Application Note

Food Testing and Agriculture

A Comparison of Amino Acid Profiles of Plant-Based Alternative Proteins and Meat Products

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Abstract

Alternate proteins are increasing in global demand due to health, economic, and ecological concerns. The comparison of amino acid profiles of plant-based proteins and their animal-meat counterparts is important in product development. This application note shows the determination and comparison of the amino acid composition of plant-based nuggets, chicken nuggets, and chicken breast meat following acidic hydrolysis. A total of 24 amino acids from the Agilent AdvanceBio amino acid analysis (AAA) standards and other standards (taurine, theanine, glucosamine, and cysteine) are included in the chromatographic method. Using the Agilent 1260 Infinity II LC connected serially to the Agilent 1260 Infinity II Diode Array Detector (DAD) and Agilent 1260 Infinity II Fluorescence Detector (FLD) allowed for comprehensive measurement of amino acids. The FLD detected all the amino acids except cysteine and cystine, which were reliably analyzed by DAD.

Introduction

To meet the protein needs of the population, meat products are consumed at an alarming rate[1]. Due to environmental factors, the ever-growing population is looking for alternative sources of protein for food consumption. In recent years, plant-based proteins have become a well-established class of alternative meat products. They are rich in proteins and amino acid content and can mimic the real meat product in terms of nutritional content. There are certain challenges[2] to plant-based products, such as low digestibility, texture, and flavor, as compared to regular meat.

In this study, the amino acid composition between plant-based nuggets, chicken meat, and chicken nuggets were studied. A chromatographic method separating 24 amino acids, which include the essential amino acids for screening and comparison was developed. Precolumn derivatization and method parameters were adopted from Agilent amino acid kit (5991-7694EN).

Experimental

Equipment

Amino acid analysis was performed using an Agilent 1260 Infinity II LC system with the following components. The LC system was operated using Agilent OpenLab CDS version 2.7.

Part Number	Component
G7112B	Agilent 1260 Infinity II Binary Pump
G7167A	Agilent 1260 Infinity II Multisampler
G7116A	Agilent 1260 Infinity II Multicolumn Thermostat
G7117C	Agilent 1260 Infinity II Diode Array Detector HS (with fixed slit)
G7121B	Agilent 1260 Infinity II Fluorescence Detector Spectra
G1321-60005	Agilent flow cell, 8 µL, 20 bar

Chemicals

All solvents used were LC grade. Acetonitrile was purchased from JT Baker (Phillipsburg, NJ, USA) and methanol was purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system (Millipak, Merck-Millipore, Billerica, MA, USA) equipped with a 0.22 µm membrane point-of-use cartridge. Sodium phosphate dibasic, disodium tetraborate decahydrate, hydrochloric acid, 37%, and phosphoric acid, 85%, were purchased from Sigma Aldrich (St. Louis, MO, USA).

The Agilent AdvanceBio Amino Acid Analysis (AAA) standards and reagents kit (part number 5190-9426) includes:

Part Number	Component
5061-3339	Borate buffer: 0.4 M in water, pH 10.2, 100 mL
5061-3337	FMOC reagent, 2.5 mg/ml in acetonitrile, 10 x 1 mL ampules
5061-3335	OPA reagent, 10 mg/mL in 0.4 M borate buffer and 3-mercaptoproprionic acid, 6 x 1 mL ampules
5061-3330	Amnio acid standard (mix of 14 standards), 1 nmol/µL, 10 x 1 mL
5061-3331	Amnio acid standard, 250 pmol/µL, 10 x 1 mL
5061-3332	Amnio acid standard, 100 pmol/µL, 10 x 1 mL
5061-3333	Amnio acid standard, 25 pmol/µL, 10 x 1 mL
5061-3334	Amnio acid standard, 10 pmol/µL, 10 x 1 mL
5062-2478	Amino acids supplement kit containing L-asparagine, L-glutamine, L-tryptophan, L-4-hydroxyproline, L-norvaline (IS), and sarcosine (IS)), 1 g each

Four other amino acids that were added to the amino acid analysis method were purchased from Sigma-Aldrich.

Part Number	Amino Acids
SMB00395	L-Theanine
PHR1109	Taurine
PHR1199	Glucosamine hydrochloride
168149	L-Cysteine

Preparation of solvents and reagents

- Mobile phase A contained 10 mM Na_2HPO_4, and 10 mM $Na_2B_4O_7$, pH 8.2.
- Mobile phase B contained acetonitrile, methanol, and water (45/45/10, v/v/v).
- 0.1N HCl was prepared by appropriate dilution of concentrated HCl using water
- Diluent: 10 mL of mobile phase A and 200 µL of phosphoric acid (85%)

Note: *After opening an OPA or FMOC ampoule, the reagents are aliquoted to amber vials (part number 5182-0716) with inserts (part number 5181-1270) and screw caps (part number 5190 7024). They are stored for no longer than a week.*

Note: *Borate buffer and injection diluent are transferred to vials without inserts. All reagents are at 4 °C. Reagents in the autosampler are exchanged daily.*

Preparation of amino acid standard solutions

1. Extended amino acid (EAA) stock solution: 1.8 nmol/µL each of asparagine, glutamine, and tryptophan, theanine, taurine, glucosamine, and 18 nmol/µL of cysteine in 0.1 M HCl.

