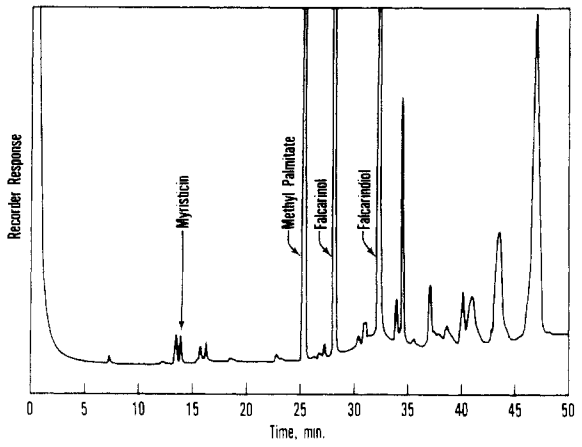

Figure 2. GLC recording of separation of carrot (commercial) fraction on 3% SE-52: myristicin, 0.8 mg/kg; falcarinol, 20.0 mg/kg; falcarindiol, 44.9 mg/kg.

Table II. Precision of GLC Analysis of Aliquots of a Pooled Carrot Root (Red Cored Chantenay) Extract with Added Myristicin

	myristicin, mg/kg	falcarinol, mg/kg	falcarindiol, mg/kg
mean of 6 aliquots	34.4	30.3	83.5
standard deviation	0.6	0.7	2.6

For the most accurate results, the ratio of toxin to internal standard should be 0.5 to 2.0. Ratios of 0.33 to 3.0 are acceptable, but the standard curves were not linear beyond this range. Also, both the OV-17 and SE-52 columns irreversibly absorb the toxins. Therefore, each sample injected should contain at least 10 μg of the toxicant or the analysis may show smaller amounts of the toxins than are actually present.

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Gas Chromatographic Analysis of the Free Amino Acid Pool of the Potato and Gas Chromatography–Mass Spectrometry Identification of γ-Aminobutyric Acid and Ornithine

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The free amino acid pool of the potato has been studied by using the *N*-heptafluorobutyryl isopropyl ester derivatives. Directly coupled gas chromatography–mass spectrometry allowed positive identification of the amino acids, where earlier work has relied solely on GC retention times. The nonprotein amino acids γ-aminobutyric acid and ornithine were shown to be present. γ-Aminobutyric acid was relatively abundant, at 27% of the free amino pool. The analytical procedure described is rapid and simple and, in combination with an established library of mass spectra of amino acid derivatives, provides a routine technique for the identification of free amino acids.

The amino acid composition of the free amino acid (FAA) pool of the potato was reviewed recently by Synge (1977). A few nonprotein amino acids were reported to occur in this fraction: α-aminobutyric acid (α-ABA), γ-aminobutyric acid (GABA), β-alanine, ornithine, L-pipecolic acid, and *S*-methylmethionine. Only GABA among these amino acids was present in significant quantity [10% (Thompson et al., 1953)].

The only other reports available on gas chromatographic analysis of the FAA pool of the potato are those of Hoff et al. (1971) and March (1975). In these studies, the identification of the amino acids was based on retention times.

In this report the *N*-heptafluorobutyryl isopropyl ester derivative of amino acids, as introduced by Golan-Goldhirsh and Wolfe (1979a), was used for the gas chromatographic analysis of the FAA extract of the potato. The

