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Amino acid determination in some edible Mexican insects

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Summary. The amino acid contents of edible insects from different provinces of Mexico and reference proteins were analysed by reversed-phase high-performance liquid chromatography and ion exchange chromatography. The insect amino acid contents were higher than the adult requirements indicated by the WHO/FAO pattern.

Keywords: Amino acids – Amino acid determination – Edible insects

Introduction

The nutritional value of food largely depends on the quality of the protein it contains. This in turn, is determined to a great extent, by the amino acid composition and digestibility of the protein. The importance of essential amino acids in the diet is emphasized in several studies (FAO, 1957, WHO/FAO, 1965, 1973; Food and Nutrition Board, 1980). They are further supported by the latest report, of the International Committee formed by the Food and Agricultural Organization, the World Health Organization and the United Nations University (WHO/FAO/UNU, 1985). The essential amino acids for human adult nutrition, specified by Munro and Crim (1988) are the following: isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and histidine.

Many conventional food sources which contain essential amino acids, such as eggs, (Lunven et al., 1972) milk and beef (FAO, 1970) are inaccessible to a large portion of the world population. Nevertheless, some uncommon sources form part of the daily diet of many communities all over the world (e.g. Bergier, 1941; Bodenheimer, 1951; Ramos-Elorduy et al., 1982). Among these foods are certain insect species which are eaten in several countries (Quinn, 1959; DeFoliart, 1989; Dufour, 1981; Katz, 1993; Ramos-Elorduy et al., 1984; Ramos-Elorduy and Pino, 1992; Ramos-Elorduy, 1993; Taylor, 1975). Indeed, 1386 edible insect species have been reported: 527 in Africa, 513 in América, 235 in Asia, 83 in Australia and 28 in Europe (Dufour,

1981; Ramos-Elorduy, 1993). They are consumed at all stages of development: as eggs, larvae, pupae or adults, most of them in immature stages. (Meyer-Rochow, 1973; Ramos-Elorduy, 1987a,b, 1993). Sold often by weight or volume, they are also exported to the world's capitals where they become "gourmet dishes" at high prices, (e.g. white agave worm for 250 US Dlls/Kg or the ant eggs "escamoles" for 200 US Dlls/Kg. (Ramos-Elorduy, 1993; Conconi, 1993). In México, insect consumption varies greatly according to the geographical region, village or town and season. Until now 306 species of edible insects have been reported in this country. (Ramos-Elorduy and Pino, 1992).

Among the preferred specimens we find the following: *Liometopum apiculatum* (Hymenoptera-Formicidae) "Escamol": the eggs, larvae and pupae of the reproductive cast are eaten. The flavor being considered similar to nuts, they are cooked with butter and pepper or in omelette. *Myrmecosistus melliger* (Hymenoptera-Formicidae) "Hormiga mielera": certain ants of this species store large quantities of nectar for the colony. The ancient Mexicans used them to produce a sacred wine, the honey being harvested as sweetener (Ramos-Elorduy and Pino, 1989). *Atta mexicana* (Hymenoptera-Formicidae) "Chicatana": the most common edible ant prepared with garlic and parsley or simply roasted. *Brachygastra azteca* (Hymenoptera-Vespidae) "Avispa seguidora": these wasps are eaten widely by the Mayan ethnia They produce much honey (from 2 to 3 Kg/hive). The adult insects are cooked with onion and chili. *Parachartegus apicalis* (Hymenoptera-Vespidae) "Avispa ala blanca": these wasps are eaten complete with hive, including immature stages. They are cooked roasted or in a special sauce producing a flavour that reminds of sesame seed. *Sphenarium purpurascens* (Orthoptera-Acrididae) "Chapulín": eaten at the stages of larvae and adults. They are considered delicious when fried. For storage purposes they are dried in large quantities. *Hoplophorion monograma* (Homoptera-Membracidae) "Periquito de aguacate": these are eaten raw or cooked, fried, served with avocado. *Ascalapha odorata* (Lepidoptera-Noctuidae) "Mariposa del Muerto": eaten only at larval stage. Preserved for many months dehydrated in large amounts. *Ephydra hians* (Diptera-Ephydridae) "Gusano del agua": these are cooked at the larval stage with olive oil and garlic, also called "Mexican eel brood" (Angulas mexicanas).

