Journal of Food Composition and Analysis 92 (2020) 103522

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Determination of free amino acids in five commercial sweetpotato cultivars by hydrophilic interaction liquid chromatography-mass spectrometry☆

Xiao Qiu[a], Rong Reynolds[b], Suzanne Johanningsmeier[b,*], Van-Den Truong[b]

[a] Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, 400 Dan Allen Drive, Raleigh, NC 27695, United States
[b] USDA-ARS, SEA Food Science Research Unit, North Carolina State University, 322 Schaub Hall, Box 7624, Raleigh, NC 27695, United States

ARTICLE INFO

Keywords:
Amino acid profile
Sweetpotato
Ipomoea batatas
Ultra-high-performance liquid chromatography
Asparagine
GABA
Triple-quadrupole
Food analysis
Food composition

ABSTRACT

A sensitive and rapid method was developed for the determination of free amino acid profiles in sweetpotatoes. The method utilized an ultra-high-performance liquid chromatography system with hydrophilic interaction liquid chromatography (HILIC) separation coupled with tandem mass-spectrometry (MS/MS) detection without the need for chemical derivatization. Separation of 36 amino acid standards was achieved on a silica HILIC column in a single MS/MS run of 19 min. This validated method was applied for the analysis of the free amino acid composition of five commercial sweetpotato cultivars (Covington, Beauregard, Hatteras, Murasaki-29 and O' Henry) from two planting lots grown in experimental fields. Analysis of variance with Tukey's test was used to determine the differences in individual free amino acid content among the analyzed samples, and principle component analysis (PCA) was implemented to evaluate the variation in amino acid profiles of different sweetpotato cultivars. Asparagine was the most abundant free amino acid and its content varied significantly among the cultivars ($p < 0.05$). Fifteen additional amino acids also varied among cultivars and contributed to the differentiation of the genotypes by PCA based on their complete amino acid profiles. The efficient, reliable, sensitive method described herein could be used in quantifying amino acids in food matrices similar to sweetpotatoes.

1. Introduction

Amino acids in foods provide essential nutrients for the synthesis of protein and participation in biochemical reactions in the human body. The analysis of amino acids is of great importance due to nutritional values and labeling requirements, control of process operating conditions, and identification of food origin as used in various products (Gökmen et al., 2012; Pereira et al., 2008). In sweetpotato, the physiological levels of amino acids impact the formation of acrylamide in thermally processed products such as fried chips and French fries, consumer products that are growing in popularity (Bond, 2017; Truong et al., 2014). Acrylamide is a human neurotoxin and is classified as a probable human carcinogen (IARC, 1994). The chemical mechanism leading to the formation of acrylamide in foodstuffs, including sweetpotatoes, derives from the Maillard reaction that occurs between reducing sugars and proteins or amino acids (Stadler et al., 2002). Asparagine, containing an amide group, seems to be the main contributing amino acid due to having the same carbon backbone as the acrylamide molecule (Zyzak et al., 2003). Many researchers have reported on the effects of asparagine and total amino acid contents on acrylamide formation in various food matrices (Elmore et al., 2015; Truong et al., 2014; Zhang et al., 2011). However, more studies have shown the complex relationship that exists between the complete free amino acid profiles (asparagine and other amino acids) and acrylamide-forming potential in potato chips and French fries (Halford et al., 2012; Muttucumaru et al., 2014). Therefore, it is important to have a method to quantify the complete profiles of free amino acids in sweetpotatoes.

Various analytical methods have been developed for the determination of amino acids in foods, such as ion-exchange liquid chromatography and gas chromatography (Lim et al., 2014; Mu et al., 2009; Yeoh and Truong, 1996). However, high-performance liquid chromatography (HPLC) is by far the most implemented technique. Amino acids have

☆ Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.

* Corresponding author at: USDA-ARS, SEA Food Science Research Unit, North Carolina State University, 322 Schaub Hall, Box 7624, Raleigh, NC 27695, United States.
E-mail address: suzanne.johanningsmeier@usda.gov (S. Johanningsmeier).

https://doi.org/10.1016/j.jfca.2020.103522
Received 30 September 2019; Received in revised form 4 May 2020; Accepted 7 May 2020
Available online 13 May 2020
0889-1575/ Published by Elsevier Inc.

high polarity and low ultraviolet (UV) absorbance; hence, their detection and accurate quantitation are difficult due to weak retention, poor separation, and limited detection limits using conventional reversed-phase (RP) HPLC-UV methods. To overcome these challenges, HPLC methods frequently employ a pre- or post-column chemical derivatization in order to enhance photometric detection sensitivity and chromatographic separation (Fish, 2012; López-Cervantes et al., 2006; Pereira et al., 2008). The derivatization reagents must include strong chromophore groups, such as ninhydrin or o-phthalaldehyde (Bartolomeo and Maisano, 2006; Joyce et al., 2016; Shimbo et al., 2009). There are many drawbacks of derivatization methods, including long derivatization time, low stability, insufficient reproducibility of derivative yield, reaction limitations to secondary amine groups, and chromatographic interference caused by the reagent (Callejón et al., 2010). Pre-column chemical derivatization of amino acids with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) coupled with LC−MS has been used for faster and more reliable derivatization and analysis of amino acids in various biological samples (Armenta et al., 2010; Salazar et al., 2012; Zhou et al., 2019). However, this method still requires additional reagents and the potential need for optimization for individual matrices.

Coupling of HPLC with mass spectrometry (MS) detection could provide better sensitivity for direct detection of free amino acids, but the difficulty in separating amino acids by reverse-phase HPLC has been a limiting factor. The introduction of perfluorinated acids as ion-pairing agents improved the separation of these polar compounds on C18-reversed phase columns, eliminating certain drawbacks related to derivatization methods (Armstrong et al., 2007; Piraud et al., 2003). Unfortunately, the use of ion-paring reagents is limited by two major disadvantages: retention time instability and a reduction in sensitivity with MS detection (De Person et al., 2008; Qu et al., 2002). In contrast to RP-HPLC, hydrophilic interaction liquid chromatography (HILIC) offered good retention and separation of highly polar compounds such as amino acids and carbohydrates (Buszewski and Noga, 2012; Greco and Letzel, 2013; Guo et al., 2013). HILIC separation is based on the strong hydrophilic interaction of polar compounds with a polar stationary phase (Jandera, 2011). More importantly, the use of an aqueous organic solvent mobile phase in HILIC separation enhances the analyte ionization for MS detection (Guo et al., 2013). Therefore, utilizing HILIC stationary phases for LC–MS, amino acid analysis in foods (Gökmen et al., 2012; Guo et al., 2013; Huang et al., 2018; Langrock et al., 2006; Prinsen et al., 2016; Zhou et al., 2013) and in human samples (Langrock et al., 2006; Prinsen et al., 2016) became accessible without pre-or post-column derivatization.

So far, there have not been any published HILIC methods for free amino acid profile analysis in sweetpotatoes. This study aimed to develop an analytical method for the rapid determination of amino acids in sweetpotato roots using hydrophilic interaction liquid chromatography and to determine similarities and differences in amino acid profiles among commercial sweetpotato cultivars. The method coupled a HILIC silica column with triple-quadrupole MS for reliable and sensitive detection and quantification of amino acids. The method requires no derivatization step, which reduced the sample preparation time, and a series of tests were performed for conducting single-laboratory validation.

2. Materials and methods

2.1. Reagents

Water, acetonitrile, hydrochloric acid (HCl), and formic acid were all of LC/MS grade and purchased from Fisher Scientific (Hampton, NH, USA). Ammonium formate (analytical grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and chemicals were of analytical grade (purity ≥ 99%).