2. Diluted EAA stock solution:

- 0.9 nmol/µL,
- 0.45 nmol/µL,
- 0.18 nmol/µL,
- 90 pmol/µL,
- 45 pmol/µL,
- 18 pmol/µL
- 9 pmol/µL with 0.1 M HCl.

Cysteine concentration in diluted EAA:

- 9 nmol/µL,
- 4.5 nmol/µL,
- 1.8 nmol/µL,
- 900 pmol/µL,
- 450 pmol/µL,
- 180 pmol/µL
- 90 pmol/µL with 0.1 M HCl.

3. Internal standard (IS) stock solution: 1.0 nmol/µL each of norvaline and sarcosine in 0.1 M HCl.

4. EAA solutions + IS: mix EAA and IS in 1:1 ratio to obtain amino acid concentrations of 4.5 to 900 pmol/µL (cysteine was at 45 to 9000 pmol/µL) and IS concentrations of 500 pmol/µL.

5. Final concentration of amino acid targets in calibration solutions:

- 0.45 (L1), 0.90 (L2), 2.25 (L3), 4.5 (L4), 9.0 (L5), 22.5 (L6), 45 (L7), and 90 (L8) pmol/µL of amino acids (for all except cysteine) with IS 50 pmol/µL
- For cysteine, the calibration level concentrations were 4.5 (L1), 9.0 (L2), 22.5 (L3), 45 (L4), 90 (L5), 225 (L6), 450 (L7), and 900 (L8) pmol/µL with IS 50 pmol/µL

Samples

Plant-based (soy-based) nuggets, chicken nuggets, and chicken breast meat were obtained from a local supermarket.

Sample preparation

For the extraction of amino acids in the plant-based nuggets, chicken nuggets, and chicken breast meat, the samples were hydrolyzed using 6 N HCl following a procedure described by Dai et al[3] as shown in Figure 1. Approximately five nuggets (~50 g), with the flour coating removed, were homogenized using a mechanical shaker. For chicken breast meat, ~ 50 g of meat was homogenized using a mechanical shaker. The 500 mg of the homogenized sample was weighed into a 15 mL glass tube for extraction. Water and fuming hydrochloric acid (37%) were added to the sample to result in 10 mL 6 N HCl. The tubes were capped and kept at a temperature of 110 °C for 24 hours for digestion. The tubes were gently shaken after 2 hours to facilitate the efficient digestion.

After digestion, the tubes were cooled to room temperature and centrifuged at 3000 rpm for 5 min. 1 mL of the supernatant was filtered using Agilent Captiva premium syringe filters (regenerated cellulose, 15 mm, 0.2 µm, part number 5190-5108). 10 µL of the filtered supernatant was diluted with 990 µL water to get a 100x dilution. Later, 950 µL of the diluted sample homogenate was added with 50 µL of the IS stock solution and analyzed on the Agilent 1260 Infinity II LC system.

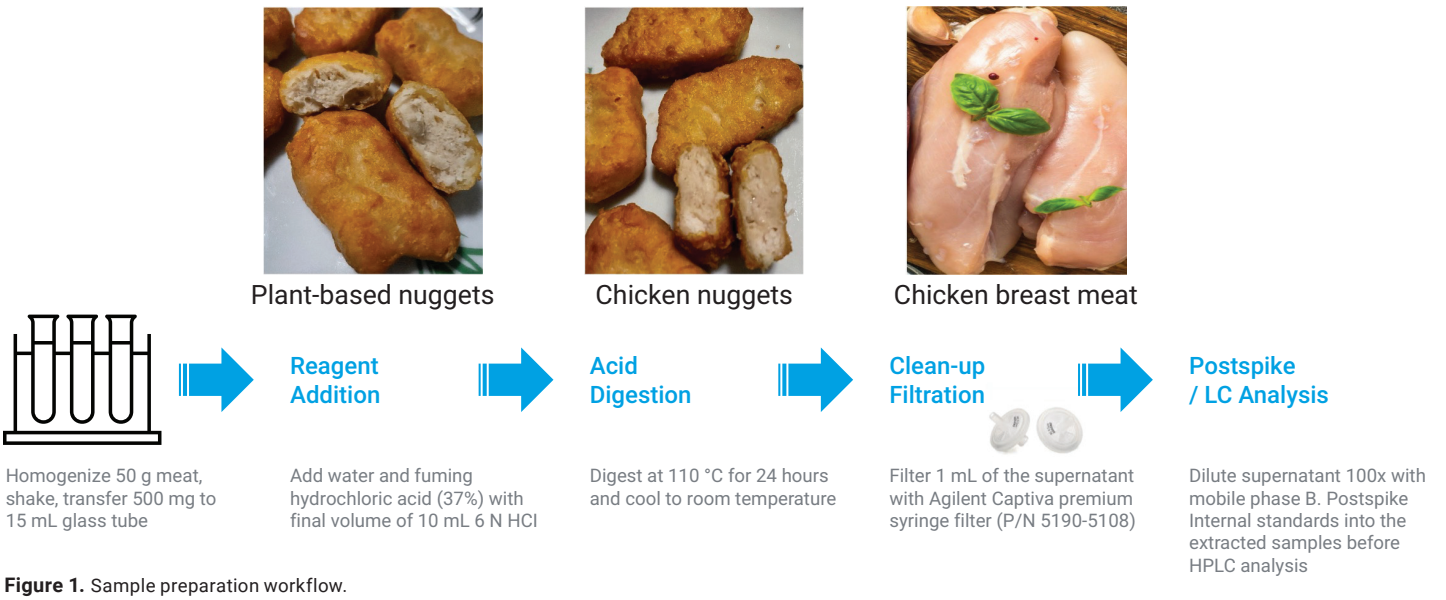

Figure 1. Sample preparation workflow.