Insects have been qualified as "protein concentrates", their content ranging from 30% in wood worms, to 81.6% in the wasps of *Polybia* sp. (Ramos-Elorduy et al., 1982; Conconi, 1993), their digestibility being from 33% in *Atta mexicana* to 96% in *Cossus redtembacheri*. They tend to have low concentrations of structural carbohydrates: 3 to 4% in immature stages and 15% in adults (Ramos-Elorduy et al., 1981). Their lipids (4.2 to 8.1% in content) are usually unsaturated, resembling olive or sunflower oil (DeFoliart, 1991).

In this study, the amino acid contents of several Mexican insect species are presented in order to demonstrate the characteristics of the technique and in advance to a more comprehensive study. The HPLC technique presented in this study is a modified version of those by Fürst et al. (1990) and Ladrón

de Guevara, et al. (1990). An evaluation of the precision of this method, from sample preparation and hydrolysis to HPLC technique, is included as well herein.

Material and methods

Apparatus

A System Gold liquid chromatographic system from Beckman (San Ramón, Ca, U.S.A.) was combined with A) a model 126 Programmable Solvent Module with an Altex 210A injection valve, B) a Nec PC 8300 Controller System, C) an Integrator (Model 427) and D) a Fluorometer (Model 121) from Gilson (excitation filter 360 nm and emission filter 450 nm); an Ultrasphere XL ODS column (3 μm) 70 mm × 4.6 mm I.D.) (237500), protected by an Ultrasphere XL ODS (3 μm) guard column (237520) both from Beckman.

A speed Vac Concentrator from Savant, a Multi-Flame burner from Fisher, a Multi-Block heater (No. 2093) from Lab-Line Instruments, Inc. (Melrose Park, ILL, USA) and a PHM84 research pH meter from Radiometer-Copenhagen were used. Samples and solvents were filtered through 0.22 μm porous membranes (XX3001200 and GVWPO4-700) from Millipore (Milford, Ma. USA).

Reagents

HPLC grade water was obtained using deionized water filtered through a Norganic Cartridge from Millipore (Cat. No. CC 1512000). Methanol (HPLC Grade) was obtained from Baker (J. T. Baker, S. A. de C. V., Xalostoc, Mex.), sodium acetate from Sigma (Cat. No. 54386). O-phthaldehyde (OPA) from Beckman (Fluo-RTM part No. 338048); ingredients: water 91.5%, potassium hydroxide 2%, potassium borate 5%, methanol 1%, mercaptoethanol 0.3%, OPA 0.1% and Brij 35 0.1%. Amino acid standard kit 22 was obtained from Pierce (Cat. No. 20065). Certified fish protein CPSP-90 labeled by "Cooperative de traitement des produits de la pêche". (Essential amino acids were determined with an LKB 4400–001 Amino Acid Analyzer). Iodoacetic acid was obtained from Sigma (Cat. No. 1-4386). Hydrochloric Acid (6N) from Pierce (Cat. No. 24309) and Methanesulfonic Acid (4N) (Cat. No. 25600) were obtained from Pierce (P.O.B. 117, Rockford, Illinois 61105 U.S.A.).

Chromatographic procedure

Individual amino acid standard stock solutions (1 μg/ml) were dissolved in water, except for glutamic acid, aspartic acid, and tyrosine, which were dissolved in water with hydrochloric acid (20 μl HCl 6N). A standard mixture of 16 amino acids was prepared at a concentration of 80–150 picomol/ml and stored at 4 °C. This standard mixture was further diluted to 1:10 v/v with iodoacetic acid solution (Cooper and Turnell, 1982) or diluted with water if cysteine was not determined. 50 μl of OPA reagent (Ali et al., 1984; Fürst et al., 1990) in an Eppendorf tube at room temperature were vortex-mixed. After 120s (optimun derivatization time for the reaction) a 5 µl aliquot was injected (3 to 7 picomoles of each amino acid). The OPA amino acid adduct eluted from the column by a gradient elution performed in 45 mins, from 15% to 70% of eluent B in four linear steps (see Fig. 1) at a flow-rate of 1.5 ml/min. The gradient elution program was followed by a 10 min washing step, programmed to 100% B, so that any residual sample components would be cleaned from the column, before and after every run. The column was equilibrated with 10% eluent B for 10 min.