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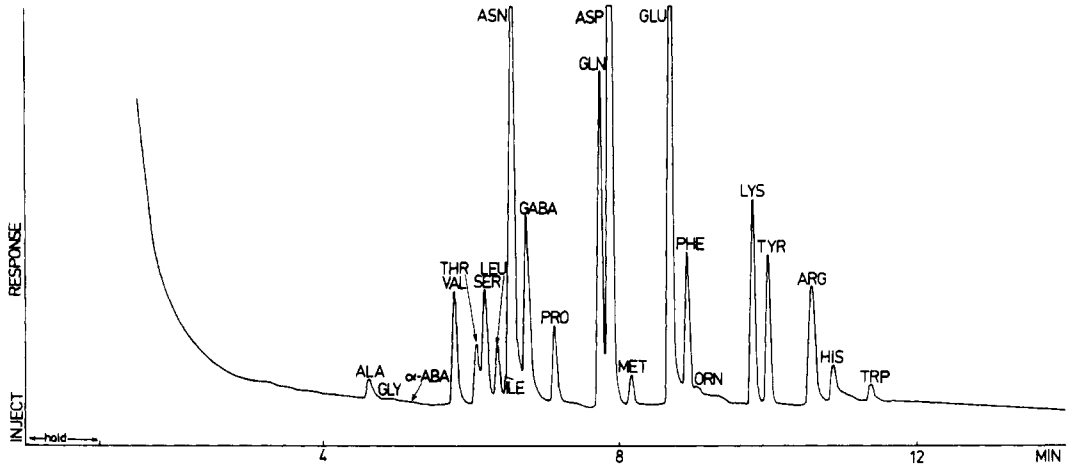

Figure 1. Gas chromatogram of the *N*-HFB isopropyl ester derivatives of a cation-exchange purified free amino acid extract of the peeled potato. The sample analyzed represents approximately 0.4 mg fresh weight of the potato. Chromatographic conditions: isothermal for 1 min, program from 70 to 225 °C at a rate of 20 °C/min, and hold at 225 °C.

identification of GABA and ornithine was obtained by an integrated GC–MS system.

MATERIALS AND METHODS

Free Amino Acid Extraction. The procedure used by Jaswal (1973) was adapted with modifications. Peeled, fresh potato samples of the Netted Gem cultivar, corresponding to approximately 5 g (dry weight basis), were triturated in 250 mL of 70% ethanol for 2 min, then transferred to an Erlenmeyer, and shaken for 8 h on a Burrell Wrist-Action shaker (Burrell Corp., Pittsburgh, PA). The mixture was then centrifuged for 10 min at 10000*g* on a Beckman Model J-21 centrifuge, the precipitate was discarded, and the supernatant was passed through a membrane filter (Millipore) with a pore size of 0.45 μm. The clear filtrate was evaporated to dryness on a rotary evaporator (Büchi/Brinkmann) under reduced pressure with intermittent nitrogen flushes. The residue was dissolved in 0.1 N HCl; final volumes varied between 10 and 20 mL. Each sample was divided into three vials and kept frozen until used for amino acid analysis.

Preparation for Gas Chromatographic (GC) Analysis. Standard amino acids were obtained from Pierce (Rockford, IL), except GABA, which was from Sigma Chemical Co. (St. Louis, MO).

Prior to derivatization of a potato sample, it was passed through a small column made of 100 mg of cation-exchange resin Rexyn 101(H), exchange capacity 5.5 mequiv/g (Fisher Scientific Co.), as a cleanup step, similar to the procedure described by Adams et al. (1977).

Sample preparation for the cleanup step involved mixing 100 μL of the thawed free amino acid extract diluted 10 times. A solution of 25% acetic acid (200 μL) was added in order to adjust the pH to between 2.0 and 2.5. The mixture was transferred to the moist resin by using a Pasteur pipet and allowed to pass through the column at about 1 drop/5 s. The sample tube was washed with 200 μL of distilled water, and the washings were transferred to the column, followed immediately by 0.5 mL of distilled water. The amino acids were eluted by passing 1 mL of 2 N NH_4OH through the column at about 1 drop/s. The eluate was collected in a reacti-vial (Pierce, Rockford, IL USA), evaporated to dryness, and kept desiccated until derivatization. Complete elution of amino acids was verified by treating a standard amino acid mixture identically.

Derivatization and Gas Chromatographic Conditions. All derivatization steps were carried out in the reacti-vials. Esterification reagent was made up by slow pipetting of acetyl chloride (200 μL) into dried 2-propanol (1 mL) at 0 °C (Felker and Bandurski, 1975; Pearce, 1977). The esterification reagent (200 μL) was pipetted onto the dried sample and the esterification reaction conducted at 80 °C for 120 min. After evaporation of the esterification solvent at room temperature, acylation was done in 100 μL of ethyl acetate and 200 μL of heptafluorobutyric anhydride (HFBA) at 110 °C for 10 min.

