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Rapid gas chromatographic screening of edible seeds, nuts and beans for non-protein and protein amino acids

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Abstract

Water extraction with subsequent picric acid treatment and solvent washing (ethyl acetate and diethyl ether) was employed for the rapid isolation of free amino acids from non-aqueous food samples. The isolated amino acids were subjected to N(O,S)-isobutyloxycarbonylation followed by solid-phase extraction and *tert.*-butyldimethylsilylation for direct analysis by gas chromatography and gas chromatography–mass spectrometry. When the present method was applied to nineteen food samples (common beans, seeds, and nuts), seventeen protein amino acids and fifteen non-protein amino acids were simultaneously screened. Eleven non-protein amino acids including γ-aminobutyric acid, pipecolic acid, pyroglutamic acid, α-aminobutyric acid, α-aminoadipic acid, and β-alanine were tentatively identified, and four compounds assumed to be non-protein amino acids remained unidentified.

1. Introduction

Free amino acids increase the nutritional value of food and also enhance its flavor. In contrast to these beneficial effects, some non-protein amino acids are known to be toxic to humans as well as to domestic animals [1–4]. The toxicity of a wide variety of plant products may originate from low concentrations of toxic amino acids. Therefore, rapid amino acid profiling to screen food products for toxic non-protein amino acids will become important.

With the advent of high-resolving columns gas chromatography (GC) played an important role in amino acid analysis [5]. However, the main disadvantages recognized for GC techniques include the troublesome sample clean-up step and preconversion of amino acids into volatile derivatives [6,7]. Recently, we reported a simple method suitable for routine profiling and screening for free amino acids [8–10]. The major advantage of our method is the recovery of zwitterionic amino acids in the form of N(O,S)-isobutyloxycarbonyl (isoBOC) carboxylic acids by a two-step solid-phase extraction (SPE) from aqueous samples after isoBOC reaction of amino functions directly in the basified aqueous media. The carboxylic functions of the solid-phase extracted amino acids are then silylated to stable *tert.*-butyldimethylsilyl (TBDMS) derivatives in a

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single step with subsequent analysis by a dual capillary column GC system. Each amino acid is positively identified by computer searching using a home-retention index (*I*) library which currently contains nineteen protein amino acids and thirty six non-protein amino acids [9].

The present work was undertaken to investigate the usefulness of our amino acid profiling method in routine screening of commonly consumed beans, seeds, and nuts for toxic non-protein amino acids. For the successful application of this method to non-aqueous plant matrices, conditions of preextracting free amino acids into aqueous phases and removing co-extracted proteins and organic acids were to be optimized.

2. Experimental

2.1. Materials

Nineteen food products examined in this study are listed in Table 1. One lot of each product was purchased locally and desiccated at −20°C until used. The non-protein amino acids were obtained from various companies, such as Aldrich (Milwaukee, WI, USA), Sigma (St. Louis, MO, USA), Calbiochem (San Diego, CA, USA), and RBI (Natick, MA, USA). Tetrahydrofuran (THF), N-methyl-(trimethylsilyl)-trifluoroacetamide (MSTFA), and N-methyl-N - (*tert.* - butyldimethylsilyl)trifluoroacetamide (MTBSTFA) of silylation grade were obtained from Pierce (Rockford, IL, USA) and isobutyl chloroformate (isoBCF) from Sigma. *n*-Hydrocarbon standards (C_{12}–C_{36}, even numbers only) were obtained from Polyscience (Niles, IL, USA) and Chromosorb P (acid washed, 80–100 mesh) from Supelco (Bellefonte, PA, USA). Chromosorb P columns (6 mm I.D.) were prepared and activated as described previously [8,9]. All other chemicals were of analytical-reagent grade.

2.2. Sample preparation

Each food sample was ground to fine powder (80–100 mesh) in a porcelain bowl and 500 mg of the ground sample was extracted with double distilled water (4 × 1 ml) by vortex-mixing. To the supernatant (1 ml), 200 μl of saturated picric

Table 1
Edible seeds, nuts and beans screened for free non-protein and protein amino acids

Common name	Scientific name	Family
Adzuki bean	*Phaseolus angularis* W.F. Wight	Fabaceae
Almond	*Pinus dulcis* Mill.	Rosaceae
Cacao	*Theobroma cacao* L.	Sterculiaceae
Capsici seed	*Capsium annuum* L.	Solanaceae
Chestnut	*Castanea deritata* Marsh.	Fagaceae
Coffee bean	*Coffea arabica* L.	Rubiaceae
Ginkgo nut	*Ginkgo biloba* L.	Ginkgoaceae
Green pea	*Pisum sativum* L.	Fabaceae
Hazelnut	*Corylus avellana* L.	Corylaceae
Kidney bean	*Phaseolus vulgaris* L.	Fabaceae
Mung bean	*Phaseolus radiatus* L.	Fabaceae
Peanut	*Arachis hypogaea* L.	Fabaceae
Perilla seed	*Perilla frutescens* Britton	Laminaceae
Pine nut	*Pinus koreaiensis* Sieb. et Zucc	Pinaceae
Sesame seed	*Sesamum indicum* L.	Pedaliaceae
Sunflower seed	*Helianthus annuus* L.	Asteraceae
Walnut	*Juglans regia* L.	Juglandaceae
Watermelon seed	*Citrullus vulgaris* Schrad	Cucurbitaceae
Wultari bean	*Phaseolus vulgaris* L.	Fabaceae

