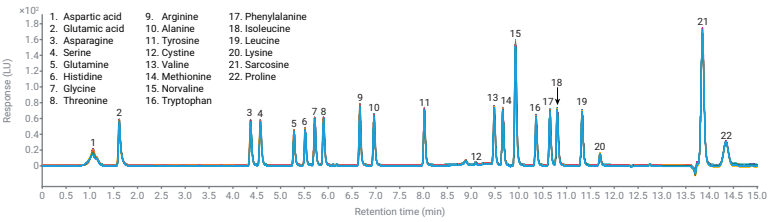
Application Note

Food & Beverage Testing

Comparison of Plant-Based Meat Alternatives and Meat

Analysis of amino acid profiles using an Agilent 1260 Infinity II LC



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Abstract

Increasing numbers of plant-based meat alternatives are being developed in response to consumer demand for sustainable food supplies and a healthy diet. The comparison of the amino acid profile of proteins from meat and plant-based meat alternatives is of interest from a nutritional quality perspective.

This application note shows the determination of the amino acid composition of beef burger patties and different plant-based burger patties following acidic hydrolysis of sample proteins. Amino acids are analyzed with an Agilent 1260 Infinity II LC using reversed-phase LC with fluorescence detection (FLD) and automated precolumn derivatization using the injector program available with the Agilent 1260 Infinity II Multisampler.

Introduction

In recent years, there has been increasing consumer interest in meat alternatives. Reasons for this trend include negative impressions of the health impact of meat, environmental stress associated with animal meat production, and animal welfare.[1] Meat alternatives include plant-based, cell-based (*in vitro* or cultured meat), and fermentation-based (mycoproteins) products. Plant-based meat alternatives represent a primary sector of this industry, and their market has grown exponentially in recent years.[1] The transition towards a more plant-based diet is considered to reduce a person's environmental footprint compared with the consumption of animal-based foods. This transition appears to be supported by plant-based products that directly mimic meat, and thus do not lead to a fundamental change in dietary habits.[2] Most plant-based meat alternatives in development are protein-based, and considering their availability, cost, and processing functionality, soy and pea proteins as well as wheat gluten are most widely used.[1] From a nutritional quality perspective, the comparison of the amino acid profile of meat and plant-based meat alternatives is of interest.

The amino acid profile of a protein is typically analyzed following hydrolysis with 6 M HCl at 110 °C for 24 hours.[3] Analysis of amino acids can be performed using various analytical methods, such as LC with fluorescence or UV detection following derivatization, LC/MS, or CE/MS.[3,4] Precolumn derivatization with o-phthalaldehyde (OPA) and 3-mercaptopropionic acid for primary as well as 9-fluorenylmethylchloroformate (FMOC) for secondary amino acids overcomes the insufficient analyte retention on reversed-phase columns and the weak fluorescence and ultraviolet absorbance of amino acids.[4] Furthermore, derivatization of amino acids followed by LC with FLD increases selectivity of the analysis.

This application note demonstrates the analysis of the amino acid profile of beef burger patties and different plant-based burger patties using a 1260 Infinity II LC with FLD. The injector program available with the 1260 Infinity II Multisampler enables automated precolumn derivatization of amino acids[4], avoiding manual liquid handling steps and saving time and cost generated by manual work. Possible errors resulting from manual work are also prevented.

Experimental

Equipment

The Agilent 1260 Infinity II LC System comprised the following modules:

- Agilent 1260 Infinity II Binary Pump (G7112B)
- Agilent 1260 Infinity II Multisampler (G7167A)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Fluorescence Detector Spectra (G7121B) with flow cell, 8 µL, 20 bar (G1321-60005)

Software

Agilent OpenLab CDS version 2.6, or later versions

Columns

Agilent AdvanceBio AAA LC column, 3.0 × 100 mm, 2.7 µm (part number 695975-322) with Agilent AdvanceBio AAA guard column, 3.0 × 5 mm, 2.7 µm (part number 823750-946)

Chemicals

All solvents were LC grade. Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). Sodium phosphate dibasic, disodium tetraborate decahydrate, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid, 37%, and phosphoric acid, 85%, were obtained from Merck (Darmstadt, Germany), and hydrochloric acid, 6 N, was obtained from Fluka (Steinheim, Germany).