A Varian 2100 gas chromatograph, equipped with a flame ionization detector coupled to a Varian recorder, Model A-25, was used. A u-shaped glass column, 3.5 m × 2 mm i.d. (thick wall), was packed with 3% SE-30 on 80–100-mesh Gas-Chrom Q (Applied Science Laboratories, State College, PA). The column was attached to the gas chromatograph through a glass column connector (Golan-Goldhirsh and Wolfe, 1979b). The single-column mode of operation was used. A flow rate of 30 mL/min was used for hydrogen and the carrier gas (nitrogen). The air flow rate was 300 mL/min. Temperature programming was as follows: hold at 70 °C for 1 min following sample injection, then temperature programming from 70 to 225 °C at a rate of 20 °C/min, and hold at 225 °C until elution of the sample was obtained. Injection and detector temperatures were maintained at 225 and 280 °C, respectively. For further details concerning derivatization reagents and conditions and gas chromatographic procedures, see Golan-Goldhirsh (1979).

GC–MS Analysis. The GC–MS data were obtained on a Varian 1400 gas chromatograph, oven coupled via a porous ceramic Watson/Biemann helium separator to an AEI MS12 mass spectrometer. A coiled glass column identical in every other respect with the u-shaped column used with the conventional gas chromatograph was employed. The helium flow rate was 30 mL/min, and the temperature program was identical with that described above. The injector was maintained at 225 °C and the interconnecting lines, separator, and ion source were maintained at >225 °C.

Data acquisition and processing were by means of an AEI DS50S data system with spectra acquired every 7 s.

Pure derivatives were also introduced via a direct probe inlet into an AEI MS50 high-resolution mass spectrometer, and elemental compositions of all ions were determined by using the AEI DS50S data system.

RESULTS AND DISCUSSION

Potato Free Amino Acids. The gas chromatographic pattern of separation of the potato FAA extract is shown in Figure 1. All amino acids were well resolved, except for isoleucine, which appears as a shoulder on the as-

Table I. Relative Molar Response (RMR) of the *N*-HFB Isopropyl Ester Derivative of Standard Amino Acids

amino acid	RMR[a]
Ala	0.459 ± 0.026
Gly	0.430 ± 0.031
Val	0.473 ± 0.059
Thr	0.738 ± 0.078
Ser	0.809 ± 0.092
Leu	1.043 ± 0.120
Ile	0.461 ± 0.059
GABA	0.543 ± 0.065
Pro	0.916 ± 0.090
Asp	0.988 ± 0.074
Met	0.725 ± 0.051
Glu	1.101 ± 0.073
Phe	1.429 ± 0.160
Lys	1.079 ± 0.051
Tyr	1.313 ± 0.121
Arg	0.821 ± 0.129
His	0.639 ± 0.063
Trp	0.730 ± 0.064
Cys_2	0.875 ± 0.065

[a] Internal standard ornithine was assigned a value of unity, 1. $RMR_{aa/is}$ = (amino acid molar response)/(ornithine molar response). aa = amino acid. is = internal standard.

cending side of the asparagine peak, due to its broad size. A small peak corresponding to the position of α-aminobutyric acid (after glycine) was observed at high sensitivity. This could not be identified by the GC–MS system, because of the small amount present as explained later. No other unexpected amino acids were identified in the FAA pool of the potato.

The amino acid analysis reported here by using the *N*-HFB isopropyl ester derivative yields good separation at faster elution time compared to previous reports on GC analysis of free amino acids of the potato (Hoff et al., 1971; March, 1975). GABA was eluted within 7 min, while the *n*-propyl derivative of this amino acid was eluted after approximately 28 min, with low resoluiton from norleucine (March, 1975).

The partial conversion of the amides (asparagine and glutamine) to their corresponding amino acids (aspartic acid and glutamic acid) upon derivatization prevented their direct quantitative determination by the GC method. However, it can be seen from the large peaks of these amino acids (Figure 1) that they constitute the largest relative amount of the FAA pool of the potato. The relative molar response (RMR) values used for quantitative determination are shown in Table I. These are based on 24 chromatograms and at least 8 replicates for each amino acid, obtained by analysis of equimolar mixtures of standard amino acids.