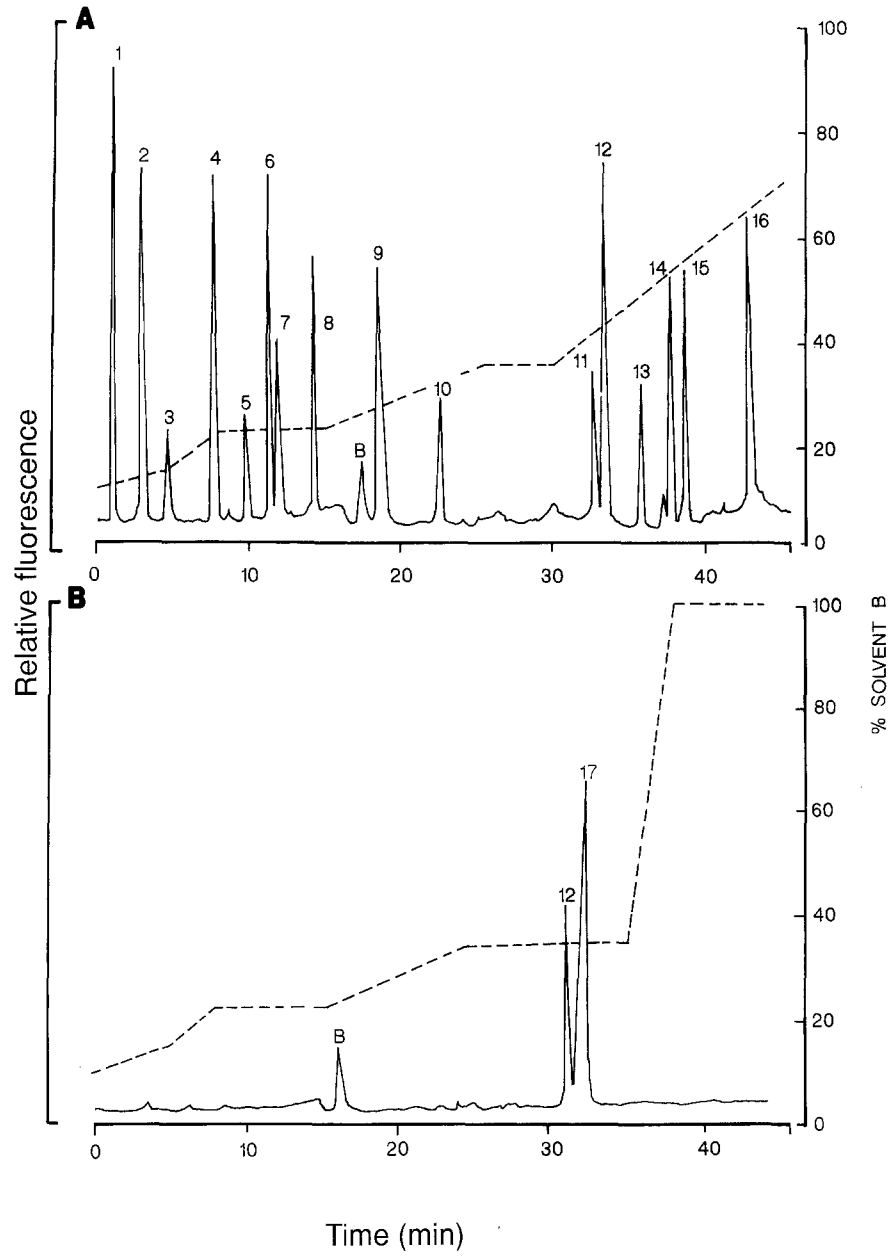

Fig. 1. **A** Chromatogram of an amino acid standard containing 16 amino acids. Peaks: *1* aspartic acid; *2* glutamic acid; *3* cysteine; *4* serine; *5* histidine; *6* glycine; *7* threonine; *8* arginine; *9* alanine; *10* tyrosine; *11* methionine; *12* valine; *13* phenylalanine; *14* isoleucine; *15* leucine; *16* lysine; *B* OPA artifact. Chromatographic conditions are described in the text (see Material and methods). The gradient program used is shown in the record trace. **B** Chromatogram of an amino acid standard containing tryptophan (*17*) and valine (*12*). Chromatographic conditions as in **A** except the eluent *B*; acetonitrile-methanol (75:25); the gradient program used is shown in the record trace

Eluent A was a 50 mM sodium acetate buffer, pH 6.8, and eluent B was methanol, both were degassed by vacuum filtration through a porous membrane (0.22 μm).