acid solution was added, gently shaken, and centrifuged (350 *g*, 2 min), then the resulting solution was acidified (pH ≤ 2) with a few drops of 10% sulfuric acid, followed by sequential washing with ethyl acetate (3 × 2 ml) and diethyl ether (2 × 2 ml). The aqueous layer was basified to pH 11 with sodium carbonate and subjected to N(O,S)-isoBOC and SPE as described elsewhere [9]. Briefly, isoBCF was added and vortex-mixed, then the mixture was washed with diethyl ether. The aqueous layer was acidified (pH 1–2) with 10% sulfuric acid, and saturated with sodium chloride. The mixture was then loaded onto a Chromosorb P column and the N(O,S)-isobutyloxycarbonyl (isoBOC) amino acids were eluted with diethyl ether. The ether eluate was dried under a gentle stream of nitrogen. The residue was reacted with MTBSTFA in THF to form TBDMS derivatives. To form trimethylsilyl (TMS) derivatives of N(O,S)-isoBOC amino acids, THF (20 μl) and MSTFA (20 μl) were added to the residue. The reaction mixture was directly analyzed by GC and GC–MS. The measurement of arginine was excluded in this study. The effects of picric acid treatment and solvent washing on amino acid recoveries were examined using 500 mg of cashew nut powder spiked with twenty-six non-protein amino acids each at 0.5 μg/mg. L-*allo*-Isoleucine as the internal standard was added at the same concentration.

2.3. Gas chromatography

GC analyses were conducted with a Hewlett-Packard HP 5890A gas chromatograph, equipped with a split/splitless inlet system, two flame ionization detectors (FID), and a HP 3392A integrator which was interfaced to a HP 5895A GC Chemstation (Hewlett-Packard, Avondale, PA, USA). For screening purposes, samples (about 0.8–1.0 μl aliquots) were injected in a split mode (30:1), and for the cashew nut sample the splitless mode was employed.

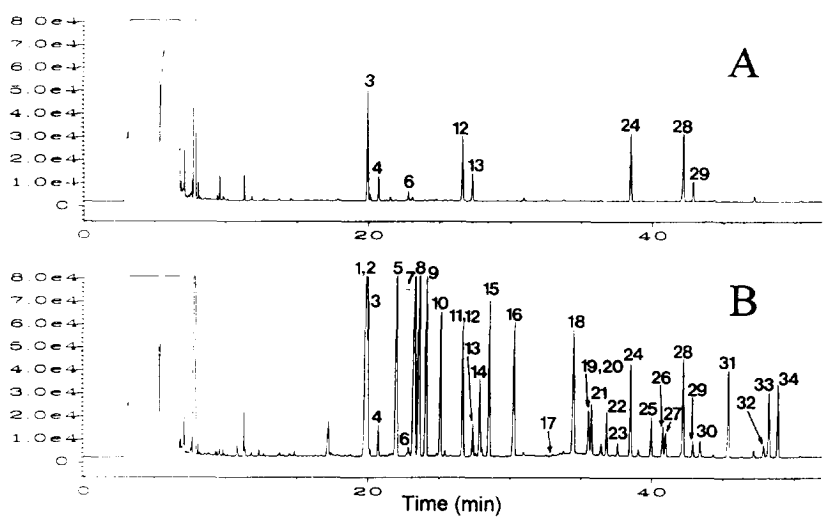

Fig. 1. Chromatograms of non-protein and protein amino acids as their N(O,S)-isoBOC TBDMS derivatives from (A) cashew nut, and (B) cashew nut spiked with non-protein amino acids separated on DB-5 (30 m × 0.25 mm I.D., 0.241 μm film thickness) fused-silica capillary column. GC conditions are described in the text. Peaks: 1 = α-aminoisobutyric acid; 2 = sarcosine; 3 = alanine; 4 = glycine; 5 = α-aminobutyric acid; 6 = valine; 7 = β-alanine; 8 = β-aminoisobutyric acid; 9 = norvaline; 10 = L-*allo*-isoleucine (internal standard); 11 = D-norleucine; 12 = proline; 13 = γ-aminobutyric acid; 14 = cycloleucine; 15 = pipecolic acid; 16 = *S*-methylcysteine; 17 = 4-hydroxyproline; 18 = ε-aminocaproic acid; 19 = ethionine; 20 = selenomethionine; 21 = *o*-aminobenzoic acid; 22 = DL-homoserine; 23 = selenoethionine; 24 = aspartic acid; 25 = β-N-methylamino-L-alanine; 26 = 2,3-diaminopropionic acid; 27 = *m*-aminobenzoic acid; 28 = glutamic acid; 29 = asparagine; 30 = *p*-aminobenzoic acid; 31 = α-aminoadipic acid; 32 = ornithine; 33 = α-aminopimelic acid; 34 = kainic acid.