Thirty-six reference compounds: tryptophan (Trp), phenylalanine (Phe), tyrosine, (Tyr), isoleucine (Ile), leucine (Leu), methionine (Met), cysteine (Cys), valine (Val), threonine (Thr), γ-aminobutyric acid (GABA), serine (Ser), alanine (Ala), trans-4-hydroxyproline (Hpro), glycine (Gly), asparagine (Asn), proline (Pro), glutamic acid (Glu), glutamine (Gln), citrulline (Cit), aspartic acid (Asp), histidine (His), arginine (Arg), ornithine (Orn), lysine (Lys), carnosine (Car), aminoethanol, aminoadipic acid, 2-aminobutyric acid, 3-aminoisobutyric acid, cystathionine, anserine, cystine, methylhistidine, hydroxylysine, creatinine, and homocystine were purchased from Pierce Biotechnology (Rockford, IL, USA) and Sigma-Aldrich (St. Louis, MO, USA). The purity of each compound was more than 98%.

Four isotopically labeled amino acids were used as internal standards for accurate quantification of amino acids expected to be important in sweetpotatoes. Isotopes $^{15}N_2$-d_8-L-asparagine and 2,3,3,4,4,-d_5-L-glutamine were purchased from Cambridge Isotope Laboratories (Tewksbury MA, USA). Isotopes 2,3,3-d_3-L-phenyl-d_5-alanine and 2,3,3,3-d_4-L-alaninewere purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of standard solutions

Individual standard stock solutions containing each reference compound were prepared by dissolution in 0.1 N HCl, except asparagine and glutamine, which were dissolved in LC/MS grade water since these compounds are more stable at neutral pH. For preparation of calibration standards, twenty five of the 36 amino acids were selected as the target free amino acids for sweetpotato roots. The concentrations of these 25 target analytes were adjusted in the stock solution between 1.53 μmol L^{-1} for Cit and 200.13 μmol L^{-1} for Ala to account for differences in sensitivity on the MS (Table 1). The standard stock solution containing all 25 compounds was freshly mixed and then serial diluted with 0.1 N HCl to five different levels for construction of calibration

Table 1
Preparation of 25 target amino acid standard solutions and 4 internal standards.

Amino acid	Concentration in individual amino acid stock solution (μmol/L)	Concentration in mixed standard stock solution (μmol/L)
Trp	100.07	0.40
Phe	49.86	1.99
Tyr	10.39	0.62
Ile	5.27	0.47
Leu	10.71	1.07
Met	10.04	0.64
Cys	9.99	0.80
Val	50.43	1.21
Thr	100.24	0.60
GABA	100.95	0.81
Ser	10.07	0.81
Ala	200.13	2.00
Hpro	10.36	0.04
Gly	101.69	1.22
Asn	100.30	12.04
Pro	20.45	0.41
Glu	99.97	2.00
Gln	50.38	1.01
Cit	1.53	0.31
Asp	40.29	3.22
His	20.85	0.46
Arg	10.06	0.40
Orn	10.00	0.02
Lys	5.21	0.21
Car	10.10	0.20
Internal standards (IS)	Concentration in individual IS stock solution (μmol/L)	Concentration in mixed IS stock solution (μmol/L)
L-phenyl-d_5-alanine-2,3,3-d_3	50.83	12.71
L-alanine-2,3,3,3-d_4	100.19	12.52
L-asparagine-$^{15}N_2$, d_8	102.19	42.58
L-glutamine-2,3,3,4,4-d_5	39.63	8.26

Table 2
MRM-parameters used for the analysis of 36 amino acid standards and 4 labeled internal standards.

Amino acid	RT (min)	MRM-transition (m/z)	Dwell Time (msec)	Q1 Pre Bias(V)	Collision energy (V)	Q3 Pre Bias(V)	Internal standard
Trp	5.6	205.00 > 188.10	10	−11	−9	−20	/
Phe	5.9	166.00 > 120.10	10	−18	−12	−12	IS-1
Tyr	6.1	182.00 > 136.20	10	−27	−21	−27	–
Ile	6.3	132.10 > 86.15	10	−14	−10	−21	–
Leu	6.5	132.10 > 86.05	10	−14	−11	−21	–
Met	6.5	149.90 > 56.10	10	−17	−17	−21	–
Cys	6.7	122.00 > 76.10	10	−14	−23	−22	–
Val	7.0	118.00 > 72.05	10	−13	−13	−22	–
Thr	7.5	120.10 > 74.05	10	−13	−14	−30	–
GABA	7.7	104.20 > 87.15	10	−12	−12	−23	–
Ser	7.7	106.00 > 60.05	10	−12	−12	−23	–
Ala	7.8	89.90 > 44.05	10	−27	−10	−20	IS-2
Hpro	7.8	132.00 > 68.05	10	−13	−23	−20	–
Gly	8.0	76.00 > 30.20	100	−14	−10	−15	–
Asn	8.0	132.95 > 74.10	10	−15	−16	−29	IS-3
Pro	8.1	116.05 > 70.10	10	−15	−14	−11	–
Glu	8.2	147.90 > 84.10	10	−16	−17	−23	–
Gln	8.2	146.95 > 84.15	10	−16	−16	−15	IS-4
Cit	8.6	176.00 > 159.10	10	−10	−11	−16	–
Asp	8.6	134.00 > 74.00	10	−15	−16	−29	–
His	9.8	156.00 > 110.10	10	−17	−13	−12	–
Arg	9.9	174.95 > 70.10	10	−14	−23	−13	–
Orn	10.2	133.00 > 70.10	10	−15	−18	−28	–
Lys	10.4	147.00 > 84.00	10	−16	−18	−23	–
Car	11.1	226.90 > 110.10	10	−20	−25	−20	–
Aminoethanol*		62.00 > 44.15	100	−12	−14	−19	–
Aminoadipic acid*		162.00 > 98.10	10	−16	−15	−10	–
2-Aminobutyric acid*		104.20 > 58.05	10	−11	−12	−24	–
3-Aminoisobutyric acid*		104.20 > 86.00	10	−11	−12	−16	–
Cystathionine*		223.00 > 88.15	10	−20	−18	−11	–
Anserine*		241.00 > 109.30	10	−15	−13	−22	–
Cystine*		241.00 > 151.95	10	−23	−31	−17	–
Methylhistidine*		170.00 > 124.15	10	−18	−18	−13	–
Hydroxylysine*		163.00 > 81.95	10	−10	−16	−26	–
Creatinine*		114.05 > 44.10	10	−11	−16	−19	–
		114.05 > 86.15	10	−10	−15	−30	–
L-Homocystine*		269.10 > 136.05	10	−14	−15	−14	–
Internal standards (IS)							
1. L-phenyl-d_5-alanine-2,3,3-d_3	5.9	174 > 128.20	10	−18	−14	−26	
2. L-alanine-2,3,3,3-d_4	7.8	94.0 > 48.20	10	−11	−15	−19	
3. L-asparagine-$^{15}N_2$, d_8	8.0	138.00 > 76.00	10	−15	−17	−30	
4. L-glutamine-2,3,3,4,4-d_5	8.2	151.90 > 88.10	10	−12	−18	−24	

* 11 amino acids were applicable for this method but not present in the sweetpotato samples. Retention time was not shown because these 11 amino acids were not analyzed with the final optimized gradient program for sweetpoato samples.

curves. The dilution ratios were 1–2, 1–3.33, 1–5, 1–10, and 1–20. All solutions were stored at 4 °C until analysis. The stock solution mixture was brought to ambient temperature prior to making calibration standards which were discarded after 3 d of use.

2.3. Experimental samples

Five different sweetpotato cultivars grown at the experimental fields of the Sweetpotato Breeding Program (North Carolina State University, Clinton, NC, USA) in 2015 were analyzed in the study. All the sweetpotatoes were planted in June and harvested in October in two different locations. Of those genotypes, three were orange-fleshed (Covington, Beauregard, and Hatteras) and two were white/yellow/cream-fleshed (Murasaki-29 and O'Henry). For each sample, 7–12 freshly harvested roots were washed, sliced, stored at −20 °C, and then freeze-dried for 3 d using a VirTis 25 L Genesis freeze dryer (Gardiner, NY, USA) with shelf temperature set at −20 °C; pulverized using a Mr. Coffee precision coffee grinder (Sunbeam, Boca Raton, FL, USA); and stored at 4 °C in 50 mL conical culture tubes until analysis.