LC analysis

Precolumn derivatization is employed based on o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) chemistry for primary and secondary amino acids. The derivatization procedures were automated using the LC autosampler injector program function. The precolumn derivatization program on the Agilent 1260 Infinity II Multisampler is shown in Table 1 and the HPLC method is shown in Table 2.

Table 1. Injector program for precolumn derivatization of amino acids. Where location 1 is borate buffer, 2 is OPA reagent, 3 is FMOC reagent and 4 is injection diluent.

Function	Parameter
Draw	Draw 5.00 µL from location "1" with default speed using default offset
Wash	Wash needle as defined in method
Draw	Draw 1.00 µL from sample with default speed using default offset
Wash	Wash needle as defined in method
Draw	Draw 1.00 µL from location "2" with default speed using default offset
Wash	Wash needle as defined in method
Mix	Mix 7.00 µL from air with default speed 10 times
Draw	Draw 0.40 µL from location "3" with default speed using default offset
Wash	Wash needle as defined in method
Mix	Mix 7.40 µL from air with default speed 10 times
Draw	Draw 32.00 µL from location "4" with maximum speed using default offset
Wash	Wash needle as defined in method
Mix	Mix 20.00 µL from air with maximum speed five times
Inject	Inject

Table 2. HPLC method for analysis of amino acids.

Parameter	Value
Column	Agilent AdvanceBio AAA LC column, 3.0 × 100 mm, 2.7 µm (part number 695975-322) Agilent AdvanceBio AAA guard column, 3.0 × 5 mm, 2.7 µm (part number 823750-946)
Solvent	Mobile phase A: 10 mM Na_2HPO_4, and 10 mM $Na_2B_4O_7$, pH 8.2 Mobile phase B: Acetonitrile, methanol, and water (45/45/10, v/v/v)
Gradient	0.0 minute – 2% B 0.4 minute – 2% B 2.0 minute – 15% B 5.0 minute – 15% B 12.0 minute – 38% B 14.0 minute – 57% B 16.0 minute – 57% B 16.5 minute – 100% B 20.0 minute – 100% B 20.5 minute – 2% B 24.0 minute – 2% B Stop time: 24 minute Post time: 2 minute

Table 2. HPLC method for analysis of amino acids.

Flow Rate	0.6 mL/minute
Temperature	40 °C
Detection (DAD)	338 nm, 10 nm bandwidth, and reference wavelength 390 nm, 20 nm bandwidth
Detection (FLD)	Excitation: 345 nm; emission: 455 nm 15.20 minute: change excitation: 265 nm; change emission: 315 nm PMT gain: 10 Peak width: >0.025 min (18.52 Hz)
Injection	1 µL, use vial/well bottom sensing Draw speed 100 µL/minute; ejection speed 400 µL/minute
Needle Wash	Flush port, 20 seconds acetonitrile: 0.1 M HCl (50:50; v:v)

Results and discussion

Neat standard mix

The existing Agilent AdvanceBio AAA HPLC method was improved for the analysis of the extended list of 24 amino acids that includes taurine, theanine, cysteine, and glucosamine. Reversed-phase LC with DAD, and FLD, detection was employed for the analysis. Figure 2 shows the separation of the 24 amino acids standard mixture together with two internal standards, norvaline and sarcosine. All the amino acids showed better sensitivity in FLD than in DAD except for cysteine and cystine (Figures 3 and 4). The lower sensitivity in FLD for cysteine and cystine is due to the low fluorescence of the adducts, which are formed with the OPA reagent[4]. Hence, for cysteine and cystine, DAD data was used for result reporting whereas FLD data was used for the rest of the amino acids.

The limit of detection, signal to noise (LOD, s/n): ≥3 and the limit of quantification, signal to noise (LOQ, s/n): ≥10 for the amino acid analyzed was 0.45 pmol/µL and 0.90 pmol/µL respectively for all the amino acids except for cysteine, which was at 4.5 pmol/µL and 9.00 pmol/µL The calibration range was from 0.90 to 90 pmol/µL for all the amino acids except for cysteine, which was in the range of 9 to 900 pmol/µL. Excellent coefficient of determination (R^2) values above 0.999 were achieved for all the amino acids.