Amino acid standards and derivatization reagents were obtained from Agilent:

- Amino acid supplement (part number 5062-2478) containing: L-asparagine, L-glutamine, L-tryptophan, L-4-hydroxyproline, L-norvaline, and sarcosine (1 g each)
- Amino acid standard, 100 pmol/µL (part number 5061-3332)
- Amino acid standard, 25 pmol/µL (part number 5061-3333)
- Amino acid standard, 10 pmol/µL (part number 5061-3334)
- Borate buffer 0.4 N in water, pH 10.2, 100 mL (part number 5061-3339)
- FMOC reagent, 2.5 mg/mL 9-fluorenylmethylchloroformate in acetonitrile, 10 × 1 mL (part number 5061-3337)

– OPA reagent, 10 mg/mL each of o-phthalaldehyde and 3-mercaptopropionic acid in 0.4 M borate buffer, 6 × 1 mL (part number 5061-3335)

Samples

Beef burger patties and different plant-based burger patties based on pea, soy, and wheat protein were obtained from a local supermarket.

Preparation of solvents and derivatization reagents

– **Mobile phase A:** Weigh 2.8 g of sodium phosphate dibasic (Na_2HPO_4) and 7.6 g of disodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10\ H_2O$), add 1.9 L of water and 1.5 mL of fuming hydrochloric acid (37%), mix until homogeneous, fill up to the total volume of 2 L with water and adjust the pH with fuming hydrochloric acid to pH 8.2. It is recommended to use an amber 2 L solvent bottle (part number 9301-6341) to avoid algae growth.

– **Mobile phase B:** Acetonitrile:methanol:water 45:45:10 (v:v:v)

– **Injection diluent:** 10 mL of mobile phase A + 200 µL of phosphoric acid (85%)

– After opening an OPA or FMOC ampoule, the reagents are distributed to amber vials (part number 5182-0716) with inserts (part number 5181-1270) and screw caps (part number 5190-7024) and stored for no longer than a week. Borate buffer and injection diluent are transferred to vials without inserts. All reagents should be stored at 4 °C and reagents in the autosampler should be exchanged daily.

Preparation of amino acid standard solutions

– An extended amino acid (EAA) stock solution containing 1.8 nmol/µL each of asparagine, glutamine, and tryptophan was prepared in 0.1 M HCl in water. The EAA stock solution was diluted to 0.9 nmol/µL, 0.45 nmol/µL, 0.18 nmol/µL, 90 pmol/µL, 45 pmol/µL, 18 pmol/µL, and 9 pmol/µL with 0.1 M HCl in water.

– An internal standard (IS) stock solution containing 1.0 nmol/µL each of norvaline and sarcosine was prepared in 0.1 M HCl in water.

– The EAA solutions were combined 1:1 with the IS stock solution to obtain amino acid concentrations of 4.5 to 900 pmol/µL and IS concentrations of 500 pmol/µL.

– Amino acid calibration solutions were prepared at 0.45, 0.90, 2.25, 4.5, 9.0, 22.5, 45, and 90 pmol/µL of amino acids and 50 pmol/µL of internal standards by combination of the EAA-IS solutions with amino acid standards and 0.1 M HCl in water.

Sample preparation

For determination of the amino acid profile, proteins contained in the samples were hydrolyzed using 6 N HCl following a procedure described by Dai *et al.*[3] Note that acidic hydrolysis leads to the conversion of asparagine, glutamine, and cysteine to aspartic acid, glutamic acid, and cystine, respectively. Tryptophan is decomposed during acidic hydrolysis.[3]

To investigate method suitability, amino acid recoveries were determined in triplicate from BSA. For this purpose, approximately 50 mg of BSA (equivalent to 0.75 µmol of protein) was weighed into a 15 mL Kimax glass tube, 10 mL of 6 N HCl was added, and the tube was gassed with nitrogen and capped.