2.4. Selection of extraction solvent

Various analytical methods have been developed for the determination of amino acids in foods, in which water (Gökmen et al., 2012; Guo et al., 2013; Truong et al., 2014), dilute HCl (Elmore et al., 2015; Halford et al., 2012), and methanol (Granby et al., 2004; Qu et al., 2002) were the most widely used as the extraction solvent in sample preparation procedures. To select the extraction solvent for this study, uncured Beauregard sweet potato samples (1 g freeze-dried sweetpotato powder) were extracted with water (5 mL s) or 0.1 N HCl (5 mL s). Initial experiments using a rapid extraction method (vortexing samples in the extraction solvent for a few minutes), showed that 0.1 N HCl sample extracts contained higher amounts of several amino acids, including arginine, asparagine, aspartic acid, citrulline, glutamate, glutamine, hydroxyproline, lysine, ornithine, serine, tyrosine, and valine ($P < 0.05$, Supplementary Table S1). However, with longer extraction methods of 1 h (with orbital shaking) or static overnight extraction, glutamate was the only amino acid in higher quantities in the 0.1 N HCl extracts (Supplementary Table S2).

2.5. Sample preparation

Each 1-gram (0.999 ± 0.004) ground freeze-dried sweetpotato sample was weighed into a 15 mL conical tube. Hydrocholoric acid (5 mL, 0.1 N) was added, vortexed, and placed in a 4 °C refrigerator for 12 h. The suspension was then centrifuged at 4830 x g for 15 min at

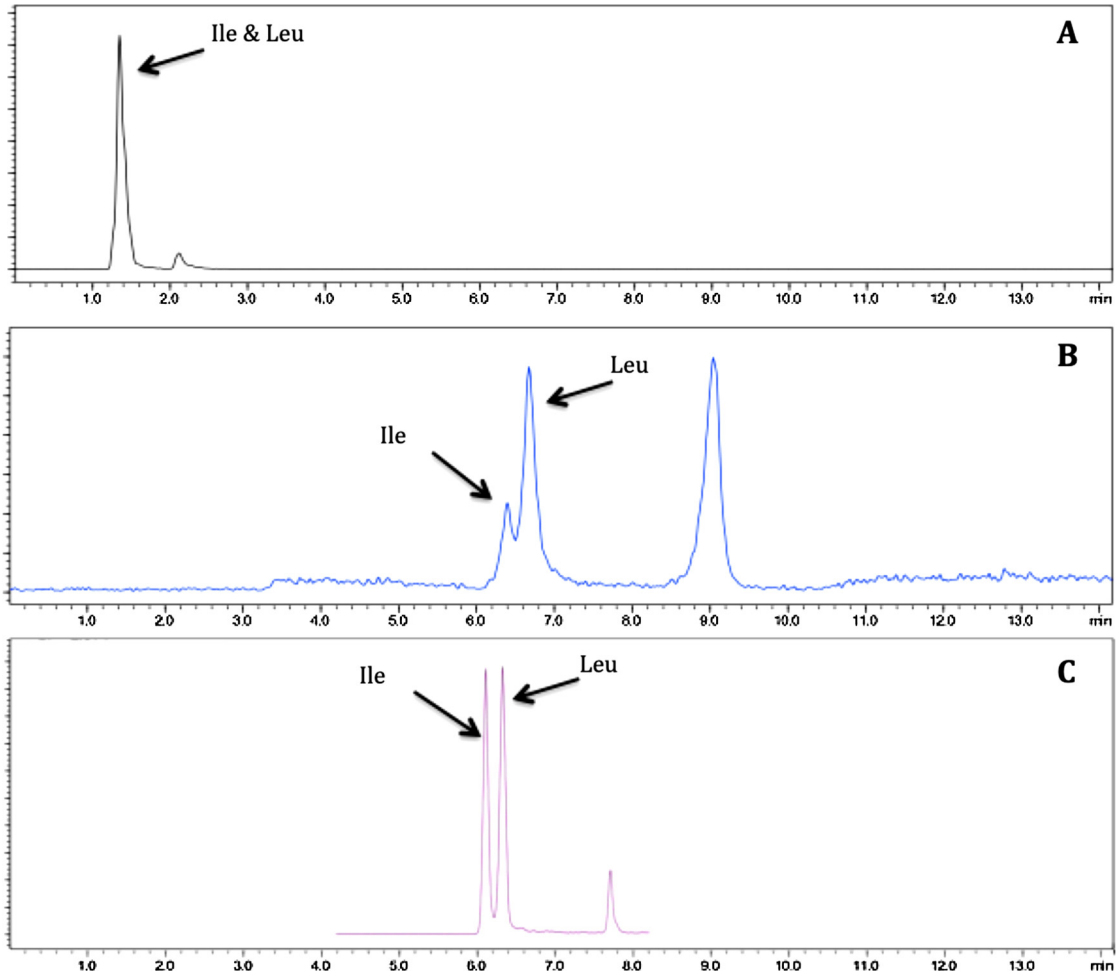

Fig. 1. Chromatographic separation of Leu and Ile using different columns and mobile phase conditions. (A) Acquity UPLC BEH amide column with a gradient of 10 mM ammonium formate in 85% acetonitrile containing 0.15% formic acid and 10 mM ammonium formate in LC–MS water containing 0.15% formic acid (Prinsen et al., 2016); (B) Atlantis Silica HILIC column with initial gradient of 10 mM ammonium formate in 85% acetonitrile containing 0.15% formic acid and 10 mM ammonium formate in LC–MS water containing 0.15% formic acid; (C) Atlantis Silica HILIC column with optimized gradient program.

4 °C. An aliquot of the supernatant (250 µL) was mixed with 12 µL of the amino acid internal standard mixture (Table 1) and 238 µL acetonitrile for a total of 500 µL solution in an eppendorf micro centrifuge tube. The mixture was vortexed for 15 s and subsequently centrifuged for 10 min at 9470 x g to remove any precipitate. The supernatant was then collected into a 0.5 mL Ultrafree-MC-GV centrifugal filter tube (0.22 µm) (Sigma-Aldrich), centrifuged for another 10 min, and the filtrate was transferred to a 1.5 mL HPLC vial for LC–MS analysis (Laboratory Supply Distributors, Corp., Vineland, NJ, USA). Recovery of amino acids from Beauregard sweetpotato with this single extraction protocol ranged from 60 to 91% with an average recovery of 78 ± 7%.

2.6. Instrument conditions

The LC–MS/MS system consisted of a Shimadzu Nexera-2 UHPLC system equipped with a SIL-30AC autosampler, DGU-20a3 degasser, LC 30AD pump, CTO-20A column oven, and CBM-20A controller connected to a Shimadzu LCMS-8030 plus Triple Quadrupole Mass Spectrometer (Shimadzu Corp., Kyoto, Japan). The chromatographic separation of amino acids was carried out on an Atlantis Silica HILIC column (4.6 mm × 100 mm, 3 µm particle size) (Waters Corporation, Midford, MA, USA). The column was maintained at a temperature of 35 °C and the sample volume injected was 2 µL. The autosampler was operated at 4 °C. The gradient system used was modified from the methods described by Prinsen et al. (2016). Separation of amino acids in sweetpotatoes was achieved at a flow-rate of 0.6 mL/min using a gradient with solvent A (10 mM ammonium formate in 85% acetonitrile containing 0.15% formic acid) and solvent B (10 mM ammonium formate in LC–MS water containing 0.15% formic acid) as follows: 0–9.6% solvent B in 3 min (0 − 3 min); 27% B in 4 min (3 − 7 min); 27% B held for 1 min; then to 37% B in 1 min (8 − 9 min); and back to 0% B in 1.5 min (9–10.5 min). The column was re-equilibrated for 8.5 min (10.5 − 19 min) under the initial conditions (0% B). Total run time was 19 min including column re-equilibration.