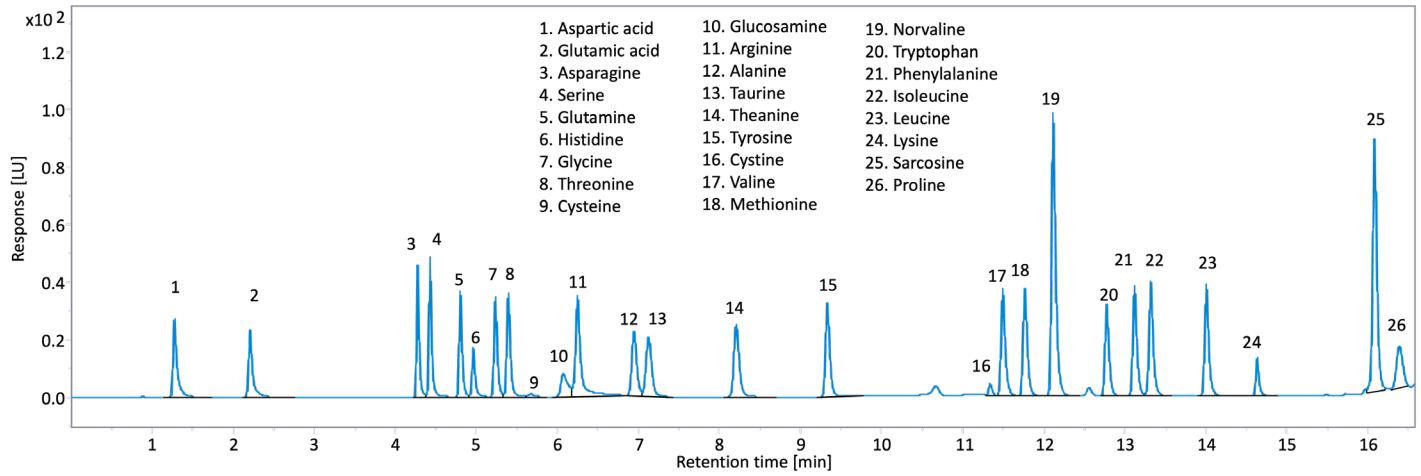

Figure 2. FLD chromatogram of amino acid calibration standard solution level 6.

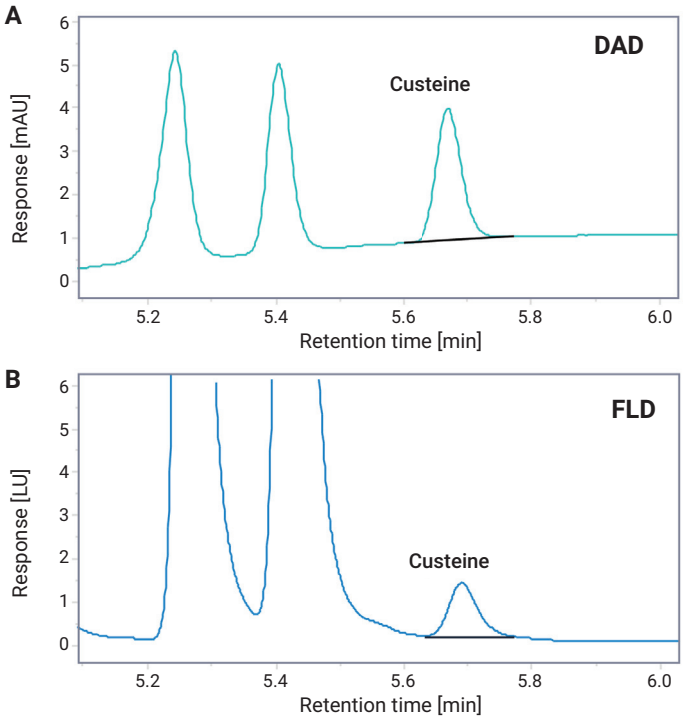

Figure 3. DAD **(A)** and FLD **(B)** chromatograms of cysteine at 225 pmol/µL.

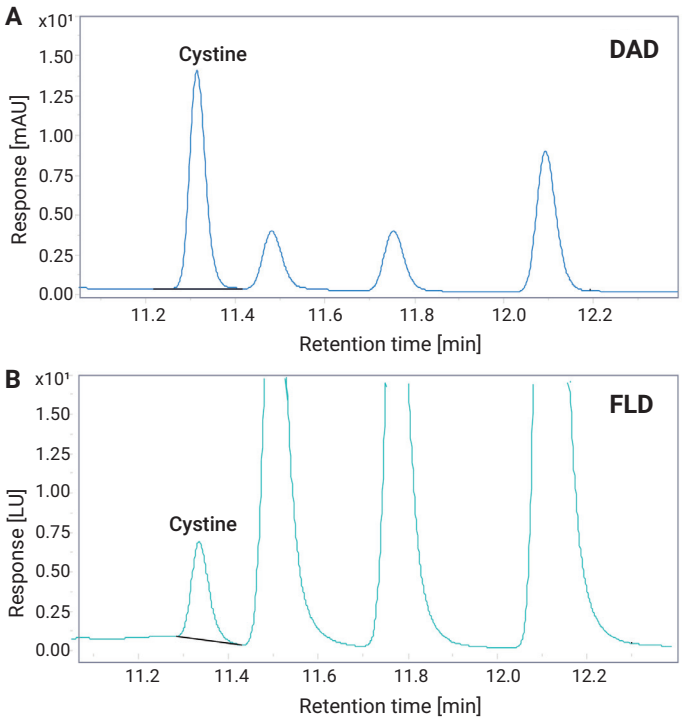

Figure 4. DAD **(A)** and FLD **(B)** chromatograms of cystine at 22.5 pmol/µL.

Table 3. Method performance summary using neat standard mix. Retention time, precision, accuracy, and resolution results are calculated at calibration level 6 concentration.