The split/splitless injector was connected to a deactivated fused-silica tubing (1 m × 0.25 μm I.D.) and a Chromfit "Y" splitter (Unimetrics, Shorewood, IL, USA) that allowed the attachment of both DB-5 (SE-54 bonded phase) and DB-17 (OV-17 bonded phase) fused-silica capillary columns (30 m × 0.25 mm I.D., 0.241 μm film thickness, J&W Scientific, Folsom, CA, USA). Oven temperature was initially held at 150°C for 2 min and then programmed to 280°C at 3°C/min rate for split injection. For splitless injection, the oven temperature was held initially at 80°C for 2 min then raised to 150°C at 30°C/min, held at 150°C for 1 min, and then increased to 280°C at a rate of 3°C/min. The purge delay time for splitless mode was 0.7 min. The split/splitless injector temperatures for split and splitless mode were 280 and 260°C, respectively. The detector temperature was 300°C. The two FID signals were processed simultaneously in dual channel mode by the GC Chemstation [8–10]. Via Chemstation BASIC programs, *I* values for the sample peaks of each channel were calculated and compared with a previously compiled *I* reference library to find matches to help in identifying the peaks [9]. The peak area of each amino acid was normalized to the largest amino acid peak in the chromatogram.

2.4. Gas chromatography–mass spectrometry

A Hewlett-Packard HP 5890A Series II gas chromatograph, interfaced to an HP 5970B MSD (70 eV, electron-impact mode) and on-line to an HP 59940A MS Chemstation system, was used with an HP-5 (SE-54 crosslinked) capillary column (25 m × 0.20 mm I.D., 0.33 μm film thickness, Hewlett-Packard, Avondale, PA, USA) to obtain mass spectra. Samples were introduced in the split injection mode (30:1) at 260°C. The oven temperature was initially 200°C for 2 min and was then raised to 280°C at a rate of 5°C/min. The interface temperature was 300°C. The mass range scanned was 60–650 amu at a rate of 1.0 scan/s. Once the total-ion chromatogram of the sample was obtained, each peak was identified by library searching using our *tbdms.l* library file containing fifty-five amino acids as N(O,S)-isoBOC TBDMS derivatives, and one hundred and fifty organic acids as TBDMS derivatives [9].

3. Results and discussion

Legume samples contain proteins in high concentrations. Therefore, the water extracts were deproteinized prior to N(O,S)-isoBOC reaction. Denaturants commonly used include picric acid [11,12], sulphosalicylic acid [13,14], trichloroacetic acid [15], and perchloric acid [16]. Sample dilution with acetic acid is also used for deproteinization. Presently, there is no precipitant which removes proteins without loss of amino acids [17]. According to preliminary experiments, picric acid treatment was most effective for the food samples.

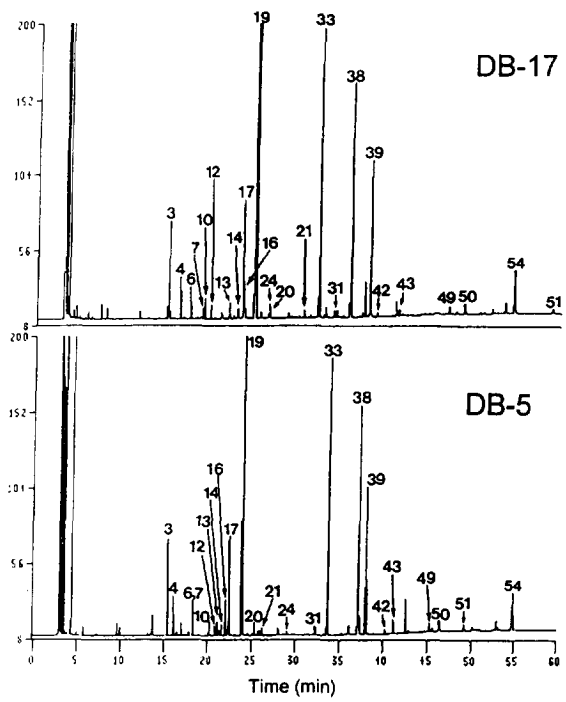

Fig. 2. Chromatograms of non-protein and protein amino acids in kidney bean (*Phaseolus vulgaris* L.) separated on DB-17 (30 m × 0.25 mm I.D.) and DB-5 (30 m × 0.25 mm I.D.) dual capillary column system. GC conditions are described in the text. Peak numbers correspond to those in Table 2.

Following deproteinization, most of the interfering organic acids co-extracted and residual picric acid were removed by washing with ethyl acetate and diethyl ether. Although methyl ethyl ketone was more effective in removing organic interfering compounds, some amino acids were lost due to the high miscibility of this solvent with water. The ion-exchange method using Dowex-50W resin caused the near complete loss of cysteine and tyrosine in our preliminary experiments. Moreover, it was time-consuming and laborious.

The effect of picric acid treatment and solvent washing on the recoveries were tested using cashew nut powder before and after addition of non-protein amino acids. Eight free amino acids including γ-aminobutyric acid were detected in cashew nut (Fig. 1A). All the non-protein amino acids added were detected together with the intrinsic amino acids in the spiked cashew nut (Fig. 1B). Overall recoveries were higher than 90%, with the exception of hydroxyproline (52%), selenomethionine (30%), *o*-aminobenzoic acid (78%), selenoethionine (66%), β-N-methylamino-L-alanine (81%), *m*-aminobenzoic acid (77%), and kainic acid (90%).