The column was coupled to a mass spectrometer for quantification. Electrospray ionization -positive ion mode was implemented with the following conditions: nebulizing gas and drying gas were nitrogen (99.998%) at a flow rate of 3.0 and 15.0 L/min, respectively; the interface voltage was autotuned to 4.5 kV; desolvation line temperature was 250 °C and the heat block temperature was 400 °C. The mass spectrometer was operated in multiple reaction monitoring mode (MRM) with argon as the collision induced dissociation gas at a pressure of 230 kPa; the detector voltage was optimized to 1.82 kV. The optimized MRM-transitions for four amino acid internal standards and 25 compounds of interest are shown in Table 2. Asparagine, glutamine, phenylalanine, and alanine were quantified using internal standard calibration curves while the other amino acids were quantified using external standard calibration curves. All the data were reported on a fresh weight (fw) basis as µmol/g fw.

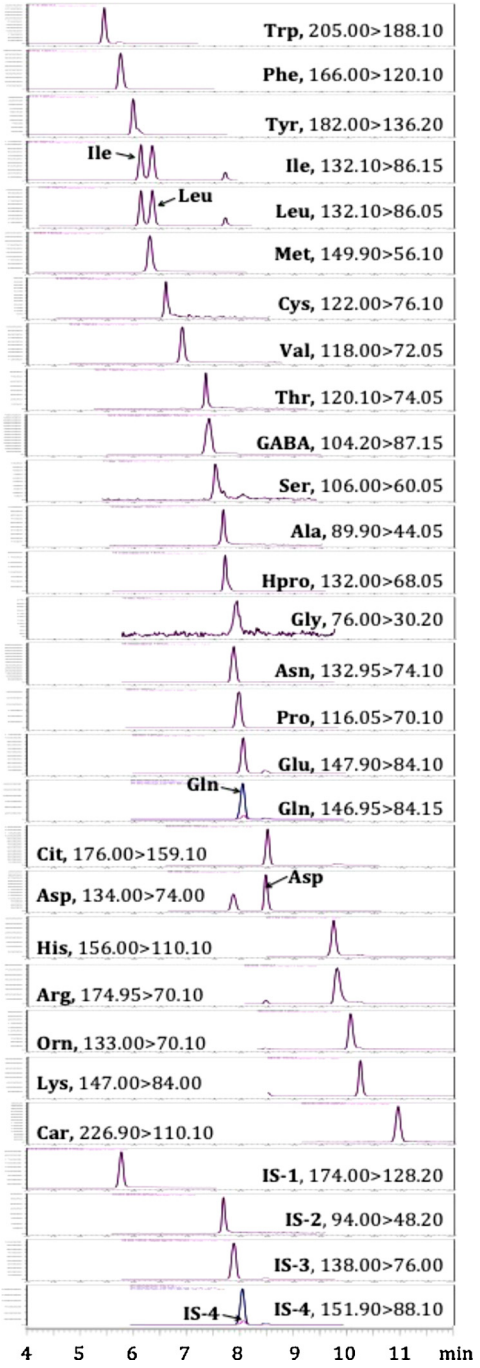

Fig. 2. MRM chromatograms of the mixed amino acid standard, including 25 target amino acids and 4 internal standards.
Notes: IS-1 to 4 were the stable isotopes of Phe, Ala, Asn, and Gln, respectively. The scale for each analyte varied based on different peak intensity.

2.7. Validation of the method

The method was validated for linearity, limits of detection (LOD), limits of quantification (LOQ), precision, and repeatability following the *International Conference on Harmonisation* (ICH) guidelines (ICH, 1996), AOAC International guidelines (AOAC International, 2013) for single-laboratory validation, and previous reports on amino acid determination (Guo et al., 2013; Prinsen et al., 2016).

The linearity for each target compound was determined by five-point standard calibration curves. The correlation coefficients, calibration equation slopes, and y-intercepts were automatically generated by the LabSolution data processing software (Shimadzu Corp.). Each calibration curve was comprised of five data points, and $R^2 \geq 0.98$ was considered acceptable. Two approaches for determining the quantitation limit were used, (1) based on signal-to-noise ratios (S/N) and (2) based on the standard deviation of the response and the slope (ICH, 1996). In the first approach, LOD and LOQ for each analyte were determined at S/N of 3 and 10, respectively. In the second approach, LOD and LOQ were calculated as 3.3 and 10 times the standard deviation of the response over the slope, respectively. The standard deviation of the response was determined based on the standard deviation of the y-intercepts of regression lines (United States Pharmacopeia (USP-NF, 2017).

To demonstrate precision, the mid-range standard solutions were analyzed for four sequential injection replicates within-day and between-day. To assess the repeatability of the assay, four sample extracts from the same sweetpotato sample (Uncured Covington, lot1, ground, freeze-dried composite of 7–12 roots) were independently prepared via the method described above and were analyzed. All variations were expressed as the relative standard deviation (% RSD).

2.8. Statistical analysis

Principle component analysis (PCA) was performed on the amino acid profiles of the five commercial genotypes using a web tool, ClustVis (https://biit.cs.ut.ee/clustvis/). A natural log (to base e) transformation was used for the amino acid data in PCA analysis. The amino acid content data was analyzed to determine differences in each individual amino acid among the five genotypes by one way analysis of variance with Tukey range test ($\alpha = 0.05$) using a web tool, VassartStats (http://vassarstats.net/).

3. Results and discussion

3.1. Method optimization and validation

3.1.1. Optimization of LC–MS conditions

To obtain a desirable chromatogram with satisfactory retention, resolution, and peak shape without excessive peak tailing, the LC conditions were investigated under the optimized MS/MS conditions for 36 amino acid standards. Initial development of the LC method followed the 18-minute gradient program described by Prinsen et al. (2016) using an Acquity UPLC BEH amide column (2.1 mm × 100 mm, 1.7 μm particle size). Chromatographic separation of amino acids, especially leucine and isoleucine, was not satisfactory, so no further optimization was performed with this column. An Atlantis Silica HILIC analytical column (4.6 mm × 100 mm, 3 μm particle size) was then evaluated as an alternative for separation of the 36 amino acid standards. Separation among analytes was suitable on this column and further improved by a modified gradient program with longer elution times. This was especially notable for the ones with similar MRM transitions such as Leu and Ile, which require chromatographic separation even with the use of the specific MRM detection (Fig. 1). Further, the gradient elution procedure was optimized to acquire desirable peak shape without excessive shoulders and tailing for some amino acids, such as His and Ser. The individual MRM chromatograms of the 25 target amino acids are presented in Fig. 2. The sensitivities among standard compounds varied more than 100-fold in this LC–MS method, which required the ratio of sample and extraction solution, injection volume, and linear range for each calibration curve (Table 3) to be individually optimized. For example, the aliphatic amino acids, such as Ser and Ala, had lower LOQs between 0.13 – 0.30 micromolar compared with the much more sensitive detection of aromatic amino acids, Phe and Trp, with lower LOQs between 0.0017 and 0.0037 micromolar (Table 3).

Table 3
Linearity, LOD, LOQ, precision, and repeatability of 25 target free amino acids.