For tryptophan determinations a standard mixture of valine and tryptophan was prepared at a concentration of 10–20 picomol/μl and stored at 4 °C. A different run was

required. The same conditions were used except for eluent B, which was methanol-acetonitrile 75:25 from 15% to 100% by a gradient elution performed in 40 min.

The fluorescence intensity of OPA-amino acid derivatives was detected at 360 nm of excitation and 450 nm of emission range 0.05 R.F.U. (Fürst et al., 1990; Cooper and Turnell, 1982). An OPA blank was run before each amino acid standard run. The external standard procedure was employed to quantify the amino acids.

Sample preparation

Insects were collected in different states of the Mexican Republic. They were placed in containers with dry ice and transferred to the Entomological Laboratory at the Institute of Biology, UNAM, where they were dried at 50 °C and grinded until powdered and homogeneized (Horwitz, 1975). A 3 g uniformly blended sample was placed in a pre-weighed Whatman cartridge with 600 ml of hexane in a Soxhlet flask during 10 hours to remove the lipid content. 1 mg freeze-dried insect sample (decolored and without carbohydrates) was hydrolysed with 200 μl of HCl 6N during 20 hours at 110 °C (Moore and Stein, 1963) or 400 μl of methanesulfonic acid 4N for tryptophane (Chiou and Wang, 1988) during 22 hours at 110 °C. Hydrolysis loss of amino acids is minimized, (providing an inert atmosphere, before sealing the sample tube) when the oxygen is excluded by a combination of nitrogen flushing and evacuation (10–15 millitorr). After hydrolysis the HCl was evaporated on the Speed Vac Concentrator. This dried hydrolysate was kept at −4 °C, then it was dissolved in water, diluted 1:10 and filtered through a 0.22 μm. A 50 μl sample was run as mentioned in the Chromatographic procedure. The hydrolysate for tryptophan determination was kept at −4 °C without drying since the methanesulfonic acid is non volatile.

Results

The linearity of the detector response for each of seventeen amino acids and their respective detection limits were measured with the individual standard of every amino acid in the concentration ranges shown on Table 1. Within these concentration ranges, standard curves are linear and therefore the correlation coefficients are nearly ideal.

Fig. 1A,B shows the elution profile of the standard amino acid mixture. Adequate separation was achieved with the best obtained gradient solvent strength (buffer:methanol) for sixteen amino acids (chromatogram A). To improve the separation between valine and tryptophan (chromatogram B) a small change was necessary in the gradient solvent strength (buffer:methanol/acetonitrile).

Most Resolution (Rs) matches were good peak pairs (Table 2). An exception was that of thr/gly that is enough for inequivocal identification (Snyder and Kirkland, 1979).

Table 3 shows the statistical analysis of the RP-HPLC method (comprising all steps from sample preparation to area peak integration) performed only on essential amino acids in order to compare with the certified data of the studied fish protein. Figures 2A,B and 3A,B, and Table 4 present the amino acid composition (essential and non-essential) of two edible insects.

The essential amino acid contents of some edible insects are presented in Table 5 and are compared with the WHO/FAO/UNU (1985) values. It should

Table 1. Linearity of detector response and detection limit for amino acid standards

Amino acid	Range of concentration (pmol/μl)	Regression equation	Correlation coefficient (r)	Detection limit[a] (pmol/μl)
Essential				
Lysine	8.5 – 170	y = 1202X – 2255	0.99994	3.1
Leucine	8.6 – 172	y = 2066X – 3471	0.99998	1.7
Isoleucine	8.4 – 169	y = 2649X – 2357	0.99998	1.5
Methionine	9.6 – 191	y = 2437X + 1711	0.99990	4.5
Valine	12.0 – 245	y = 2588X – 1497	0.99998	2.3
Phenylalanine	7.6 – 151	y = 1930X – 1631	0.99999	1.7
Threonine	14.0 – 279	y = 2211X – 6202	0.99998	2.9
Histidine	8.0 – 159	y = 1717X – 3784	0.99998	2.7
Tryptophan	6.7 – 123	y = 1387X + 540	0.99980	3.3
Non-essential				
Aspartic acid	7.7 – 153	y = 2587X – 1609	0.99999	0.9
Serine	9.0 – 179	y = 2849X – 2977	0.99998	1.6
Cysteine	0.3 – 2.91	y = 19020X – 1532	0.99982	0.1
Glutamic acid	7.0 – 140	y = 2472X – 2109	0.99998	1.5
Tyrosine	8.5 – 170	y = 2380X – 3073	0.99999	1.4
Glycine	17.0 – 343	y = 1943X – 4246	0.99995	5.9
Alanine	15.0 – 310	y = 2295X – 6325	0.99997	4.1
Arginine	7.7 – 154	y = 3647X – 3625	0.99999	1.3