Peak Number	Compound	Retention Time (min)	RT RSD (%)	Area RSD (%)	Accuracy (%)	Resolution	Calibration Range (pmol/µL)	Calibration Type	R^2
1	Aspartic acid	1.30	0.3	0.3	95		0.90 to 90	Linear	1.000
2	Glutamic acid	2.34	0.8	0.3	91	12.1	0.90 to 90	Linear	0.999
3	Asparagine	4.36	0.4	0.3	98	30.1	0.90 to 90	Linear	1.000
4	Serine	4.51	0.4	0.3	96	2.9	0.90 to 90	Linear	1.000
5	Glutamine	4.88	0.3	0.6	97	6.7	0.90 to 90	Linear	0.999
6	Histidine	5.05	0.3	1.2	101	2.7	0.90 to 90	Quadratic	1.000
7	Glycine	5.33	0.3	0.2	96	4.1	0.90 to 90	Linear	1.000
8	Threonine	5.49	0.3	0.4	97	2.2	0.90 to 90	Linear	1.000
9	Cysteine*	5.77	0.3	0.2	97	3.3	9 to 900	Quadratic	0.999
10	Glucosamine	6.21	0.3	0.3	98	3.8	0.90 to 90	Linear	1.000
11	Arginine	6.36	0.3	0.6	97	1.3	0.90 to 90	Linear	1.000
12	Alanine	7.06	0.3	0.3	94	6.1	0.90 to 90	Linear	0.999
13	Taurine	7.25	0.3	0.2	93	1.5	0.90 to 90	Linear	0.999
14	Theanine	8.30	0.3	0.3	96	8.7	0.90 to 90	Linear	0.999
15	Tyrosine	9.38	0.4	0.3	96	10.3	0.90 to 90	Linear	1.000
16	Cystine*	11.28	0.3	1.8	95	25.5	0.90 to 90	Linear	0.999
17	Valine	11.46	0.3	0.2	96	2.1	0.90 to 90	Linear	1.000
18	Methionine	11.73	0.3	0.2	96	3.0	0.90 to 90	Linear	1.000
19	Norvaline	12.06	0.3	0.2	NA	3.7	NA	NA	NA
20	Tryptophan	12.70	0.2	0.5	97	7.2	0.90 to 90	Linear	1.000
21	Phenylalanine	13.03	0.2	0.5	97	3.7	0.90 to 90	Linear	1.000
22	Isoleucine	13.22	0.2	0.6	97	2.1	0.90 to 90	Linear	1.000
23	Leucine	13.89	0.1	0.7	97	7.3	0.90 to 90	Linear	1.000
24	Lysine	14.50	0.0	1.9	101	7.6	0.90 to 90	Linear	0.999
25	Sarcosine	16.02	0.1	1.7	NA	18.4	NA	NA	NA
26	Proline	16.38	0.1	1.7	94	2.6	0.90 to 90	Linear	0.999

**Cysteine and cystine results were based on DAD.*

Retention time and area precision values were below 0.4 and 1.0% respectively (n=3). At 22.5 pmol/µL, the average resolutions of all amino acid peaks were above 1.5 except for arginine. All amino acid targets exhibited a good accuracy value (back calculated using linearity equations) between 91 to 101 %. Precision (RT and area %RSD), accuracy, resolution, and calibration results obtained from the analysis of the amino acid calibration standards are presented in Table 3.

Method applicability for plant-based and meat products analysis

The FLD chromatograms of endogenous amino acids present in plant-based nuggets and chicken nuggets is shown in Figure 5. Cysteine and cystine were detectable in DAD (Figures 6 and 7).