Amino acid	Linearity $^{-1}$(μmol L)	R^2	LOD[a] (μmol L^{-1})	LOQ[a] (μmol L^{-1})	LOD[b] (μmol L^{-1})	LOQ[b] (μmol L^{-1})	Precision (% RSD)		Repeatability[#] (% RSD, $n = 4$)
							Within day, $n = 4$	Between day, $n = 7$	
Trp	0.02 – 0.20	0.99	0.00050	0.00167	0.0229	0.0692	1.58	3.00	6.97
Phe	0.10 – 1.00	0.99	0.00111	0.00371	0.0486	0.1472	2.89	2.80	3.98
Tyr	0.04 – 0.45	0.99	0.01851	0.06169	0.0873	0.2645	4.99	5.04	2.85
Ile	0.02 – 0.24	0.99	0.03160	0.10534	0.0264	0.0800	6.48	5.48	2.87
Leu	0.05 – 0.54	0.99	0.02491	0.08303	0.0410	0.1243	3.36	4.06	4.37
Met	0.03 – 0.32	0.99	0.01226	0.04087	0.0228	0.0692	6.13	4.45	3.13
Cys	0.04 – 0.40	0.99	0.02700	0.09000	0.0047	0.0144	4.08	3.56	NA
Val	0.06 – 0.61	0.99	0.00900	0.03000	0.0227	0.0688	3.25	3.71	4.23
Thr	0.03 – 0.30	0.99	0.03000	0.10000	0.0468	0.1418	4.09	6.28	3.65
GABA	0.06 – 0.61	0.99	0.00067	0.00223	0.0170	0.0514	0.52	2.20	6.77
Ser	0.04 – 0.40	0.99	0.04800	0.16000	0.0178	0.0539	7.64	6.94	5.10
Ala	0.10 – 1.00	0.99	0.03900	0.13000	0.0474	0.1435	5.55	7.21	3.34
Hpro	0.002 – 0.02	0.98	0.00089	0.00297	0.0005	0.0014	8.51	6.89	NA
Gly	0.06 – 0.61	0.99	0.09000	0.30000	0.1088	0.3297	1.97	1.88	NA
Asn	0.60 – 6.02	0.99	0.00300	0.01000	0.2964	0.8982	2.02	2.08	4.41
Pro	0.02 – 0.20	0.99	0.00121	0.00404	0.0109	0.0330	1.88	2.79	5.02
Glu	0.10 – 1.00	0.99	0.00149	0.00498	0.0206	0.0623	3.58	4.17	4.36
Gln	0.05 – 0.50	0.99	0.00110	0.00366	0.0072	0.0217	3.15	3.75	4.63
Cit	0.02 – 0.15	0.99	0.00010	0.00035	0.0003	0.0010	1.58	11.97	6.10*
Asp	0.16 – 1.61	0.99	0.02100	0.07000	0.0494	0.1497	1.53	6.01	3.31*
His	0.02 – 0.23	0.99	0.00278	0.00926	0.0085	0.0258	2.03	4.57	5.63
Arg	0.02 – 0.20	0.99	0.00009	0.00031	0.0075	0.0228	2.51	6.06	6.26
Orn	0.001 – 0.01	0.99	0.00004	0.00012	0.0004	0.0011	0.43	2.09	NA
Lys	0.01 – 0.10	0.99	0.00061	0.00204	0.0050	0.0150	2.04	1.90	4.17
Car	0.01 – 0.10	0.99	0.00001	0.00004	0.0001	0.0002	3.27	4.95	NA

* The % RSD was calculated from 3 replicates without an outlier data point (Grubbs' test, a = 0.05).
\# In repeatability test, the samples from uncured Covington in lot1 were chosen as the test samples.
[a] LOD and LOQ calculations were based on signal-to noise approach.
[b] LOD and LOQ were based on the standard deviation of response and the slope.

3.1.2. Method validation

Identification of analytes in the test materials was determined by comparing peak retention times under the specific MRM detection (Table 2). Representative chromatograms of a mixed standard solution (Fig. 2**)** and one test sample (Fig. 3) are shown. Notably, Gly, Orn, Cys, Hpro, and Car were not detected in sweet potatoes using this method. Purple sweetpotatoes were previously reported to contain ~ 0.337 μmol glycine/g fresh weight (Lim et al., 2014), but the LOD for glycine with this method was 108.8 μmol/mL, which was an order of magnitude higher than for the other amino acids. The low sensitivity of glycine in this MS system may be caused by the small *m/z* ratio of the product ion after the fragmentation. Conversely, Cys, Orn, Hpro, and Car contents were quantifiable with this method at levels of 0.004, 0.0004, 0.0005, and 0.0001 μmol L^{-1}, respectively. Therefore, the absence of these peaks indicated that these amino acids were not present in sweetpotato in significant quantities. The results of linearity, LODs, LOQs, precision and repeatability are summarized in Table 3. The extended five-point calibration curve was used for demonstrating linearity of the 25 target amino acids, in which only Asn, Phe, Gln, Ala were calibrated by internal standards. The R^2 for all 25 reference standard calibration curves was no less than 0.98. The within-day and between-day variations (RSDs) in precision of the target amino acid standards were in the range of 0.43-8.51% and 1.88-11.97%, respectively. The relative standard deviations for repeatability ranged from 2.85% to 6.97% for the 20 amino acids detected in sweetpotato. These results indicated that the developed method described herein, has proven to be selective, linear, precise, and repeatable. The limitation of this method could be the poor sensitivity in glycine detection, which may need to be measured by another method.

3.2. Free amino acid profiles of five commercial sweetpotato cultivars

To test the applicability of the method, uncured sweetpotato roots of five cultivars from two lots were analyzed to evaluate the differences in the amino acid composition of the samples. The experiment was conducted with duplicate 7–12 root samples of sweetpotato from two different lots of each genotype. The contents of amino acids varied significantly among the cultivars (Table 4). The levels of most amino acids were significantly higher in Beauregard and O'Henry than the other three cultivars ($p < 0.05$), with the exception of GABA and Glu. Asparagine content contributed most to the variation in total amino acid levels between Beauregard and O'Henry. In contrast, there were only a few of the amino acids, including Tyr, Ala, Cit, and Asp that did not differ significantly among the five cultivars ($p > 0.05$). Total amino acid content ranged from 13.2 μmol/g fw in Murasaki-29 to 41.6 μmol/g fw in Beauregard, a more than three-fold difference. Among the individual amino acids in the profile investigated, Asn was the predominant amino acid in all five cultivars of sweetpotato with the range of 4.91 μmol/g fw in Murasaki-29 to 25.8 μmol/g fw in Beauregard (~five-fold difference). The proportion of Asn to total amino acids in the 5 cultivars were between 37.1% (Murasaki-29) and 67.4% (Covington). Purple sweetpotatoes were previously reported to contain ~5.2 μmol/g fw (Lim et al., 2014), which is similar to the level of asparagine in the cream-fleshed Murasaki-29 in this study. Interestingly, the Asn content of the Covington roots in this study was approximately twice as much as previously reported (Truong et al., 2014). The deviation could be due to differences in planting fields, growing seasons, and storage time of the sweetpotato roots. Multiple studies reported that Asn can contribute to formation of acrylamide during thermal processing in the presence of reducing sugars (Elmore et al., 2015; Lim et al., 2014; Truong et al., 2014). Therefore, the processing of sweetpotatoes should be controlled to mitigate acrylamide formation considering the significant quantity of Asn in the roots.

To evaluate the overall variation in amino acid composition of the five sweetpotato cultivars, PCA was performed on the contents of the 20 free amino acids detected and quantified (Table 4). The first principal

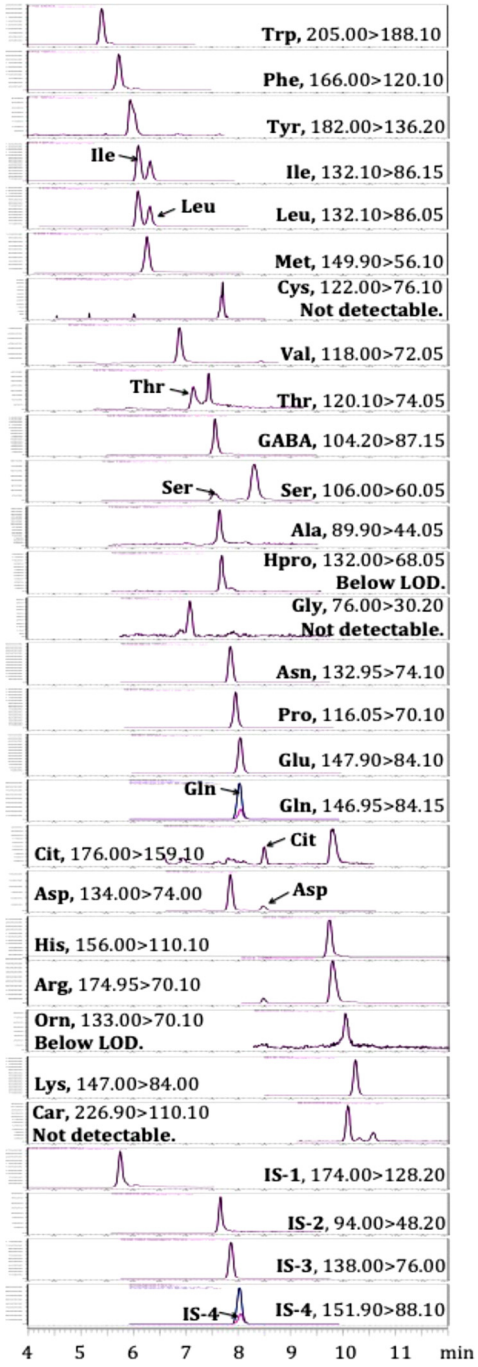

Fig. 3. MRM chromatograms of uncured Covington sweetpotato roots, showing the presence of 20 free amino acids.
Notes: Cys, Hpro, Gly, Orn, and Car were below the LOD in the sweetpotato samples. The scale for each analyte varied based on different peak intensity.

component (PC1) and the second principal component (PC2) accounted for 62.6% and 16.7% of the whole variance, respectively (Fig. 4). The sum of PC1 and PC2 with 79.3% of the total variance were extracted for analysis, since the remaining PCs had a minor effect on the model.