The usefulness of the present GC profiling method for amino-acid analysis of non-aqueous sample matrices is well demonstrated in typical dual-chromatograms of kidney bean, mung bean, and watermelon seed (Figs. 2–4). One lot of each plant sample was analyzed. Samples included commonly consumed foods such as unprocessed nuts, seeds, and protein-rich beans.

By computer comparison of *I* sets with the reference values in our amino acid *I* library, twenty-eight free amino acids, including eleven non-protein amino acids, were positively identified from the nineteen samples (Tables 2–4). The *I* sets, measured with the dual capillary

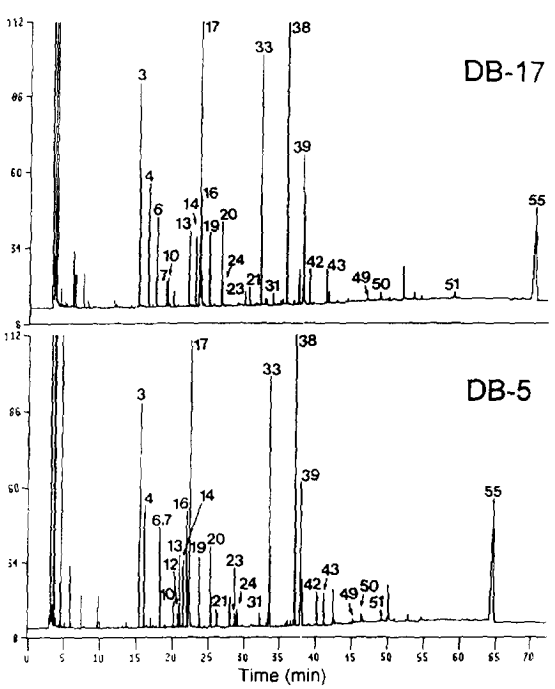

Fig. 3. Chromatograms of non-protein and protein amino acids in mung bean (*Phaseolus radiatus* L.) separated on DB-17 (30 m × 0.25 mm I.D.) and DB-5 (30 m × 0.25 mm I.D.) dual capillary column system. GC conditions are described in the text. Peak numbers correspond to those in Table 2.

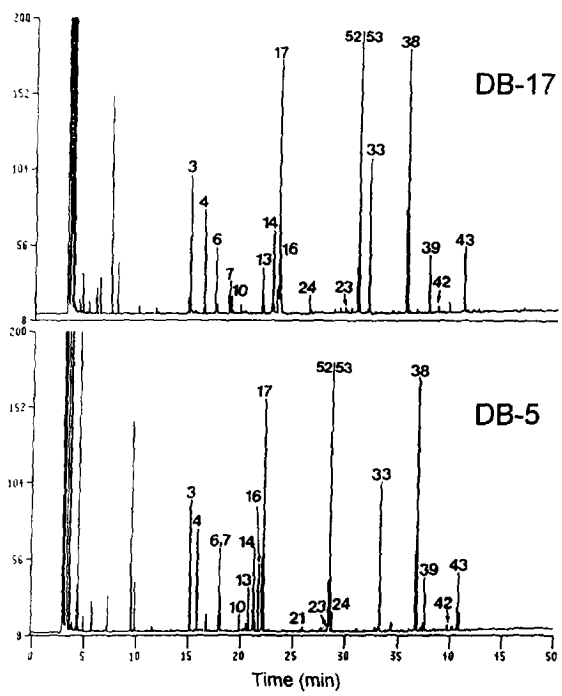

Fig. 4. Chromatograms of non-protein and protein amino acids in watermelon seed (*Citrullus vulgaris* Shrader) separated on DB-17 (30 m × 0.25 mm I.D.) and DB-5 (30 m × 0.25 mm I.D.) dual capillary column system. GC conditions are described in the text. Peak numbers correspond to those in Table 3.

column system, were useful in cross-checking each amino acid [9], which was further confirmed by GC–MS. Concentration ratios of the identified amino acids from duplicate runs were expressed as the mean percent peak areas normalized to the largest peak.

Among the identified non-protein amino acids, large amounts of γ-aminobutyric acid were found in all samples except for ginkgo nut. Pyroglutamic acid, found in eighteen samples, may have formed from glutamine during sample storage and processing [18]. α-Aminoadipic acid, pipecolic acid, and β-alanine were detected in many samples. Pipecolic acid, a higher homologue of proline [1], was most abundant in beans belonging to *Phaseolus vulgaris* (Fig. 2). In mung bean, the S-methylcysteine peak was uniquely large as compared with other samples (Fig. 3). β-Aminoisobutyric acid and 4-hydroxyproline were found only in pine nut, and ornithine was found only in walnut. Several peaks detected reproducibly remained unidentified due to the limitation of the databases in our *I* and MS libraries.