[a] At a signal to noise ratio of 3:1

Table 2. Resolution (Rs) and separation factors (alfa) for 16 OPA-amino acid derivatives (See Fig. 1)

Amino acid	Rs	alfa
Glx[a]/Asx[b]	6.07	2.47
Cys/Glx[a]	4.11	1.60
Ser/Cys	7.60	1.62
His/Ser	6.17	1.27
Gly/His	3.26	1.16
Thr/Gly	1.09	1.04
Arg/Thr	3.58	1.19
Ala/Arg	6.94	1.30
Tyr/Ala	6.56	1.20
Met/Tyr	18.94	1.42
Val/Met	1.47	1.03
Phe/Val	4.86	1.07
Ile/Phe	4.80	1.05
Leu/Ile	2.25	1.02
Lys/Leu	11.69	1.11
Trp/Val	1.69	1.04

[a] Glutamic acid + glutamine; [b] Aspartic acid + asparagine.

Table 3. Statistical analysis of the RP-HPLC method on essential amino acid determination from certified fish protein (CPSP-90)

Amino acid	Found data (g/100 g of protein) (dry basis)	Certified data (g/100 g of protein) (dry basis)
	C.L.	
Lysine	7.3 ± 0.37	7.4
Leucine	6.2 ± 0.35	6.5
Isoleucine	4.5 ± 0.27	4.3
Methionine	2.8 ± 0.16	3.0
Valine	4.8 ± 0.12	4.4
Phenylalanine	3.8 ± 0.16	3.7
Tyrosine	2.9 ± 0.11	2.8
Threonine	4.3 ± 0.25	4.2
Histidine	2.0 ± 0.06	1.7
Tryptophan	0.85 ± 0.10	0.9

C.L. Confidence limit at the 95% significance level (t = 2.78) based on 5 replicates.

