# APPLICATION OF A SIMPLE METHOD TO THE CHARACTERIZATION AND DIFFERENTIATION OF PROTEIN FOODS

*Julio S. Araújo-Neto,*[1] *Vera L. A. Costa-Carvalho, Mara B. C. Araújo and Gerson F. Pinto*

**Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, Brazil**

SUMMARY

A screening method for the characterization and differentiation of proteinaceous samples and amino acid mixtures was applied to protein foods (4 protein-rich mixtures, one product sold as dietary supplement, and 3 raw materials of frequent use in the preparation of these products). Graphic profiles which describe the relative amounts of amino acid groups in the samples were obtained and subjected to statistical analysis. According to a previously established criterion of identity or difference, the correlation coefficients showed that at least 90% of the comparisons dealt with different samples. The method is proposed as a valuable tool for the quality control of protein-rich foods and their raw materials.

*Manuscrito modificado recibido:* 4—4—79.

1 Present address: Departamento de Engenharia Bioquímica, Escola de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

## INTRODUCTION

A simple and inexpensive method for the identification and differentiation of protein systems and amino acid mixtures without developing the complete aminogram of the sample was recently proposed (1). Since the production of protein-rich mixtures is at present an active field of research in developing countries, we studied the fitness of graphic profiles in controlling products prepared with some common protein concentrates as well as the most used vehicles.

## MATERIALS AND METHODS

### *Materials*

Samples No. 1-5 are manufactured products which circulate in the Brazilian market and have the following main features:

Sample No. 1 (registered trade mark: "Gevral") – A powder with 60% crude protein; each 100 g contain 30 g of soy protein and 30 g of pure casein.

Sample No. 2 (registered trade mark: "Pó de Proteínas, Vitaminas e Minerais") – A powder with 47.5% soy proteins provided by defatted soy meal plus vitamins and mineral salts.

Sample No. 3 (registered trade mark: "Farinha Láctea") – Pre-cooked and milled flakes prepared with milk, wheat meal, sugar, vitamins, and mineral salts; contains 13.5% crude protein.

Sample No. 4 (registered trade mark: "Neston") – A pre-cooked mixture prepared with wheat meal, barley, oats, malt extract, food yeast, sugar, and salts, with a 14% content of crude protein.

Sample No. 5 (registered trade mark: "Cremogema") – A powder based on cornstarch; contains less than 1% crude protein.

Besides these manufactured products, 4 other samples were analyzed:

Sample No. 6 – Cassava meal, as purchased at local supermarkets.

Sample No. 7 – Enriched cassava meal, an experimental protein-rich mixture prepared in our laboratories. Contains cassava meal, 83.8%; dried skim milk, 6%; soy protein isolate (Sanbra), 10%; DL-methionine, 0.2%. The final protein content

(N x 6.25) on a wet basis (2), was 19.9%.

Sample No. 8 – A feed grade fish meal with 80% crude protein (wet basis) produced in Rio de Janeiro.

Sample No. 9 – Hens' eggs as purchased at local supermarkets, 13% crude protein (wet basis).

All samples were dried at 60°C and homogenized.

*Methods*

*Hydrolysis of samples*

Three ml 6N HCl prepared from double distilled analytical grade HCl were added to 4.5 – 5 mg Kjeldahl nitrogen-containing samples in 5-10 ml vials. After shaking gently and heating at 100°C in a water bath for 5 min, the vials were immediately sealed. After completion of this operation, vials were kept at 105°C in an oven for 24 hours. Vials were opened only after returning to room temperature. The hydrolysate was then centrifuged at 800 x g/10 min and 2 ml supernatant were dried on a watch glass in a vacuum desiccator over NaOH pellets and silica gel. The dry residue was suspended in 0.5 ml distilled water and dried again as stated above; the residue was resuspended in 2 ml distilled water and centrifuged at 800 x g/10 min, saving the supernatant and storing at below 0°C.

*Chromatography and quantitative evaluation of spots*

On Whatman No. 1 chromatographic paper (29 cm diameter) 10 µl samples of diluted hydrolysate avoiding spots > 0.5 cm diameter were micropipeted. After a 6-7 hr run (butanol–acetic acid-water, 4+1+1) in a circular chromatographic jar, the paper was dried overnight at room temperature and then sprayed with 0.4% (w/v) ninhydrin solution in acetone. Color was developed in an oven at 75°C for 20 min. Separation of amino acid subgroups is shown in Figure 1. Subgroups were then cut out and each one eluted with 5 ml of 10 mg% alcoholic solution of cupric sulfate. Aqueous solution of copper salt was added to ethanol immediately before use. A piece of chromatographic paper stained without showing reactive compounds was eluted and the eluate used as blank. Elution was carried out by shaking with copper solution in the dark for 30 min, and eluates read at 520 nm.