In an attempt to identify the unknown peaks 52 and 53 found at high concentrations in watermelon seed (Fig. 4), solid-phase extracted samples were analyzed both as TBDMS and TMS derivatives by GC–MS (Fig. 5). According to the previous findings on the general fragmentation of N(O,S)-isoBOC TBDMS derivatives [9,10], the ions at *m/z* 354, 296, 238, 210, and the most abundant ion at *m/z* 312 of peak 52 (Fig. 5A) were assumed to be the characteristic

Table 2
Non-protein and protein amino acids found in beans

No.[a]	Amino acid	Amount (% normalized area)[b]						
		Kidney bean	Mung bean	Green pea	Wultari bean	Adzuki bean	Cacao	Coffee
3	Alanine	11.1	25.7	8.3	11.2	20.2	46.8	24.3
4	Glycine	4.0	17.0	10.5	10.5	12.6	12.2	4.4
6	Valine	7.3	15.3	5.5	8.2	12.5	61.6	5.4
7	β-Alanine	0.7	3.8	2.8	3.6	4.4		0.7
10	Leucine	3.1	4.8	2.5	1.9	5.2	35.1	
12	Isoleucine	2.5	3.7	2.4	1.7	3.6		
13	Threonine	1.9	8.3	21.1	7.8	17.4	29.5	18.9
17	γ-Aminobutyric acid	6.9	32.7	19.9	59.2	68.8	31.7	25.2
19	Pipecolic acid	100.0	12.8	2.2	100.0	69.9		3.5
20	S-Methylcysteine	4.4	14.9		3.9			
21	Pyroglutamic acid	6.4	10.1	8.0	1.4	7.5	2.3	10.2
31	Phenylalanine	1.9	1.9	1.9	1.3			
33	Aspartic acid	69.5	53.4	4.2	10.2	65.7	100.0	45.1
38	Glutamic acid	56.5	100.0	100.0	24.7	100.0	93.0	100.0
39	Asparagine	36.3	32.0	58.8	46.3	44.7	7.7	46.6
42	α-Aminoadipic acid	1.4	5.8	1.9		9.3		1.2
43	Glutamine	3.0	3.6	3.4	2.2	3.3	6.7	1.0
49	Lysine			1.3				
50	Histidine	1.1	trace	0.6				
51	Tryptophan	1.2	1.4	0.6				
54	Unidentified	10.1			7.6			
55	Unidentified		17.0		10.7			

[a] Same numbers listed in the previous *I* reference library [9].
[b] Percentage of peak area normalized to the largest peak.

Table 3
Non-protein and protein amino acids found in seeds

No.[a]	Amino acid	Amount (% normalized area)[b]				
		Sesame seed	Sunflower seed	Capsici seed	Watermelon seed	Perilla seed
3	Alanine	43.2	37.3	0.8	23.0	33.1
4	Glycine	30.6	26.1	0.8	19.6	15.6
6	Valine	32.4	21.1		15.6	5.8
7	β-Alanine				6.7	
10	Leucine	32.1	12.2			
11	*allo*-Isoleucine		1.8			
12	Isoleucine	21.9	16.7			
13	Threonine	19.8	7.1	18.2	9.5	6.0
14	Serine	32.9	20.6		11.2	30.7
16	Proline	17.7	22.5	1.0	16.9	5.7
17	γ-Aminobutyric acid	16.5	8.7	0.8	45.8	11.3
21	Pyroglutamic acid	2.5	4.6	1.2	2.8	7.7
23	Methionine		2.1			
31	Phenylalanine	16.7	7.1			
33	Aspartic acid	76.7	100.0	84.2	57.0	65.1
38	Glutamic acid	100.0	82.5	10.1	90.8	100.0
39	Asparagine	55.3	28.9	100.0	21.2	42.8
42	α-Aminoadipic acid				2.0	1.6
43	Glutamine	11.2	4.2	2.6	21.4	1.6
49	Lysine	5.6	3.8			
50	Histidine	11.1	trace			
51	Tryptophan	2.6	5.0			
52	Unidentified				NC	
53	Unidentified				NC	

NC = not calculable due to incomplete separation between peak 52 and 53.
[a] Same numbers listed in the previous *I* reference library [9].
[b] Percentage of peak area normalized to the largest peak.

$[M-15]^+$, $[M-73]^+$, $[M-131]^+$ and $[M-159]^+$ and $[M-57]^+$ which are formed by loss of methyl, isobutyloxy, OTBDMS, COOTBDMS, and *tert.*-butyl functions from the molecular ion at *m/z* 369, respectively (Fig. 6A). The prominent ion at *m/z* 188 was assumed to be formed by the consecutive loss of $COOCHCH(CH_3)_2$ and R groups. The ions at *m/z* 212, 195, and 110 were assumed to be formed by the loss of $COOCHCH(CH_3)_2$, $CH(CH_3)_2$, and $COOCHCH(CH_3)_2$ from $[M-57]^+$, $[M-131]^+$ and $[M-159]^+$, respectively. For the corresponding TMS derivative (Fig. 5B), ions at *m/z* 312, 254, and 238 and the most abundant ion at *m/z* 210 were assumed to be $[M-15]^+$, $[M-73]^+$, $[M-89]^+$, and $[M-117]^+$ ions generated by the loss of methyl, isobutyloxy, OTMS, and COOTMS functions from the molecular ion at *m/z* 327, respectively (Fig. 6B). The ions at *m/z* 195 and 110 were assumed to be formed by the loss of isopropyl and $COOCHCH(CH_3)_2$ functions from $[M-89]^+$ and $[M-117]^+$ ions, respectively. Two ions at *m/z* 81 and 110, present in both spectra, may be rationalized as R^+ and $R\text{-}CH\text{-}NH_2^+$, respectively, indicating that the compound is an α-amino acid of molecular mass