Due to the presence of ornithine and GABA in the FAA extract of the potato, neither one of them could be used as an internal standard; therefore, data are presented on a mole percent basis (Table II). The relative amounts of the different amino acids reported here are comparable to those previously reported by Synge (1977), bearing in mind the fact that the amides and their corresponding amino acids were not included. GABA is higher than any other amino acid (27.3%), with arginine, valine, lysine, and serine found in fair quantity (Table II). Most of the other protein amino acids occur in relatively small quantities in the FAA pool of the potato. The pattern of amino acid composition renders the FAA pool of the potato nutritionally unbalanced.

GC–MS Identification of GABA and Ornithine. GABA and ornithine were first identified by their GC retention times, by comparison of the GC of derivatized

Table II. Potato Free Amino Acid Extract Composition

amino acid	rel mol %[a]
Ala	3.0 ± 0.3
Gly	1.4 ± 0.1
Val	10.0 ± 1.3
Thr	4.4 ± 0.2
Ser	7.0 ± 0.6
Leu	2.7 ± 0.2
Ile	[b]
GABA	27.3 ± 4.5
Pro	4.2 ± 0.3
Met	1.9 ± 0.2
Phe	5.7 ± 0.6
Orn	0.9 ± 0.1
Lys	8.2 ± 0.4
Tyr	5.5 ± 0.5
Arg	12.7 ± 1.4
His	3.4 ± 0.2
Trp	1.7 ± 0.1

[a] Average of independent triplicate analyses. Relative mole percent for an amino acid = $[(A_{aa}/RMR_{aa/orn})]/[\Sigma(A_{aa}/RMR_{aa/orn})] \times 100$ where A_{aa} = area of amino acid peak on chromatogram and $RMR_{aa/orn}$ = (amino acid molar response)/(ornithine molar response). [b] Detected.

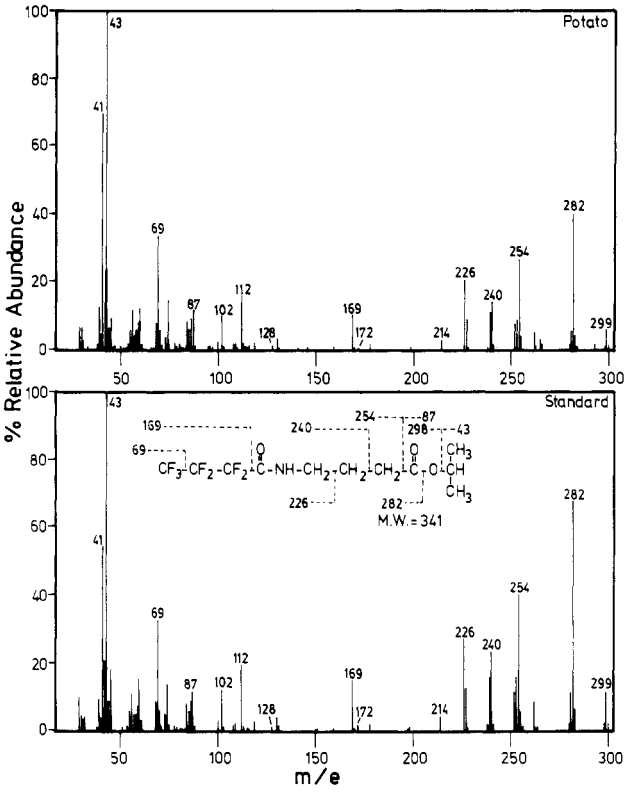

Figure 2. Mass spectra of the *N*-HFB isopropyl ester of GABA from potato and the standard amino acid mixture.

potato extract with that of a similarly derivatized mixture of pure amino acids. The same samples were then injected on a combined GC–MS and the mass spectra shown in Figures 2 and 3 obtained. The spectra from the extract and the standard mixture are identical within the limitations of the experiment.