For determination of the amino acid profiles of the samples, approximately 2 g of sample was accurately weighed and homogenized in 8 mL water using a laboratory homogenizer. Approximately 1.2 g of the resulting suspension was weighed into a 15 mL Kimax glass tube and appropriate amounts of water and fuming hydrochloric acid (37%) were added to result in 10 mL 6 M HCl. The tube was gassed with nitrogen and capped.

The tubes were placed in an oven with an inside temperature of 110 °C for 24 hours. After 2 hours, the tubes were gently shaken to ensure that the sample was completely covered by the solution. After the 24-hour period, the tubes were allowed to cool to room temperature, and the whole solution was transferred to a 100 mL flask and made up to the final volume with water. One hundred microliters of the resulting hydrolysate was combined with 50 µL of the IS stock solution and 850 µL water and filtered using a 1 mL plastic syringe with Agilent Captiva premium syringe filters, regenerated cellulose, 15 mm, 0.2 µm (part number 5190-5108).

Table 1. Method for analysis of derivatized amino acids.

Parameter	Value
Column	Agilent AdvanceBio AAA LC column, 3.0 × 100 mm, 2.7 µm with Agilent AdvanceBio AAA guard column, 3.0 × 5 mm, 2.7 µm
Solvent	A) 10 mM Na_2HPO_4 and 10 mM $Na_2B_4O_7$, pH 8.2 B) Acetonitrile:methanol:water (45:45:10, v:v:v)
Gradient	0.00 min – 2% B 0.40 min – 2% B 13.60 min – 57% B 14.00 min – 100% B Stop time: 17 min Post time: 3 min
Flow Rate	0.600 mL/min
Temperature	40 °C
Detection	Excitation: 345 nm; emission: 455 nm 13.00 min: change excitation: 265 nm; change emission: 315 nm PMT gain: 10 Peak width: >0.025 min (18.52 Hz)
Injection	Use sample preparation method (injector program) shown in Table 2 for derivatization of amino acids Injection volume: 1 µL Needle wash: 5 s in acetonitrile:0.1 M HCl in water (50:50; v:v) Draw speed: 100 µL/min Eject speed: 400 µL/min Wait time after draw: 1.2 s Use vial/well bottom sensing

Table 2. Sample preparation method (injector program) for derivatization of amino acids.

Function	Parameter
Draw	Draw 5.00 µL from location "Borate Buffer" 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 "OPA reagent" 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 "FMOC reagent" 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 "Injection Diluent" 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

Results and discussion

To enable determination of the amino acid profile of beef burger patties and different plant-based burger patties, amino acids were analyzed using reversed-phase LC with FLD and automated precolumn derivatization following acidic hydrolysis of sample proteins. The LC-FLD analysis with precolumn derivatization of amino acids using the injector program has been described in detail in a previous application note.[4]

Figure 1 shows the tenfold analysis of a calibration solution containing 22.5 pmol/µL of amino acids and 50 pmol/µL of internal standards. Twenty amino acids and the two internal standards norvaline and sarcosine were successfully separated within a run time of 17 minutes. Excellent retention time and peak area precision was obtained, showing values below 0.1% RT RSD and below 1.0% area RSD for most compounds (N = 10; see Table 3).

Repeatability, sensitivity, and calibration results obtained during the analysis of amino acid calibration solutions are presented in Table 3. Excellent sensitivity with limit of detection (LOD) values below 0.2 pmol on column was observed for all amino acids except cystine. The higher LOD obtained for cystine can be explained by low fluorescence of the adduct formed with the OPA reagent.[5] Calibration was performed in the range of 0.45 to 90 pmol/µL and showed excellent R^2 values above 0.999 for all compounds.

