food material, the limit of 80ng/mL is orders of magnitude lower than needed.

Figure 3. Limit of Quantitation of Asparagine



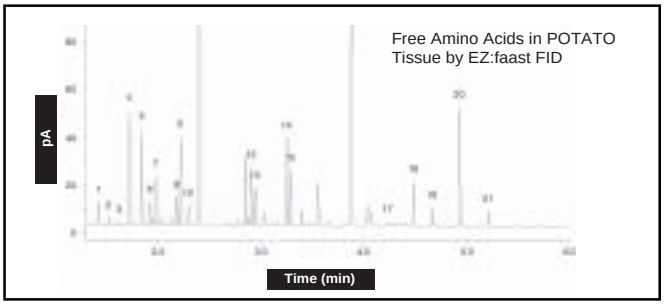
The repeatability of the EZ:faast® procedure was evaluated based upon multiple injections of the same preparation of a standard mixture, and also upon multiple preparations of the same potato extract. The RSD for injections of the same preparation was 0.72% (for 9 consecutive runs; *Figure 4*). The RSD for four preparations of the same extract was 3.1%.

Figure 4. Repeatability in Asparagine Derivative Quantitation



The accuracy of asparagine analysis using the EZ:faast® procedure was evaluated based on % recovery calculated from spiked potato extracts, at three different levels of concentration. First 50µL aliquots of each potato extract (prepared at 0.1g: 4, 40, or 400 mL extraction ratios) were prepared and analyzed as described above. A typical chromatogram for the analysis of amino acids in potato is shown in Figure 5. Next, 50µL aliquots were spiked with 20nmols of ASN. The recoveries at all three levels were 99-102% *(Table 1).*

Figure 5. Free Amino Acids in Potato Tissue by EZ:faast FID



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1 – ALA; 2 – GLY; 3 – ABA; 4 – VAL; 5 – IS; 6 – LEU; 7 – ILE; 8 – THR; 9 – SER; 10 – PRO; 11 – ASN; 12 – ASP; 13 – MET; 14 – GLU; 15 – PHE; 16 – GLN; 17 – ORN; 18 – LYS; 19 – HIS; 20 – TYR; 21 – TRP.

Table 1. Recovery data for the analysis of asparagine in potato extracts with the EZ:faast® AAA Ki

ASN concentration level	Conc in potato extract, nmoL/mL	Conc in spiked extract, nmoL/mL	Recovery, %	RSD, %
1	59.2	259.2	100	0.53
2	554.9	759.8	102	1.86
3	5796	5996	99.2	1.38

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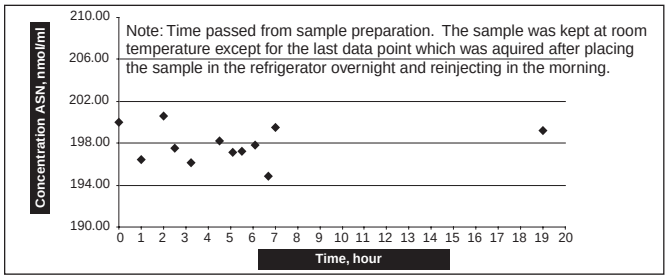
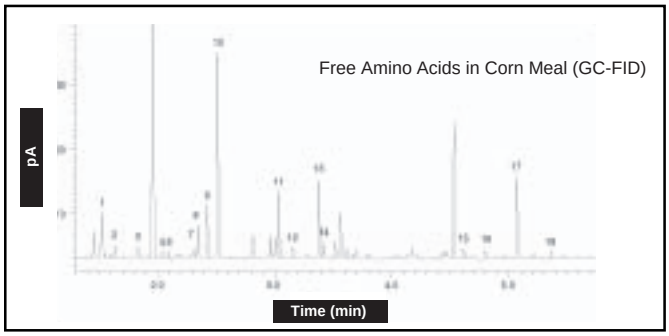
Derivative stability is an issue for several methods of analysis for amino acids. Ortho-phthalaldehyde (OPA) derivatives are notoriously unstable to such an extent that they have to be prepared and analyzed with careful timing, shortly after their preparation, otherwise analytical precision is influenced by the rate of decay of the prepared derivatives. The EZ:faast® ASN derivative is stable over many hours, at room temperature, as shown in *Figure 6*. The same sample prepared early in the day was analyzed repeatedly during a period of 7 hours (11 times). The sample was stored at room temperature during the day, then placed in the refrigerator overnight, and re-analyzed in the morning. No change in ASN concentration was recorded for over 20 hours passed from the time of sample preparation.

Figure 6. Asparagine Derivative Stability in Time



Other food products besides potato were found to contain high levels of acrylamide, although significantly less than potato. Corn and tomato have been shown to also generate acrylamide when cooked at high temperatures. The applicability of the EZ:faast® procedure for the analysis of ASN and of other amino acids in food products in general is exemplified in *Figure 7*. The major amino acids present in corn are ASN, GLU, SER and ASP, while in tomato GLU, GLN, ASP, ASN and SER.

Figure 7. Free Amino Acids in Corn Meal (GC-FID)



1 – ALA; 2 – GLY; 3 – VAL; 4 – IS; 5 – LEU; 6 – ILE; 7 – THR; 8 – SER; 9 – PRO; 10 – ASN; 11 – ASP; 12 – UNK; 13 – GLU; 14 – PHE; 15 – LYS; 16 – HIS; 17 – TYR; 18 – TRP

Conclusions

The EZ:faast® amino acid analysis procedure is a sensitive, accurate and reliable method for the analysis of asparagine and other amino acids in food products. It requires generic instrumentation available in most laboratories. The kit developed based on this procedure is the most rapid and cost effective product on the market.

References

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