PC1 had large positive associations with most of the analytes (coefficients = 0.16–0.28) with the exception of GABA, Glu, and Asp (coefficients ≤ 0.05), whereas PC2 exhibited large positive associations with GABA, Glu, and Asp (coefficients ≥ 0.36). The results suggested that all 20 amino acids may contribute to the classification of the samples. Sweetpotatoes from different lots of the same genotypes grouped similarly, but also showed some variations, indicating different fields may affect the overall amino acid composition of the roots. Data from the potato national fry and chip processing trials also indicated that variance from genotype, genotype by location, and genotype by year occurred on total yield, vine maturity, and chemical composition, including glucose, asparagine, and acrylamide (Schmitz Carley et al., 2019; Wang et al., 2016). Similar variance also was shown in canola (Cullis et al., 2010). No clustered domains were found in the genotype scatter plot (Fig. 4), indicating that these commercial sweetpotato genotypes have markedly different amino acid compositions. Free amino acids play a key role in flavor development in foods subjected to thermal processes (Mottram, 2007). It has also been shown that other amino acids besides asparagine can influence acrylamide formation in model systems (Koutsidis et al., 2009). Yet, we do not know how variations in free amino acid profiles among sweetpotato genotypes will influence acrylamide formation or flavor of fried products. Further research is warranted to identify the amino acid composition that contributes to the desired quality of processed sweetpotato products.

4. Conclusions

A method is presented for quantification of underivatized amino acids in sweetpotatoes using hydrophilic interaction liquid chromatography separation coupled with triple-quadrupole mass spectrometry. The method is fast, simple, reliable, sensitive, and applicable for sweetpotatoes or other similar types of foods containing various levels of amino acids. In this method, the sample preparation procedures were very simple, greatly reducing the laborious and time-consuming derivatization procedures required by most other current methods. This method provides an efficient and reliable process for the quantitation of amino acids in sweetpotato samples and could be valuable for determining amino acids in other foods as well. For sweetpotatoes, the quantification of amino acid profiles in a larger set of genotypes would help determine various precursors in acrylamide formation and could be used to determine the role of the complete amino acid composition in selecting appropriate genotypes for producing healthier chips and fries with low acrylamide content.

Funding

This study was funded by the United States Department of Agriculture, Agricultural Research Service, Southeast Area, Food Science Research Unit with partial support from PepsiCo, Inc. and North Carolina State University [NCSU RADAR # 2016-1172SOW # 2016-4364555]. The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc.

CRediT authorship contribution statement

Xiao Qiu: Investigation, Methodology, Validation, Formal analysis, Writing - original draft. **Rong Reynolds:** Methodology, Writing - review & editing. **Suzanne Johanningsmeier:** Conceptualization, Resources, Writing - review & editing, Supervision. **Van-Den Truong:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Declaration of competing interest

Authors declare no conflict of interests.

Acknowledgements

The authors would like to thank Dr. Craig Yencho, Mr. Ken Pecota and Mr. Ragy Ibrahem of the Dept. of Horticultural Science, North Carolina State University, for providing the sweetpotato materials used

Table 4
Contents of 20 free amino acids detected and quantified (μmol/g fresh weight) in uncured sweetpotatoes.

Free amino acid	Sweetpotato cultivar				
	Covington	Murasaki-29	Beauregard	O'Henry	Hatteras
Trp	0.200 ± 0.059^{ab}	0.171 ± 0.037^{a}	0.776 ± 0.124^{c}	0.672 ± 0.084^{c}	0.370 ± 0.098^{b}
Phe	0.653 ± 0.081^{b}	0.403 ± 0.011^{a}	1.30 ± 0.166^{d}	1.48 ± 0.042^{d}	0.874 ± 0.113^{c}
Tyr	0.843 ± 0.522^{a}	0.394 ± 0.319^{a}	1.38 ± 0.767^{a}	1.34 ± 0.634^{a}	0.947 ± 0.713^{a}
Ile	0.317 ± 0.059^{a}	0.296 ± 0.099^{b}	0.425 ± 0.083^{ab}	0.665 ± 0.092^{b}	0.367 ± 0.019^{a}
Leu	0.413 ± 0.018^{ab}	0.331 ± 0.047^{a}	0.929 ± 0.084^{c}	1.20 ± 0.161^{d}	0.587 ± 0.034^{b}
Met	0.575 ± 0.100^{b}	0.237 ± 0.034^{a}	0.846 ± 0.194^{c}	0.867 ± 0.092^{c}	0.430 ± 0.073^{ab}
Val	0.472 ± 0.044^{a}	0.345 ± 0.039^{a}	0.992 ± 0.251^{b}	1.06 ± 0.080^{b}	0.608 ± 0.083^{a}
Thr	0.271 ± 0.055^{a}	0.277 ± 0.066^{a}	0.429 ± 0.110^{ab}	0.550 ± 0.104^{b}	0.319 ± 0.044^{a}
GABA	0.132 ± 0.026^{a}	0.499 ± 0.183^{b}	0.444 ± 0.044^{ab}	0.336 ± 0.225^{ab}	0.434 ± 0.156^{ab}
Ser	0.465 ± 0.047^{a}	0.517 ± 0.090^{a}	1.17 ± 0.389^{c}	0.955 ± 0.130^{bc}	0.613 ± 0.067^{ab}
Ala	1.05 ± 0.351^{a}	1.201 ± 0.404^{a}	1.98 ± 0.714^{a}	1.25 ± 0.082^{a}	1.12 ± 0.554^{a}
Asn	16.5 ± 1.10^{d}	4.91 ± 0.334^{a}	25.8 ± 2.03^{e}	12.8 ± 0.753^{c}	8.50 ± 2.052^{b}
Pro	0.099 ± 0.005^{a}	0.140 ± 0.018^{ab}	0.174 ± 0.016^{b}	0.269 ± 0.061^{c}	0.127 ± 0.018^{ab}
Glu	1.12 ± 0.256^{a}	2.05 ± 0.033^{b}	1.47 ± 0.312^{a}	2.05 ± 0.271^{b}	1.21 ± 0.229^{a}
Gln	0.346 ± 0.043^{b}	0.099 ± 0.035^{a}	1.56 ± 0.119^{d}	0.621 ± 0.090^{c}	0.342 ± 0.118^{b}
Cit	0.099 ± 0.084^{a}	0.064 ± 0.026^{a}	0.175 ± 0.163^{a}	0.108 ± 0.092^{a}	0.145 ± 0.141^{a}
Asp	0.612 ± 0.177^{a}	1.02 ± 0.275^{a}	0.801 ± 0.321^{a}	1.33 ± 0.912^{a}	1.03 ± 0.642^{a}
His	0.229 ± 0.021^{a}	0.141 ± 0.030^{a}	0.502 ± 0.111^{b}	0.409 ± 0.067^{b}	0.244 ± 0.049^{a}
Arg	0.048 ± 0.016^{a}	0.061 ± 0.018^{a}	0.303 ± 0.075^{b}	0.264 ± 0.034^{b}	0.135 ± 0.061^{a}
Lys	0.048 ± 0.008^{a}	0.068 ± 0.008^{a}	0.138 ± 0.019^{ab}	0.219 ± 0.099^{b}	0.087 ± 0.007^{a}
Total	24.6 ± 0.661^{b}	13.2 ± 0.726^{a}	41.6 ± 4.24^{c}	28.5 ± 1.67^{b}	18.5 ± 3.04^{a}

mean ± standard deviation, $n = 4$*.
*4 replicates include duplicate root samples from each of two lots.
#Values within rows with non-overlapping superscript letters are significantly different at $p < 0.05$.