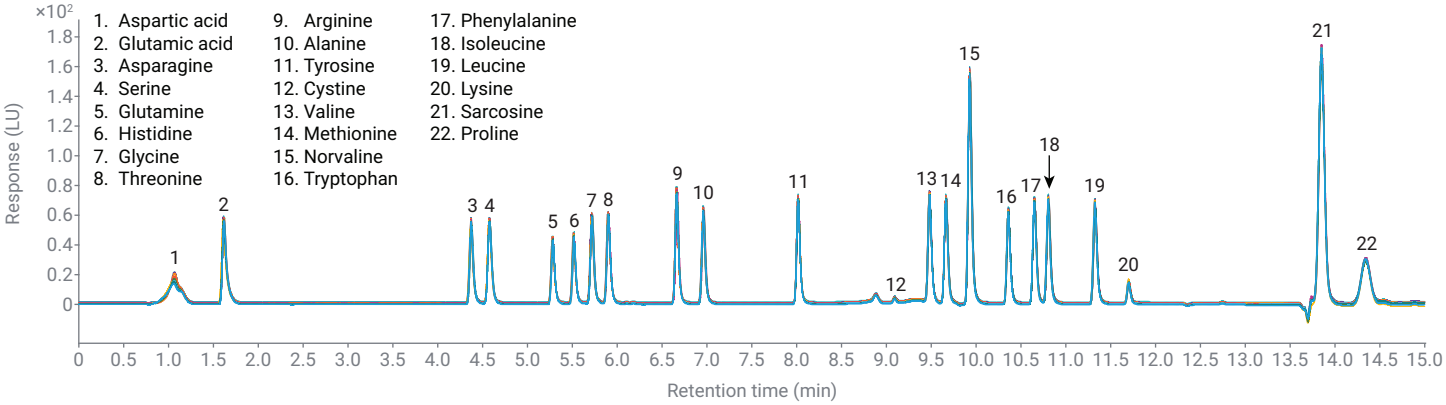

Figure 1. Tenfold analysis of a calibration solution containing 22.5 pmol/µL of amino acids and 50 pmol/µL of internal standards.

Table 3. Repeatability, sensitivity, and calibration results obtained during amino acid analysis. Repeatability calculations are based on 10 consecutive analyses of a calibration solution containing 22.5 pmol/µL of amino acids and 50 pmol/µL of internal standards. Limit of detection (LOD) is calculated for an S/N value of 3.

Peak No.	Compound	Retention Time (min)	RT RSD (%)	Area RSD (%)	LOD (pmol on column)	Calibration Range (pmol/µL)	Calibration Type	R^2
1	Aspartic acid	1.07	0.60	0.58	0.12	0.45 to 90	Linear	0.99999
2	Glutamic acid	1.63	0.20	0.30	0.07	0.45 to 90	Linear	0.99999
3	Asparagine	4.38	0.05	0.26	0.06	0.45 to 90	Linear	1.00000
4	Serine	4.58	0.05	0.24	0.06	0.45 to 90	Linear	0.99999
5	Glutamine	5.29	0.04	0.21	0.08	0.45 to 90	Linear	0.99999
6	Histidine	5.53	0.03	0.60	0.07	0.45 to 90	Quadratic	0.99999
7	Glycine	5.73	0.03	0.32	0.05	0.45 to 90	Linear	0.99999
8	Threonine	5.91	0.03	0.24	0.06	0.45 to 90	Linear	1.00000
9	Arginine	6.67	0.04	0.27	0.05	0.45 to 90	Linear	0.99999
10	Alanine	6.97	0.03	0.23	0.06	0.45 to 90	Linear	0.99998
11	Tyrosine	8.02	0.02	0.26	0.05	0.45 to 90	Linear	0.99999
12	Cystine	9.10	0.01	1.08	1.98	4.5 to 90	Quadratic	0.99989
13	Valine	9.49	0.01	0.24	0.05	0.45 to 90	Linear	0.99999
14	Methionine	9.67	0.01	0.23	0.06	0.45 to 90	Linear	0.99993
15	Norvaline*	9.93	0.01	0.23	NA	NA	NA	NA
16	Tryptophan	10.37	0.01	0.44	0.06	0.45 to 90	Linear	0.99993
17	Phenylalanine	10.66	0.01	0.28	0.06	0.45 to 90	Linear	0.99999
18	Isoleucine	10.81	0.01	0.32	0.05	0.45 to 90	Linear	0.99996
19	Leucine	11.33	0.01	0.40	0.05	0.45 to 90	Linear	0.99996
20	Lysine	11.71	0.01	3.22	0.17	0.45 to 90	Quadratic	0.99925
21	Sarcosine*	13.85	0.01	2.57	NA	NA	NA	NA
22	Proline	14.35	0.01	3.05	0.04	0.45 to 90	Quadratic	0.99997