Table 4
Non-protein and protein amino acids found in nuts

No.[a]	Amino acid	Amount (% normalized area)[b]						
		Chest-nut	Ginko nut	Pine nut	Walnut	Hazel-nut	Peanut	Almond
3	Alanine	53.3		3.5	13.5	39.0	17.5	26.8
4	Glycine	13.3	3.9	3.6	5.0	22.7	5.8	28.5
5	α-Aminobutyric acid				0.9		trace	
6	Valine	28.4			9.1	58.7	4.1	
7	β-Alanine			0.7			1.3	
8	β-Aminoisobutyric acid			0.7				
10	Leucine	11.5			5.4	7.1	0.8	trace
11	*allo*-Isoleucine				5.7		0.8	
12	Isoleucine	13.2						
13	Threonine	12.5	trace	1.4	1.3	18.3	2.1	16.9
14	Serine	38.8	17.4	4.4	5.5	39.6	5.3	46.7
16	Proline	34.3	trace	49.3	11.1	17.4	12.3	58.5
17	γ-Aminobutyric acid	24.1		12.7	10.3	6.4	5.8	39.9
19	Pipecolic acid				5.5		35.0	trace
21	Pyroglutamic acid	7.1	8.3	trace	15.6		5.0	8.5
22	4-Hydroxyproline			2.0				
23	Methionine	1.4						
31	Phenylalanine	10.0			5.9			
33	Aspartic acid	10.1	100.0	25.8	25.7	71.3	40.0	100.0
38	Glutamic acid	100.0	56.0	13.9	100.0	100.0	100.0	72.8
39	Asparagine	72.8	17.2	100.0	4.8	40.2	28.6	trace
42	α-Aminoadipic acid			0.9			1.0	trace
43	Glutamine	2.7	38.1	1.3	5.7	15.4		
46	Ornithine				1.5			
49	Lysine	trace			3.8			
50	Histidine	trace			trace			
54	Unidentified						trace	

[a] Same numbers listed in the previous *I* reference library [9].
[b] Percentage of peak area normalized to the largest peak.

155, and that 81 is the mass of the R group.

In the same manner, ions at *m/z* 418, 376, 360, 320, 302, and 276 were proposed to be the characteristic $[M-15]^+$, $[M-57]^+$, $[M-73]^+$, $[M-113]^+$, $[M-131]^+$, and $[M-157]^+$ ions, respectively, for the TBDMS derivative (Fig. 5C), while for the corresponding TMS derivative (Fig. 5D) ions at *m/z* 376, 302, and 274 were proposed to be $[M-15]^+$, $[M-89]^+$, and $[M-117]^+$ ions, respectively, indicating that the unknown compound at peak 53 is a non-protein amino acid of molecular mass 219.

Two unknown peaks 54 and 55 were found in wultari bean, while one unknown peak 55 was detected in mung bean (Fig. 3) and peak 54 in kidney bean and peanut. The ions at *m/z* 574 and 532 for the TBDMS derivative of peak 54 (Fig. 7A) were assumed to correspond to the characteristic pairs of $[M-57]^+$ and $[M-15]^+$ ions formed from the molecular ion of odd mass 589, indicating that the compound is a non-protein amino acid. In the same manner, ions at *m/z* 577, 535, 519, 461, and 435 of peak 55 (Fig. 7B) were proposed to correspond to the characteristic $[M-15]^+$, $[M-57]^+$, $[M-73]^+$, $[M-131]^+$, and $[M-157]^+$ ions formed from the