The GABA spectra (Figure 2) show only small variations in relative intensities of the ions, which are explainable in terms of the sample pressure change in the ion source during the 3.5 s required for the MS scan of the fairly sharp GC peak.

The ornithine spectra (Figure 3) are somewhat less similar because, in addition to the effect of sample pressure changes, the potato extract contains such a small amount of ornithine that some of the low-abundance ions, including

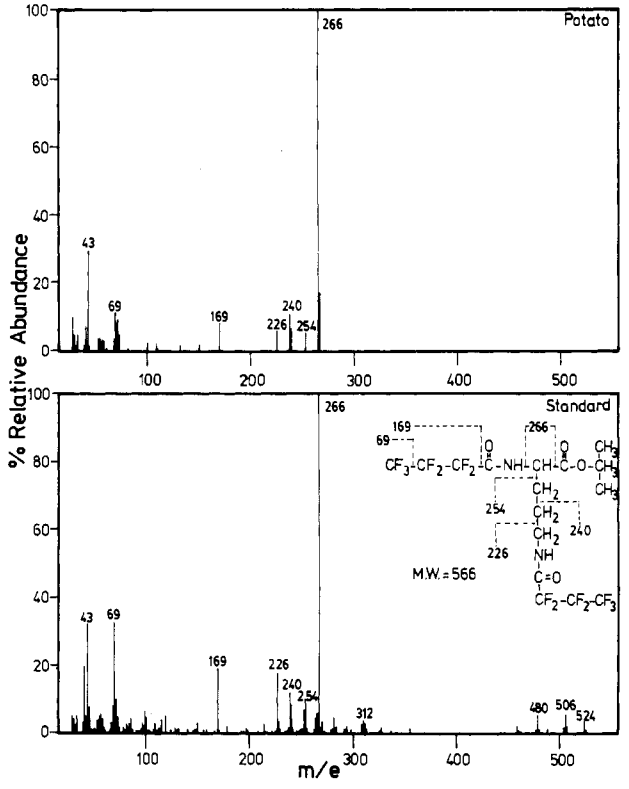

Figure 3. Mass spectra of the *N*-HFB isopropyl ester of ornithine from potato and the standard amino acid mixture.

all those in the high-mass region, fall below the detection limits of the mass spectrometer.

As part of a more complete study of *N*-HFB isopropyl derivatives of protein and nonprotein amino acids, standards were prepared from 23 pure amino acids, and a small library for the DS50S data system was created from their low-resolution spectra. When the spectra from the two GC peaks of interest in the potato extract were compared with the library, the data system selected GABA and ornithine as the closest matches. In future studies in this laboratory of *N*-HFB isopropyl derivatives of amino acids extracted from food product sources, it should thus be possible to conduct rapid automatic identifications by using this spectral library. The structural interpretation of the ions in the mass spectra of *N*-HFB isopropyl amino acids based on the study of both high- and low-resolution spectra will be published elsewhere (Golan-Goldhirsh et al., 1979). J. Eagles and J. F. March have made available mass spectra of the corresponding *n*-propyl esters (Eagles et al., 1980). In essence, both these series are very similar to the spectra of *N*-TFA *n*-butyl amino acids which have been exhaustively reported by Leimer et al. (1977). While molecular ions are generally either absent or of low abundance, the major ions in the high-mass region are due to fragmentation of the derivatizing groups and contain the diagnostically significant amino acid skeleton.

SUMMARY AND CONCLUSIONS

Complete gas chromatographic analysis of the free amino acid extract of the potato was obtained within 12 min by using the *N*-HFB isopropyl derivative of amino acids. The method reported here can be used to analyze any amino acid extract with considerable saving on time of analysis. At the present state of development of the method, the amides (asparagine and glutamine) of aspartic and glutamic acids cannot be directly determined quantitatively. The analytical potential of gas chromatography analysis of amino acids was demonstrated by the use of an integrated GC–MS system for the direct and fast identification of GABA and ornithine in the potato extract.

It was shown that in addition to the amides and their corresponding amino acids GABA is a major constituent of the FAA pool of the potato. Most of the protein amino acids occur in small amounts in this fraction.

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