* Internal standard

To investigate suitability of the method for the analysis of the amino acid profile of a protein, BSA was hydrolyzed, and the recovery rates of the individual amino acids were determined. Figure 2 and Table 4 show the analysis and the determined recovery rates.

As mentioned previously, acidic hydrolysis using 6 M HCl at 110 °C for 24 hours leads to the conversion of asparagine, glutamine, and cysteine to aspartic acid, glutamic acid, and cystine, respectively, and tryptophan is decomposed during acidic hydrolysis.[3] Tryptophan could be recovered using an alkaline hydrolysis and the determination of asparagine and glutamine could be accomplished using enzymatic hydrolysis.[3]

Recovery rates determined for the individual amino acids range between 85% and 115% for all compounds except cystine (see Table 4). These results demonstrate the suitability of the method for the analysis of the amino acid profile of a protein.

Figure 3 and Table 5 show the results of the amino acid profile analysis of a beef burger patty and three different plant-based burger patties that were obtained from a local supermarket. Differences between the amino acid profiles of the individual samples can be clearly observed. Cystine could be detected in all samples analyzed but was not quantified, as peak areas were below the calibration range.

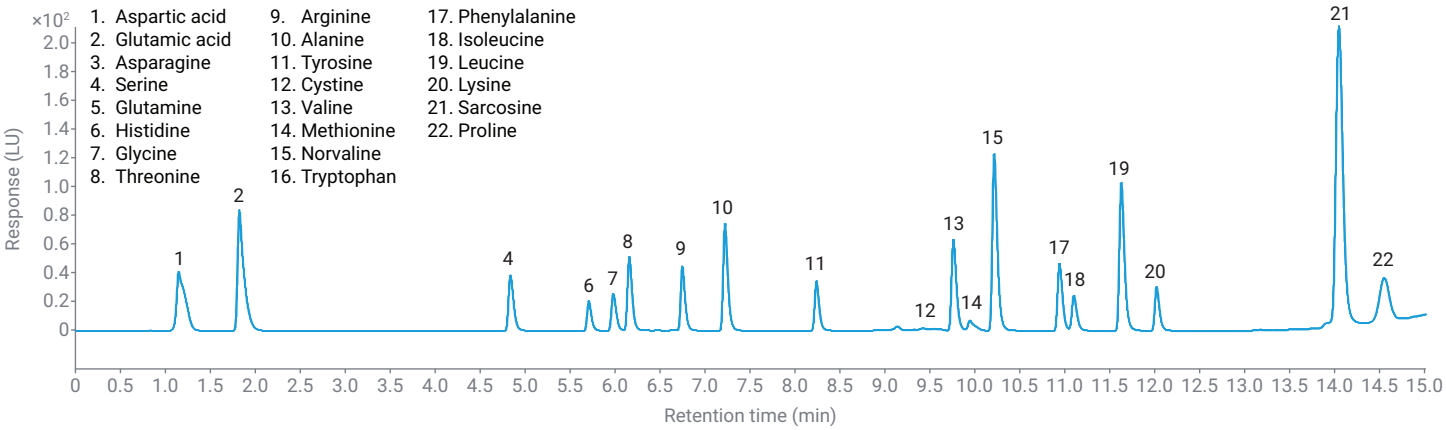

Figure 2. Analysis of the amino acid profile of a hydrolysate of bovine serum albumin (BSA).