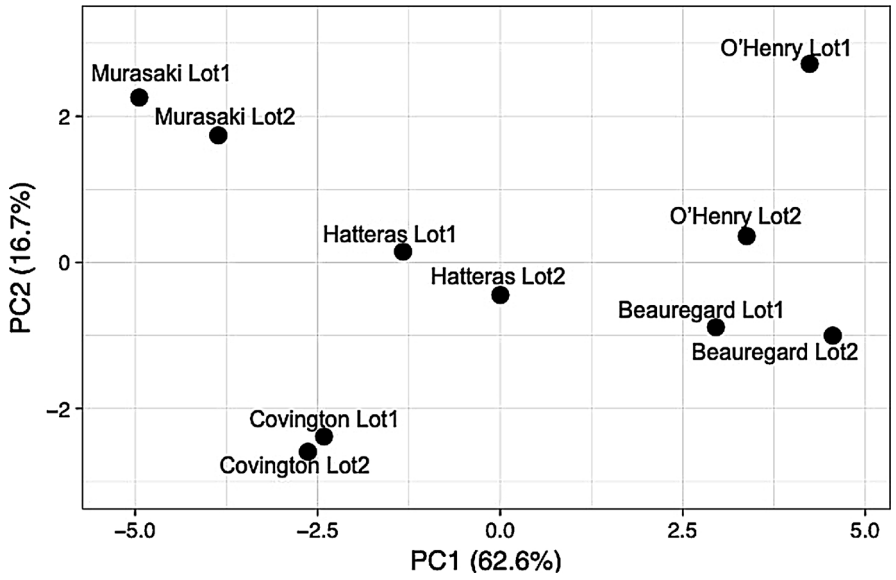

Fig. 4. Principle component analysis (PCA) of free amino acid contents for five commercial sweetpotato cultivars, showing the clear differentiation of the genotypes based on their overall amino acid profiles.

in this study and Mrs. Sandra Parker of the USDA-ARS, SEA Food Science Research Unit for her excellent editorial assistance.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2020.103522.

References

AOAC International, 2013. Appendix K: Guidelines for Dietary Supplements and Botanicals - Part I AOAC Guidelines for Single-laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals. Official Method of Analysis AOAC Int., pp. 1–15. https://doi.org/10.1007/s00170-006-0780-3.

Armenta, J., Cortes, D., Pisciotta, J., Shuman, J., Blakeslee, K., Rasoloson, D., Ogunbiyi, O., Sullivan Jr, D., Shulaev, V., 2010. A sensitive and rapid method for amino acid quantitation in malaria biological samples using AccQ•Tag UPLC-ESI-MS/MS with multiple reaction monitoring. Anal. Chem. 82 (2), 548–558. https://doi.org/10.1021/ac901790q.

Armstrong, M., Jonscher, K., Reisdorph, N.A., 2007. Analysis of 25 underivatized amino acids in human plasma using ion-pairing reversed-phase liquid chromatography/time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom.. 21, 2717–2726. https://doi.org/10.1002/rcm.3124.

Bartolomeo, M.P., Maisano, F., 2006. Validation of a reversed-phase HPLC method for quantitative amino acid analysis. J. Biomol. Techn. 17, 131–137. https://doi.org/10.2139/ssrn.410441.

Bond, J.K., 2017. U.S. SweetPotato Production Swells. Trade, USDA Econ. Reserach Serv.

Buszewski, B., Noga, S., 2012. Hydrophilic interaction liquid chromatography (HILIC)-a powerful separation technique. Anal. Bioanal. Chem. 402, 231–247. https://doi.org/10.1007/s00216-011-5308-5.

Callejón, R.M., Troncoso, A.M., Morales, M.L., 2010. Determination of amino acids in grape-derived products: a review. Talanta 18, 1143–1152. https://doi.org/10.1016/j.talanta.2010.02.040.