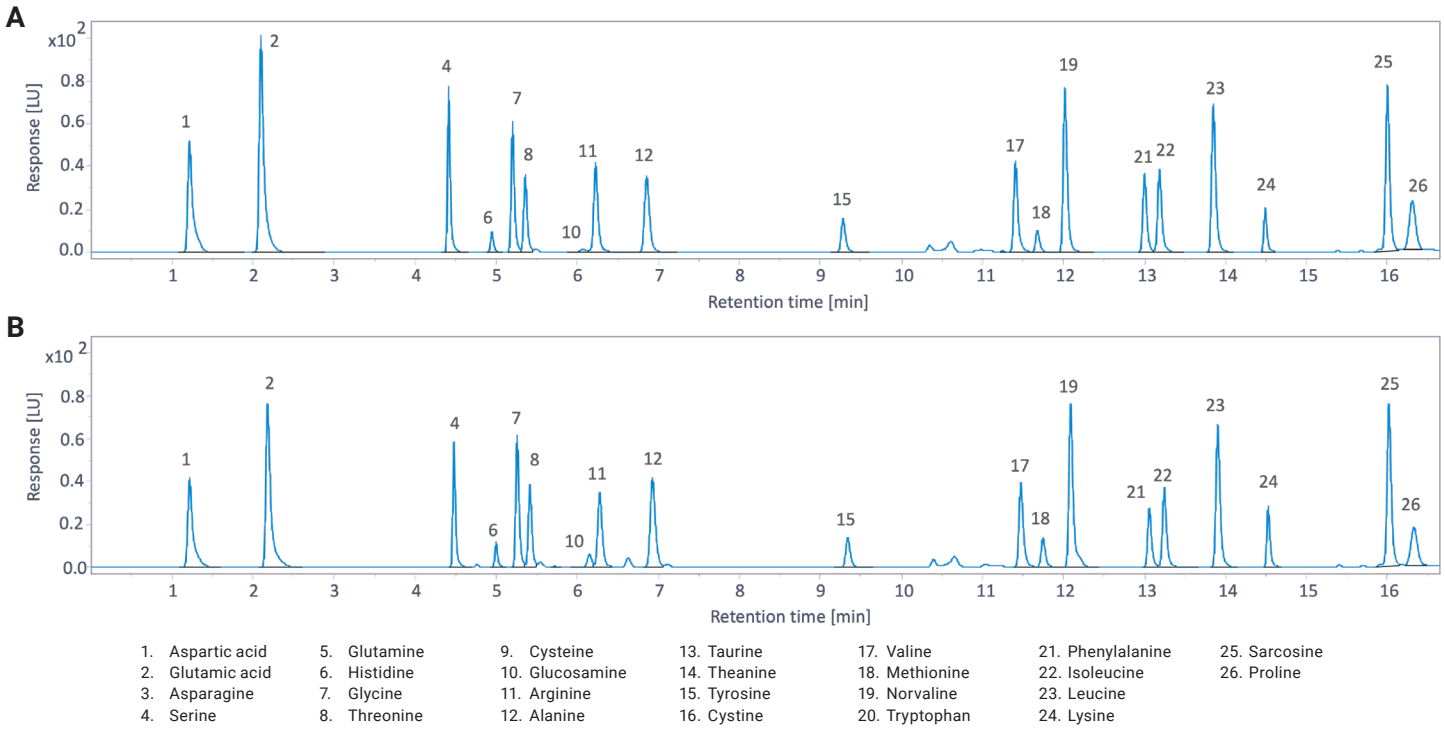

Figure 5. FLD chromatograms of endogenous amino acids separation in plant-based **(A)** and chicken nuggets **(B)**.

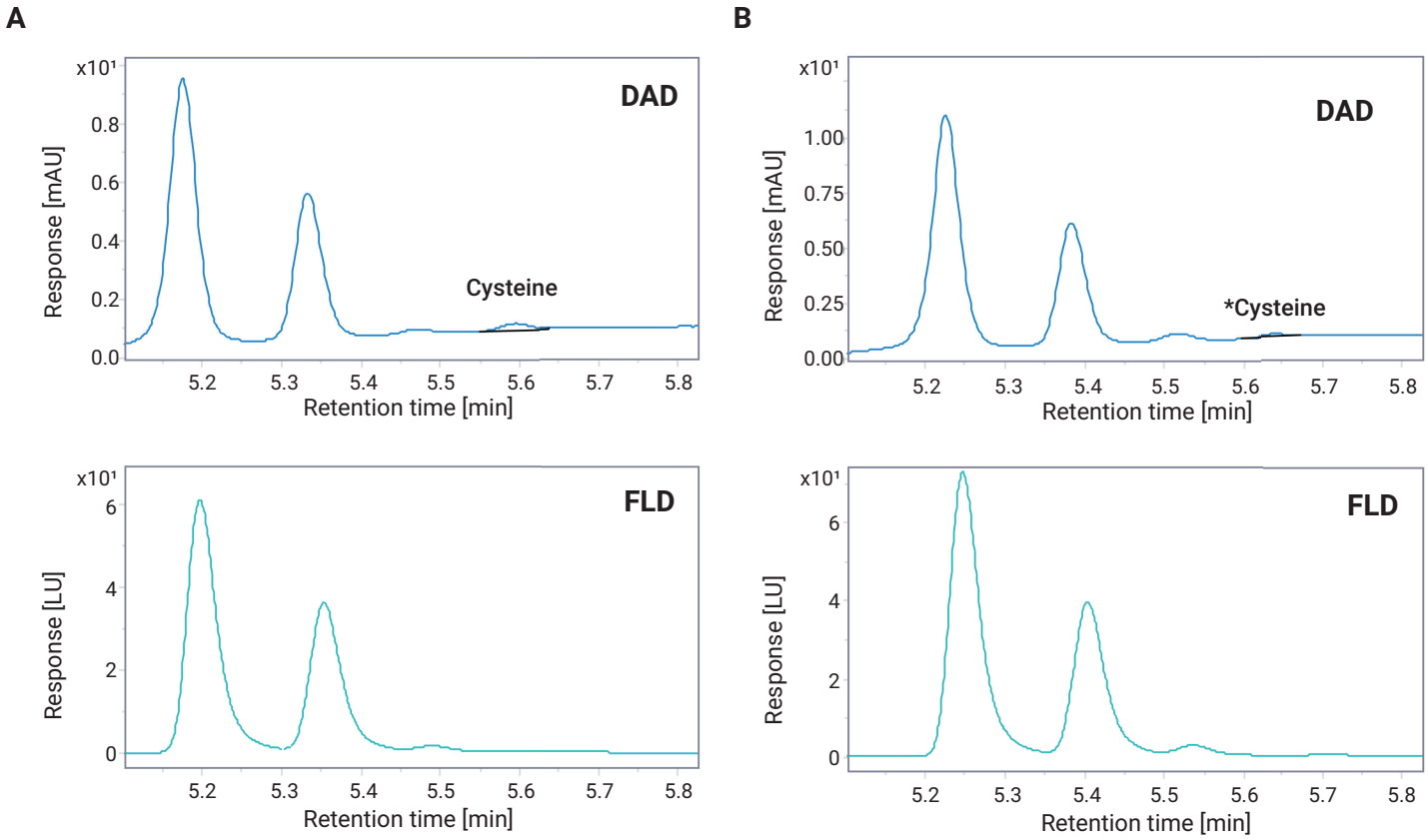

Figure 6. DAD and FLD chromatograms of endogenous cysteine in plant-based nuggets **(A)** and chicken nuggets **(B)**. *Cysteine in DAD of chicken nuggets is below the limit of quantification (LOQ) but above the limit of detection (LOD).