Table 4. Recovery rates of the individual amino acids determined during the analysis of a hydrolysate of bovine serum albumin (BSA) (N = 3).

Peak No.	Compound	Recovery Rate (%)
1	Aspartic acid*	115.3
2	Glutamic acid*	104.7
3	Asparagine	Converted to aspartic acid
4	Serine	85.2
5	Glutamine	Converted to glutamic acid
6	Histidine	90.6
7	Glycine	94.3
8	Threonine	86.8
9	Arginine	92.6
10	Alanine	90.6

Peak No.	Compound	Recovery Rate (%)
11	Tyrosine	86.6
12	Cystine	45.5
13	Valine	88.2
14	Methionine	109.5
16	Tryptophan	Not recovered
17	Phenylalanine	91.2
18	Isoleucine	91.0
19	Leucine	89.7
20	Lysine	95.2
22	Proline	95.0

* Aspartic acid and glutamic acid originate from the sum of aspartic and glutamic acid contained in the sample and the conversion of asparagine and glutamine to their respective acids during acidic hydrolysis.

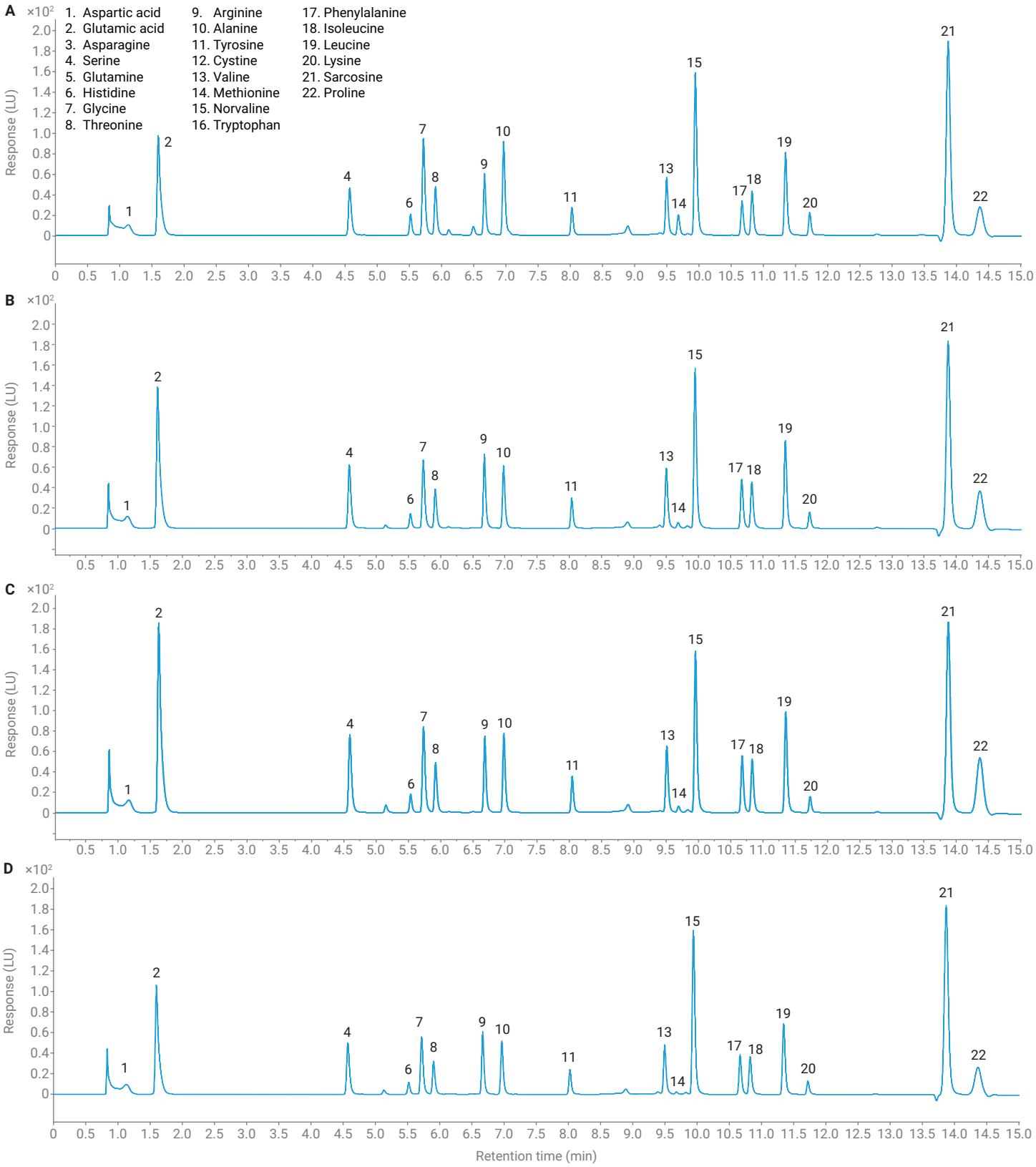

Figure 3. Analysis of the amino acid profile of hydrolysates of beef burger patties (A) and different plant-based burger patties (B–D).

Table 5. Determined amino acid profile of beef burger patties and different plant-based burger patties.

Peak No.	Compound	Percentage of Amino Acids (%)			
		Beef Burger	Plant-Based Burger B	Plant-Based Burger C	Plant-Based Burger D
1	Aspartic acid*	8.8	10.0	9.1	10.3
2	Glutamic acid*	12.2	17.3	19.4	16.5
3	Asparagine	Converted to aspartic acid			
4	Serine	5.5	7.3	7.4	7.3
5	Glutamine	Converted to glutamic acid			
6	Histidine	3.0	2.1	2.2	2.1
7	Glycine	10.7	7.4	7.7	7.7
8	Threonine	5.3	4.2	4.4	4.3
9	Arginine	5.2	6.1	5.1	6.3
10	Alanine	9.7	6.2	6.5	6.6
11	Tyrosine	2.5	2.7	2.7	2.8
12	Cystine	Not quantified			
13	Valine	5.1	5.2	4.7	5.3
14	Methionine	1.8	0.5	0.4	0.3
16	Tryptophan	Not detected			
17	Phenylalanine	3.3	4.5	4.3	4.5