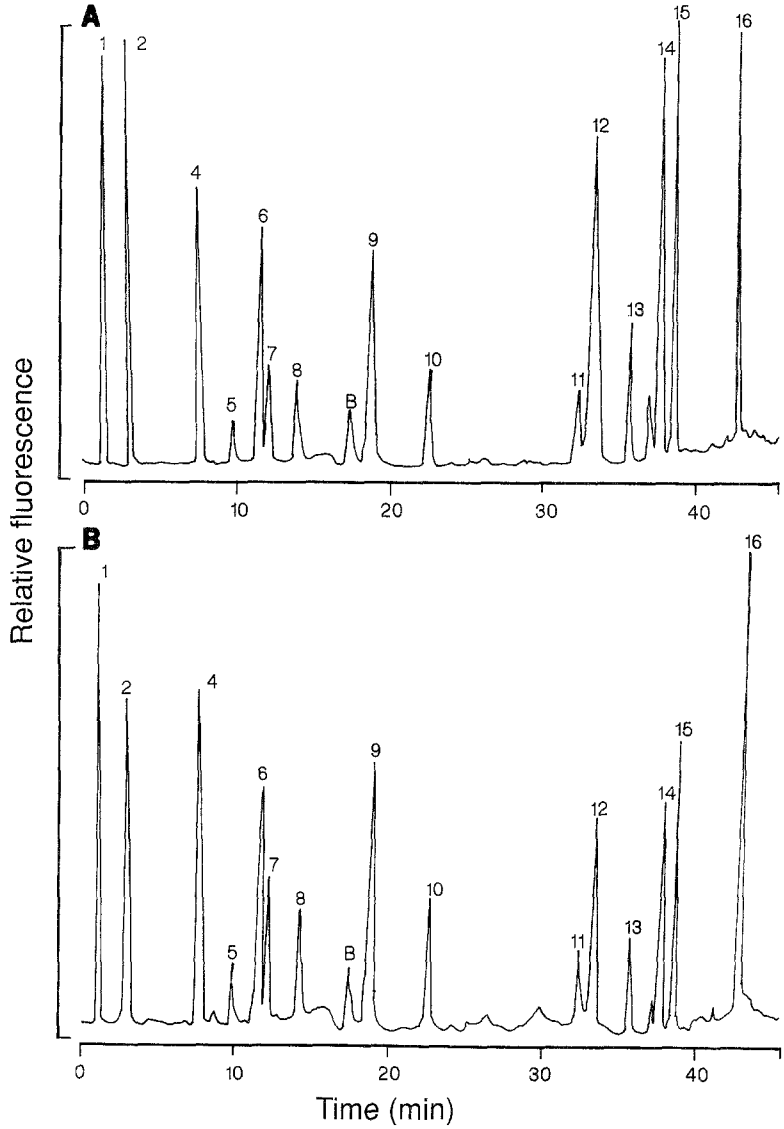

Fig. 2. Chromatograms of two species of edible insects hydrolysate with HCl 6N. **A** Axayacatl; **B** "Gusano blanco de maguey". Peaks are labelled and the chromatographic conditions are as indicated in Fig. 1A

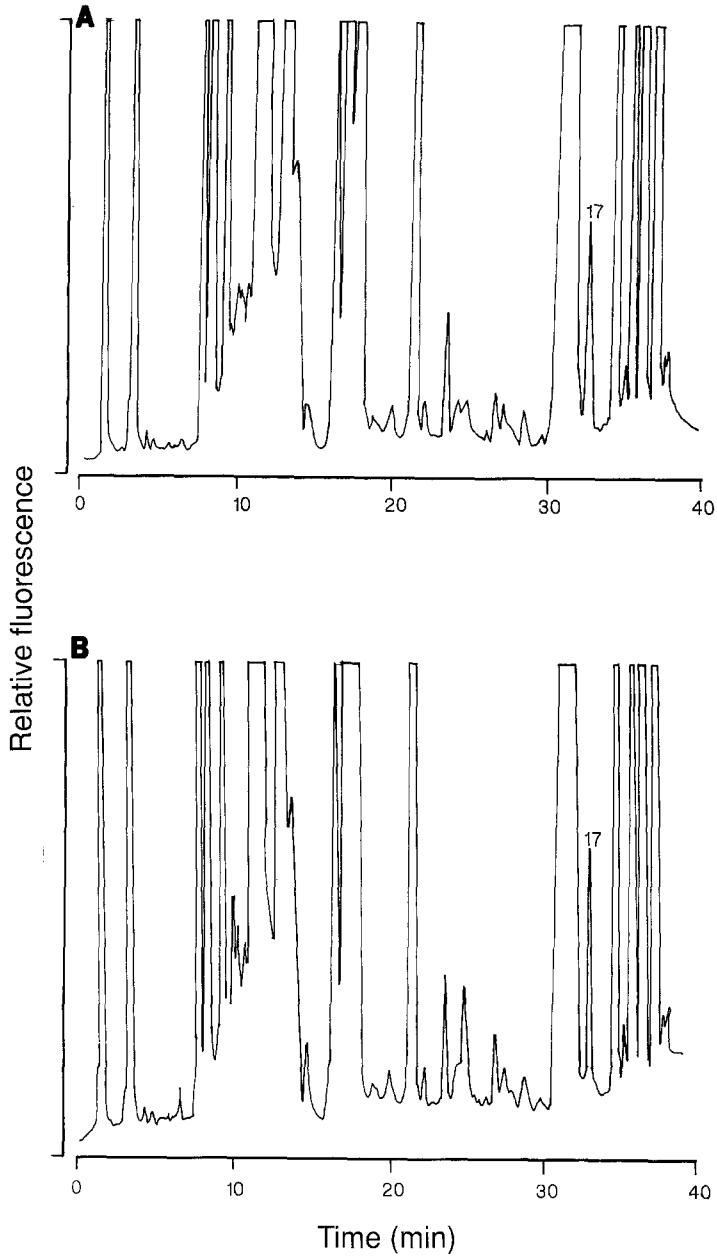

Fig. 3. Chromatograms of two species of edible insects hydrolysate with 4N methanesulfonic acid. **A** Axayacatl; **B** "Gusano blanco de maguey". Peaks are labelled and the chromatographic conditions are as indicated in Fig. 1B

be noted that these differ from those presented in 1973 due to the increase of the safe level of reference protein from 0.57 to 0.75 gram per kg. and that the corresponding requirements of the essential amino acid requirements are reduced.