Cullis, B.R., Smith, a.B., Beeck, C.P., Cowling, W.A., 2010. Analysis of yield and oil from a series of canola breeding trials. Part II. Exploring variety by environment interaction using factor analysis. Genome 53, 1002–1016.
De Person, M., Chaimbault, P., Elfakir, C., 2008. Analysis of native amino acids by liquid chromatography/electrospray ionization mass spectrometry: comparative study between two sources and interfaces. J. Mass Spectrom.. 43, 204–215. https://doi.org/10.1002/jms.1287.
Elmore, J.S., Briddon, A., Dodson, A.T., Muttucumaru, N., Halford, N.G., Mottram, D.S., 2015. Acrylamide in potato crisps prepared from 20 UK-grown varieties: effects of variety and tuber storage time. Food Chem. 185, 1–8. https://doi.org/10.1016/j.foodchem.2015.02.103.
Fish, W.W., 2012. A reliable methodology for quantitative extraction of fruit and vegetable physiological amino acids and their subsequent analysis with commonly available HPLC systems. Food Nutr. Sci. 3, 863–871. https://doi.org/10.4236/fns.2012.36115.
Gökmen, V., Serpen, A., Mogol, B.A., 2012. Rapid determination of amino acids in foods by hydrophilic interaction liquid chromatography coupled to high-resolution mass spectrometry. Anal. Bioanal. Chem. 403, 2915–2922. https://doi.org/10.1007/s00216-012-5779-z.
Granby, K., Andersen, J.H., Christensen, H.B., 2004. Analysis of pesticides in fruit, vegetables and cereals using methanolic extraction and detection by liquid chromatography-tandem mass spectrometry. Anal. Chim. Acta 520, 165–176. https://doi.org/10.1016/j.aca.2004.05.088.
Greco, G., Letzel, T., 2013. Main interactions and influences of the chromatographic parameters in HILIC separations. J. Chromatogr. Sci. 51, 684–693. https://doi.org/10.1093/chromsci/bmt015.
Guo, S., Duan, J.A., Qian, D., Tang, Y., Qian, Y., Wu, D., Su, S., Shang, E., 2013. Rapid determination of amino acids in fruits of ziziphus jujuba by hydrophilic interaction ultra-high-performance liquid chromatography coupled with triple-quadrupole mass spectrometry. J. Agric. Food Chem. 61, 2709–2719. https://doi.org/10.1021/jf305497r.
Halford, N.G., Muttucumaru, N., Powers, S.J., Gillatt, P.N., Hartley, L., Elmore, J.S., Mottram, D.S., 2012. Concentrations of free amino acids and sugars in nine potato varieties: effects of storage and relationship with acrylamide formation. J. Agric. Food Chem. 60, 12044–12055. https://doi.org/10.1021/jf3037566.
Huang, Y., Keller, A., Anumol, T., Bivens, A., 2018. Quantitative analysis of underivatized amino acids in plant matrix by hydrophilic interaction chromatography (HILIC) with LC/MS detection [WWW Document]. Agil. Appl. Note URL. https://www.agilent.com/cs/library/applications/5991-8922EN_Plant_Amino_Acids_Quant_Poroshell_120_HILICZ_Application.pdf.
ICH, 1996. Guidance for industry Q2B validation of analytical procedures: methodology. U. S. Food Drug Adm. Biol. Eval. Res. 1–13 https://doi.org/62FR 27464.
International Agency for Research on Cancer, 1994. Acrylamide. IRAC monogr. Eval. Carcinoenic Risks Hum. 60, 389–433.
Jandera, P., 2011. Stationary and mobile phases in hydrophilic interaction chromatography: a review. Anal. Chim. Acta 692, 1–25. https://doi.org/10.1016/j.aca.2011.02.047.
Joyce, R., Kuziene, V., Zou, X., Wang, X., Pullen, F., Loo, R.L., 2016. Development and validation of an ultra-performance liquid chromatography quadrupole time of flight mass spectrometry method for rapid quantification of free amino acids in human urine. Amino Acids 48, 219–234. https://doi.org/10.1007/s00726-015-2076-0.
Koutsidis, G., Simons, S.P.J., Thong, Y.H., Haldoupis, Y., Mojica-Lazaro, J., Wedzicha, B.L., Mottram, D.S., 2009. Investigations on the effect of amino acids on acrylamide, pyrazines, and Michael addition products in model systems. J. Agric. Food Chem. 57 (19), 9011–9015. https://doi.org/10.1021/jf9014763.
Langrock, T., Czihal, P., Hoffmann, R., 2006. Amino acid analysis by hydrophilic interaction chromatography coupled on-line to electrospray ionization mass spectrometry. Amino Acids 30, 291–297. https://doi.org/10.1007/s00726-005-0300-z.
Lim, P.K., Jinap, S., Sanny, M., Tan, C.P., Khatib, A., 2014. The influence of deep frying using various vegetable oils on acrylamide formation in sweetpotato (*Ipomoea batatas* L. Lam) chips. J. Food Sci. 79, 115–121. https://doi.org/10.1111/1750-3841.12250.
López-Cervantes, J., Sánchez-Machado, D.I., Rosas-Rodríguez, J.A., 2006. Analysis of free amino acids in fermented shrimp waste by high-performance liquid chromatography. J. Chromatogr. A 1105, 106–110. https://doi.org/10.1016/j.chroma.2005.08.040.
Mottram, D.S., 2007. The Maillard Reaction: Source of Flavour in Thermally Processed Foods. In: Berger, R.G. (Ed.), Flavours and Fragrances. Springer, Berlin, Heidelberg.
Mu, T.H., Tan, S.S., Xue, Y.L., 2009. The amino acid composition, solubility and emulsifying properties of sweetpotato protein. Food Chem. 112, 1002–1005. https://doi.org/10.1016/j.foodchem.2008.07.012.
Muttucumaru, N., Powers, S.J., Elmore, J.S., Briddon, A., Mottram, D.S., Halford, N.G., 2014. Evidence for the complex relationship between free amino acid and sugar concentrations and acrylamide-forming potential in potato. Ann. Appl. Biol. 164, 286–300. https://doi.org/10.1111/aab.12101.
Pereira, V., Pontes, M., Câmara, J.S., Marques, J.C., 2008. Simultaneous analysis of free amino acids and biogenic amines in honey and wine samples using in loop orthophthalaldeyde derivatization procedure. J. Chromatogr. A 1189, 435–443. https://doi.org/10.1016/j.chroma.2007.12.014.
Piraud, M., Vianey-Saban, C., Petritis, K., Elfakir, C., Steghens, J.P., Morla, A., Bouchu, D., 2003. ESI-MS/MS analysis of underivatised amino acids: a new tool for the diagnosis of inherited disorders of amino acid metabolism. Fragmentation study of 79 molecules of biological interest in positive and negative ionisation mode. Rapid Commun. Mass Spectrom. 17, 1297–1311. https://doi.org/10.1002/rcm.1054.
Prinsen, H.C.M.T., Schiebergen-Bronkhorst, B.G.M., Roeleveld, M.W., Jans, J.J.M., de Sain-van der Velden, M.G.M., Visser, G., van Hasselt, P.M., Verhoeven-Duif, N.M., 2016. Rapid quantification of underivatized amino acids in plasma by hydrophilic interaction liquid chromatography (HILIC) coupled with tandem mass-spectrometry. J. Inherit. Metab. Dis. 39, 651–660. https://doi.org/10.1007/s10545-016-9935-z.
Qu, J., Wang, Y., Luo, G., Wu, Z., Yang, C., 2002. Validated quantitation of underivatized amino acids in human blood samples by volatile ion-pair reversed-phase liquid chromatography coupled to isotope dilution tandem mass spectrometry. Anal. Chem. 74, 2034–2040. https://doi.org/10.1021/ac0111917.
Salazar, C., Armenta, J.M., Cortés, D.F., Shulaev, V., 2012. Combination of an AccQ·Tag-ultra performance liquid chromatographic method with tandem mass spectrometry for the analysis of amino acids. In: In: Alterman, M., Hunziker, P. (Eds.), Amino Acid Analysis. Methods in Molecular Biology (Methods and Protocols), vol 828 Humana Press, Totowa, NJ.
Schmitz Carley, C.A., Coombs, J.J., Clough, M.E., De Jong, W.S., Douches, D.S., Haynes, K.G., Higgins, C.R., Holm, D.G., Miller, J.C., Navarro, F.M., Novy, R.G., Palta, J.P., Parish, D.L., Porter, G.A., Sathuvalli, V.R., Thompson, A.L., Yencho, G.C., Zotarelli, L., Endelman, J.B., 2019. Genetic covariance of environments in the potato national chip processing trial. Crop Sci. 59, 107–114. https://doi.org/10.2135/cropsci2018.05.0314.
Shimbo, K., Yahashi, A., Hirayama, K., Nakazawa, M., Miyano, H., 2009. Multifunctional and highly sensitive precolumn reagents for amino acids in liquid chromatography/tandem mass spectrometry. Anal. Chem. 81, 5172–5179. https://doi.org/10.1021/ac900470w.
Stadler, R.H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P.A., Robert, M.-C., Riediker, S., 2002. Acrylamide from Maillard reaction products. Nature 419, 449–450. https://doi.org/10.1038/419449a.
Truong, V..Den, Pascua, Y.T., Reynolds, R., Thompson, R.L., Palazoğlu, T.K., Atac Mogol, B., Gökmen, V., 2014. Processing treatments for mitigating acrylamide formation in sweetpotato french fries. J. Agric. Food Chem. 62, 310–316. https://doi.org/10.1021/jf404290v.
United States Pharmacopeia (USP-NF), 2017. USP40-NF35. General Chapter on Validation of Compendial Procedures < 1225 > https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c1225_1SUSP40.pdf.
Wang, Y., Bethke, P.C., Bussan, A.J., Glynn, M.T., Holm, D.G., Navarro, F.M., Novy, R.G., Palta, J.P., Pavek, M.J., Porter, G.A., Sathuvalli, V.R., Thompson, A.L., Voglewede, P.J., Whitworth, J.L., Parish, D.I., Endelman, J.B., 2016. Acrylamide-forming potential and agronomic properties of elite US potato germplasm from the national fry processing trial. Crop Sci. 56, 1–10. https://doi.org/10.2135/cropsci2015.03.0173.
Yeoh, H.H., Truong, V.D., 1996. Amino acid composition and nitrogen-to-protein conversion factors for sweetpotato. Trop. Sci. 36, 243–246.
Zhang, G., Huang, G., Xiao, L., Seiber, J., Mitchell, A.E., 2011. Acrylamide formation in almonds (Prunus dulcis): influences of roasting time and temperature, precursors, varietal selection, and storage. J. Agric. Food Chem. 59, 8225–8232. https://doi.org/10.1021/jf201595q.
Zhou, G., Pang, H., Tang, Y., Yao, X., Mo, X., Zhu, S., Guo, S., Qian, D., Qian, Y., Su, S., Zhang, L., Jin, C., Qin, Y., Duan, J.A., 2013. Hydrophilic interaction ultra-performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry for highly rapid and sensitive analysis of underivatized amino acids in functional foods. Amino Acids 44, 1293–1305. https://doi.org/10.1007/s00726-013-1463-7.
Zhou, P., Zhao, F., Chen, M., Ye, N., Lin, Q., Ouyang, L., Cai, X., Meng, P., Gong, X., Wang, Y., 2019. Determination of 21 free amino acids in 5 types of tea by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) using a modified 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) method. J. Food Compos. Anal. 81, 46–54. https://doi.org/10.1016/j.jfca.2019.05.007.
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., 2003. Acrylamide formation mechanism in heated foods. J. Agric. Food Chem. 51, 4782–4787. https://doi.org/10.1021/jf034180i.