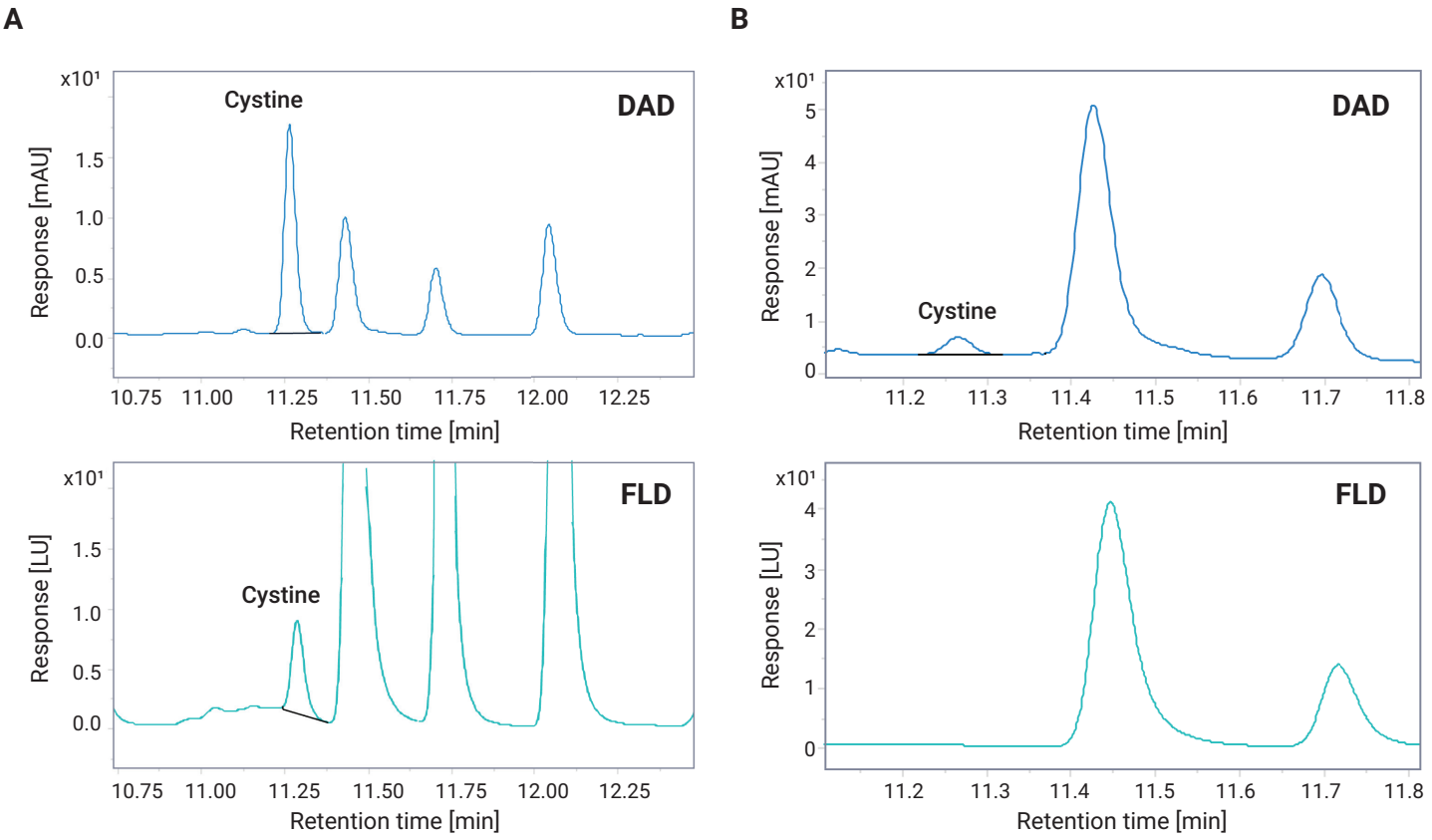

Figure 7. DAD and FLD chromatograms of endogenous cystine in plant-based nuggets **(A)** and chicken nuggets **(B)**.

The amino acid profile of plant-based nuggets, chicken nuggets, and chicken breast meat are shown in Figure 8. Chicken breast meat contains a higher concentration of amino acids than processed plant-based/chicken nuggets. Essential amino acids such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine are reported to be lower in levels or lacking in plant-based products[2]. Both plant-based and chicken nuggets had similar levels of essential amino acids in this analysis. Chicken flavor is influenced by cystine and cysteine amino acids[5]. The study finds cysteine to be well represented in plant-based nuggets compared to chicken nuggets. Other amino acids, especially the glutamic acid, threonine, serine, glycine, and alanine, which are known to add taste[6] are also represented well in both products.

The amino acids asparagine and glutamine are converted to aspartic acid and glutamic acid during acid hydrolysis and tryptophan is decomposed[7]. As a result, in all matrices, these amino acids are absent. In this amino acid profiling experiment, it was also observed that taurine and theanine are absent in all the matrices.