Table 4. Amino acid content of edible insects from Mexico (g*/100 g of protein) (dry basis)

Order	Hemiptera Complex of different species[a]	Lepidoptera *Aegiale Acentrocneme hesperiaris*
Common name	Axayacatl[b]	"Gusano blanco de maguey"
Consumption Stage	Larvae, adults	Larvae
Protein (%)	61.90	45.30
Fat (%)	8.99	44.20
Ashes (%)	17.83	2.80
Essential amino acid		
Lysine	6.10	6.60
Leucine	8.50	7.20
Isoleucine	4.75	4.60
Methionine	4.30	4.70
Valine	7.20	5.80
Phenylalanine	4.40	5.10
Threonine	4.65	7.60
Tryptophan	1.20	0.30
Histidine	3.00	3.10
Non essential amino acid		
Aspartic acid	9.00	9.90
Serine	6.40	8.30
Glutamic acid	14.20	12.30
Tyrosine	5.80	6.30
Glycine	6.10	5.90
Alanine	8.10	6.60
Arginine	6.30	5.70

g* gram of amino acid; [a] Complex of aquatic bugs from the families: *Krizousacorixa azteca, K. femorata, Corisella mercenaria, Corisella edulis, Notonecta unifasciata;* [b] living in alkaline pond waters.

Discussion

Figure 1 shows a considerable reduction of total elution time. The detection limit of each amino acid given in Table 1, indicates the increased sensitivity from nanomol to picomol level. Results for the Rs and separation factors as given in Table 2 prove the efficiency and the statistical analysis given in Table 3 shows its reliability. The low fluorescence intensity of Cys-OPA derivative is overcome by pre-treating the standard cysteine with iodoacetic acid before derivatization according to Cooper (1982). However, we found good repeatability only with standard determinations and not with protein hydrolisates. We will try to overcome this problem as suggested by Smillie and Nattriss (1991) by reducing the freeze-dried insect sample with betamercaptoethanol in the presence of a denaturant (e.g. 6M guanidine or 8M urea).

Table 5. Comparision of essential amino acid content of edible insects from Mexico against the WHO/FAO/UNU pattern (1985) (g/100 g of protein) (dry basis)

Order	Consumption stage	Protein (%)	Lys	Leu	Ile	Met	Val	Phe / Tyr	Thr	Trp	His
Orthoptera											
Boopedon flaviventris	Nymphs, adults	71.35	5.5	8.8	4.7	1.8	5.7	4.1 7.4	4.4	0.6	2.4
Homoptera											
Hoplophorion monograma	Nymphs, adults	63.77	5.5	7.7	4.1	1.9	7.4	4.7 9.0	4.5	0.96	1.5
Lepidoptera											
Ascalapha odorata	Larvae	56.10	6.3	6.9	4.1	2.3	4.8	9.5 4.4	4.0	0.44	2.8
Diptera											
Ephydra hians	Larvae	60.22	5.5	7.4	4.0	1.9	6.1	5.4 5.1	4.9	0.71	1.0
Hymenoptera											
Parachartegus apicalis	Lavae, pupae	54.59	5.8	7.7	4.2	2.0	5.7	4.3 7.1	4.7	0.62	2.9
Brachygastra azteca	Larvae, pupae and adults	62.74	6.1	8.5	5.1	1.4	6.4	4.1 6.5	4.4	0.70	2.8
Liometopum apiculatum	Eggs, larvae, pupae of reproductive cast	41.66	6.0	8.9	4.4	1.8	4.8	3.5 6.8	3.5	0.62	2.9
Atta mexicana	Adults (reproductive cast)	46.30	4.9	8.0	5.3	3.4	6.4	8.8 4.7	4.3	0.60	2.5
WHO/FAO/UNU 1985			5.8	6.6	2.8	2.5	3.5	6.3	3.4	1.1	1.9

It can be concluded that the complete method described in this paper is simple, rapid, efficient and reliable for the determination of amino acids in insect protein. It was used since 1990 to evaluate over forty edible insect species of 14 orders (Ramos-Elorduy and Pino, 1984). Still, over two hundred classified insect species are awaiting amino acid determination.

In summary, insects can be considered a realistic alternative in the battle against hunger. Since 1885, Holt thought of introducing insects as another edible resource. Presently they can be easily produced industrially at the place where they will be consumed without altering the environment, being independent of weather fluctuation. Indeed controlled culture of insects could provide an endless protein source where others are hard to procure.

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