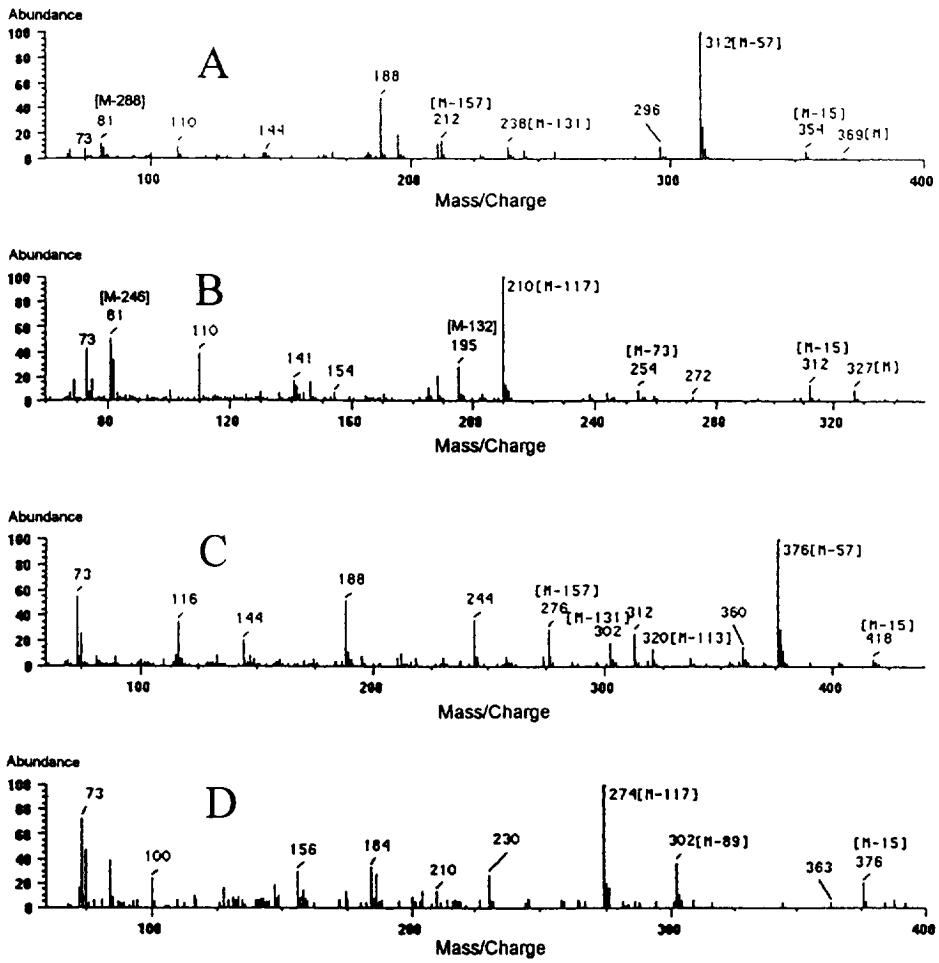

Fig. 5. Electron-impact mass spectra of N(O,S)-isoBOC TBDMS derivatives of (A) peak 52 and (C) peak 53 and of N(O,S)-isoBOC TMS derivatives of (B) peak 52 and (D) peak 53.

molecular ion of even mass 592, indicating that the compound is a non-protein amino acid containing an even number of amino functions

4. Conclusions

The water extraction with subsequent picric-acid treatment and solvent washing by ethyl acetate and diethyl ether allow efficient and rapid isolation of free amino acids into aqueous phase from non-aqueous food samples. When combined with N(O,S)-isobutyloxycarbonylation and *tert.*-butyldimethylsilylation of the isolated free amino acids, the present method provided rapid profiling and screening of nineteen samples for free amino acids. Twenty-eight amino acids including eleven non-protein amino acids were tentatively identified, and four unknown compounds assumed to be non-protein amino acids were detected. The molecular masses of the two unknown amino acids in watermelon seed were estimated by comparing mass spectra of their N(O,S)-isoBOC TBDMS and N(O,S)-isoBOC TMS derivatives. Further investigations on the

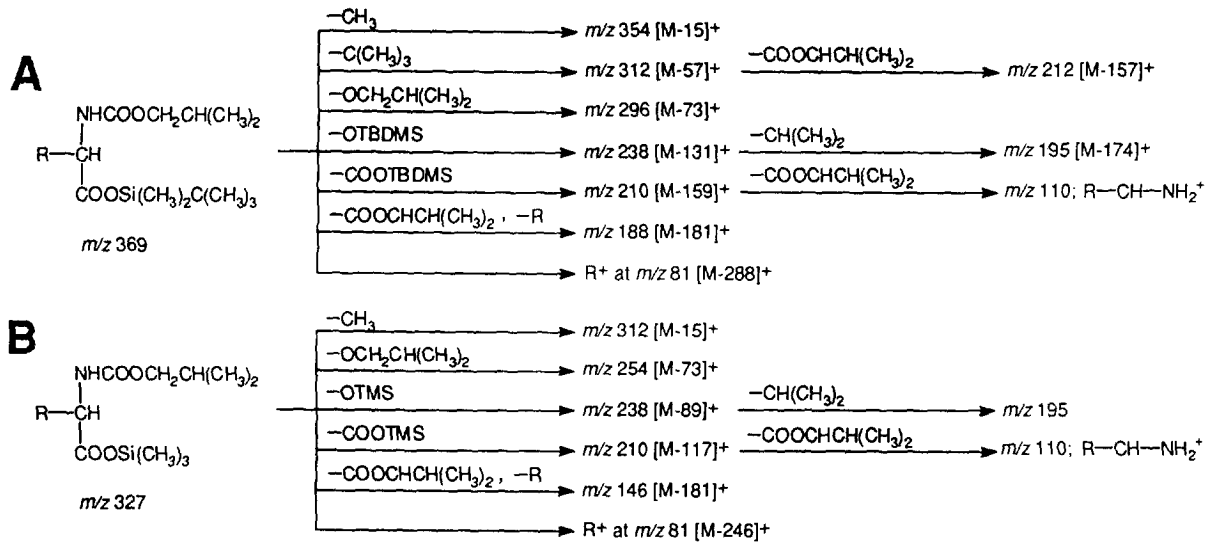

Fig. 6. Proposed mass fragmentation patterns of (A) N-isoBOC TBDMS derivative and (B) N-isoBOC TMS derivative of the unknown peak 52.