Literature suggests that glutamic acid is found to be more abundant in plant-based products[8]. In the present study, glutamic acid was found to be more abundant in plant-based nuggets. While plant-based nuggets contain less glucosamine than chicken nuggets.

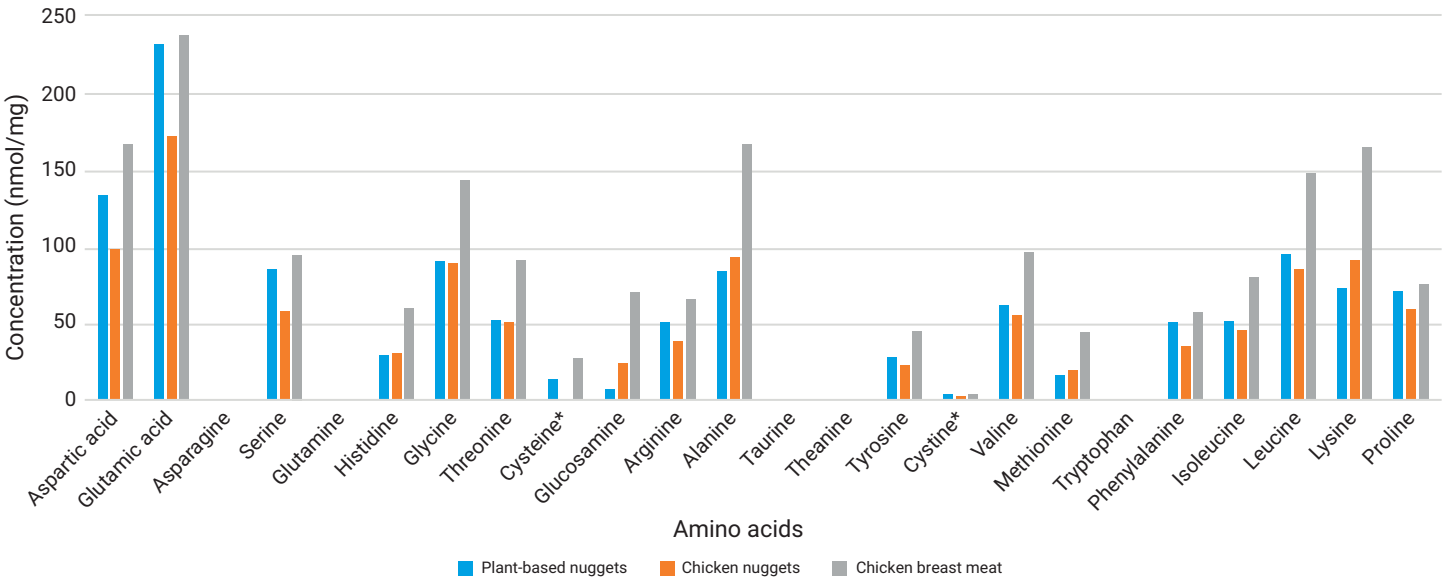

Figure 8. Comparison of amino acid profiles between plant-based nuggets, chicken nuggets, and chicken breast meat. *Cysteine and cystine results were based on DAD.

For amino acid recovery analysis, the matrix blank was spiked at level 6 concentration. The endogenous concentration of targets was subtracted from the postspiked matrix and compared with calibration level 6 and expressed as percentage (Table 4). The recovery of individual amino acid ranged between 70 to 130 % for all compounds in both matrices except for cysteine in plant-based nuggets. These results demonstrated the suitability of the method for the analysis of the amino acid profile for the nuggets.

Table 4. Spike recovery results at level 6 calibration concentration.

Peak Number	Name	% Recovery	
		Plant-Based Nuggets	Chicken Nuggets
1	Aspartic acid	111	126
2	Glutamic acid	102	126
3	Asparagine	102	96
4	Serine	108	115
5	Glutamine	98	90
6	Histidine	118	119
7	Glycine	106	121
8	Threonine	106	110
9	Cysteine*	142	128
10	Glucosamine	117	100
11	Arginine	107	95
12	Alanine	109	118
13	Taurine	106	100
14	Theanine	102	96
15	Tyrosine	107	104
16	Cystine*	111	106
17	Valine	108	110
18	Methionine	105	106
19	Tryptophan	102	97
20	Phenylalanine	108	107
21	Isoleucine	108	109
22	Leucine	110	118
23	Lysine	129	121
24	Proline	127	116

**Cysteine and cystine results were based on DAD.*

Conclusion

This application note demonstrates the effective amino acid profiling of plant-based and meat products using the Agilent 1260 Infinity II LC system with Agilent 1260 Infinity II DAD and Agilent 1260 Infinity II FLD. The present study suggests that the plant-based nuggets and chicken nuggets are comparable in amino acid content. The additional amino acids in the method such as cysteine and glucosamine are also represented in the nuggets. Our method using the Agilent AdvanceBio AAA column allows for the separation of 24 amino acids with good analytical performance. The automated precolumn derivatization saves time and removes the manual liquid handling steps with reduced human error. Agilent Captiva premium syringe filters used in sample cleanup minimizes the matrix interferences and offers good target recovery. The developed method is suitable for profiling amino acids from various alternative proteins samples.

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