18	Isoleucine	4.2	4.3	4.1	4.4
19	Leucine	8.1	8.4	7.9	8.4
20	Lysine	8.8	6.3	4.9	6.2
22	Proline	5.9	7.6	9.1	7.0

* Aspartic acid and glutamic acid originate from the sum of aspartic and glutamic acid contained in the sample and the conversion of asparagine and glutamine to their respective acids during acidic hydrolysis.

According to literature, alanine, glycine, and methionine are less abundant in plant-based burgers compared to meat burgers, whereas glutamic acid is more abundant in plant-based burgers.[6] These differences in the amino acid profile could also be observed in the current analysis (see Table 5).

Among the plant-based burger patties analyzed, burgers B and D were based on pea protein, whereas burger C was based on soy and wheat protein. For burger C, a slightly higher percentage of glutamic acid and a slightly lower percentage of lysine was determined compared to burgers B and D (see Table 5). This result is consistent with a higher amount of glutamic acid and a lower amount of lysine found in soy protein compared to pea protein.[7,8]

Conclusion

The analysis of amino acids was performed using reversed-phase LC with fluorescence detection following automated precolumn derivatization using the injector program of the Agilent 1260 Infinity II Multisampler. This enabled successful determination of the amino acid profiles of beef burger patties and different plant-based burger patties. Excellent precision and sensitivity were obtained using the Agilent 1260 Infinity II LC. Automation of derivatization also removed the need for manual liquid handling steps, reducing sources of error and saving time and cost generated by manual work.

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