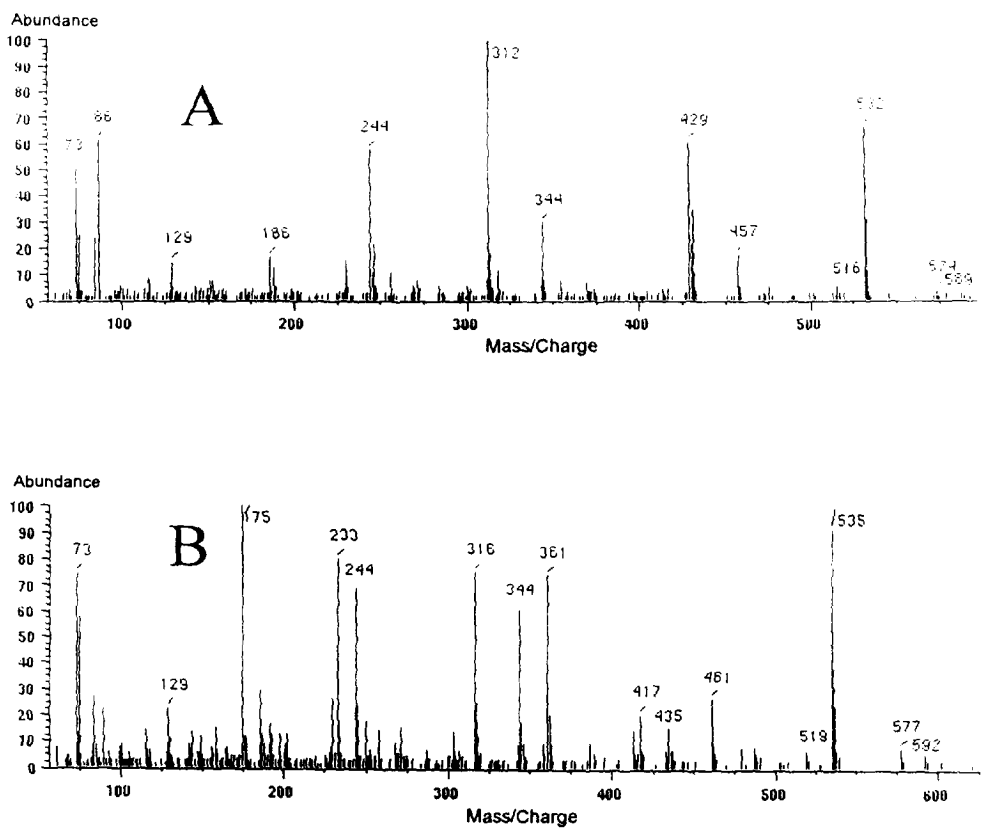

Fig. 7. Electron-impact mass spectra of N(O,S)-isoBOC TBDMS derivatives of (A) peak 54 and (B) peak 55.

positive identification of the unknown peaks are in progress.

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References

[1] E.A. Bell and P.B. Nunn, Biologist, 35 (1988) 39.
[2] M.W. Duncan, I.J. Kopin, J.S. Crowley, S.M. Jones and S.P. Markey, J. Anal. Toxicol., 13 (1989) 169.
[3] S.L. Fowden, in G. Lubec and G.A. Rosenthal (Editors), Amino Acids: Chemistry, Biology and Medicine, Escom Science Publishers, Netherlands, 1990, p. 3.
[4] B. Meldrum, Brain Res. Rev., 18 (1993) 293.
[5] S.L. Mackenzie, in R.E. Clement (Editor), Gas Chromatography: Biochemical, Biomedical, and Clinical Applications, John Wiley, New York, 1990, p. 267.
[6] H. Frank and H. Jaeyer, in H. Jaeger (Editor), Glass Capillary Chromatography in Clinical Medicine and Pharmacology, Marcel Dekker, New York, 1985, p. 73.
[7] H. Brückner and M. Hansh, Chromatographia, 28 (1989) 487.
[8] K.-R. Kim, J.-H. Kim and C.-H. Oh, Anal. Sci., 7 (suppl.) (1991) 231.
[9] C.-H. Oh, J.-H. Kim, K.-R. Kim, D.M. Brownson and T.J. Mabry, J. Chromatogr. A, 669 (1994) 125.
[10] K.-R. Kim, J.-H. Kim, C.-H. Oh and T.J. Mabry, J. Chromatogr., 605 (1992) 241.
[11] F.E. Kaiser, C.W. Gehrke, R.W. Zumwalt and K.C. Kuo, J. Chromatogr., 94 (1974) 113.
[12] D. Labadarios, I.M. Moodie and G.S. Shephard, J. Chromatogr., 310 (1984) 223.
[13] G. Gamerith, G.F.X. Zuder, A. Giuliani and H. Brantner, J. Chromatogr., 328 (1985) 241.
[14] T. Murai and H. Ogata, J. Nutr., 120 (1990) 711.
[15] M. Miyagawa, Y. Tabuchi, K. Yamane, H. Matsuda, S. Watabe, K. Hashimoto, R. Katakai and Y. Otsuka, Agric. Biol. Chem., 54 (1990) 359.
[16] S. Yamamoto, S. Kiyama, Y. Watanabe and M. Makita, J. Chromatogr., 233 (1982) 39.
[17] G. Sarwar and H.G. Bottig, J. Chromatogr., 615 (1993) 1.
[18] G. Fortier, D. Tenaschuk and S.L. Mackenzie, J. Chromatogr., 361 (1986) 253.