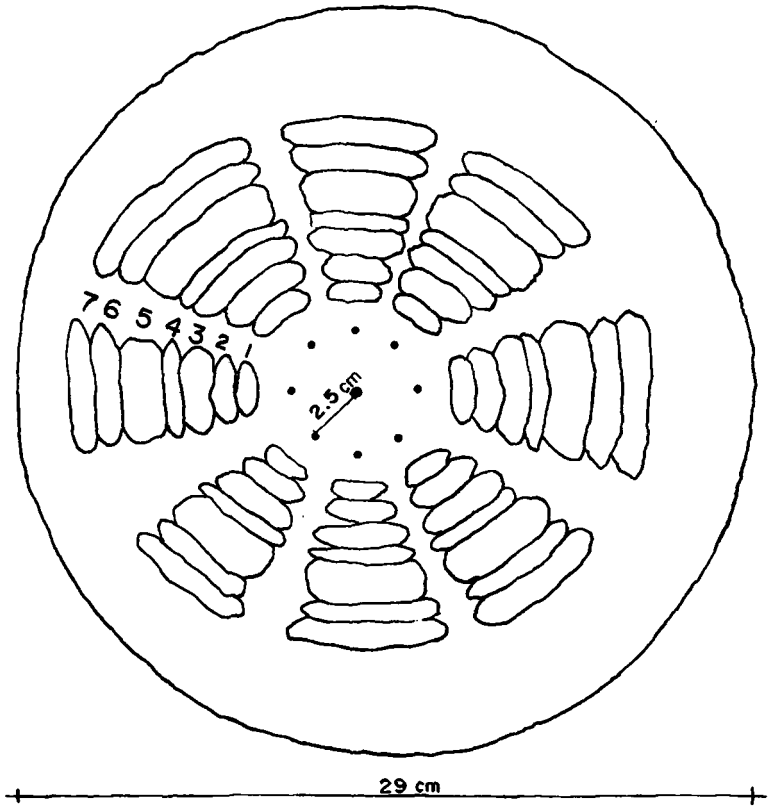



FIGURE 1

**Typical aminogram after development and staining.**

*Subgroups constituents*

Analysis by means of a Beckman Model 121 amino acid analyzer showed the following distribution of major amino acid constituents among the seven subgroups: Subgroup 1: ammonia, 1/2 cys; subgroup 2: arg, his, lys; subgroup 3: gly, ser, asp; subgroup 4: glu, thr; subgroup 5: pro, ala, tyr; subgroup 6: val, met, phe; and subgroup 7: leu, ile, phe.

*Construction of graphic profiles*

The sum of absorbances found for the 7 subgroups in each sample was taken as 100. The value for each subgroup, expressed as a percentage of the total, is used for the construction of tables and graphic profiles consisting of 7 bars, ordered according to increasing $R_f$ value. Each bar is drawn with a height proportional to its percentage value (Table 1 and Figure 2).

TABLE 1

PERCENTAGE OF ABSORBANCE USED FOR THE CONSTRUCTION OF GRAPHIC PROFILES FOR THE STUDY SAMPLES

| | Sample | Subgroup 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 1 | Gevral | 1.9 | 15.0 | 16.6 | 25.8 | 9.6 | 10.2 | 20.8 |
| 2 | Pó de proteínas | 1.5 | 15.2 | 17.0 | 26.7 | 11.6 | 9.4 | 18.6 |
| 3 | Farinha láctea | 1.1 | 9.5 | 15.2 | 34.5 | 9.7 | 10.9 | 19.2 |
| 4 | Neston | 1.4 | 9.0 | 14.8 | 38.7 | 9.8 | 9.7 | 16.6 |
| 5 | Cremogema | 0.0 | 6.9 | 19.6 | 21.8 | 19.7 | 10.7 | 21.3 |
| 6 | Cassava meal | 0.0 | 19.1 | 18.7 | 26.5 | 14.5 | 8.5 | 12.3 |
| 7 | Enriched cassava meal | 1.3 | 14.0 | 18.5 | 25.0 | 7.5 | 13.5 | 20.0 |
| 8 | Fish meal | 1.1 | 14.9 | 16.5 | 22.0 | 12.9 | 14.0 | 18.6 |
| 9 | Hens' egg | 2.1 | 12.7 | 19.7 | 20.5 | 12.8 | 13.7 | 18.5 |
| | Soybean meal* | 1.0 | 16.1 | 18.9 | 26.5 | 10.8 | 10.1 | 16.8 |
| | Casein* | 0.3 | 12.3 | 14.1 | 28.7 | 9.2 | 15.5 | 19.9 |

* Data from Pinto *et al.* (1).

*Statistical studies*

As a criterion of identity or difference, coefficients of correlation were calculated by the following formula:

$$r = \sum (x - \bar{x})(y - \bar{y}) / (N-1) \quad \sigma x \cdot \sigma y, \text{ where}$$

r = coefficient of correlation; $\sigma x$ = standard deviation for

aminogram X; $\sigma_y$ = standard deviation for aminogram Y; N = number of subgroups considered (N=6); x = value of each subgroup in aminogram X; y = value of each subgroup in aminogram Y; $\bar{x}$ and $\bar{y}$ = arithmetic average of the subgroup values in aminograms X and Y, respectively. It should be pointed out that due to the low numerical values and high standard deviations, subgroup 1 has only been used as a helpful semiquantitative aid.

From the statistical analyses performed on several pure proteins and some proteinaceous materials (1), it may be accepted that correlation coefficients $\geq$ 0.960 indicate identity of samples, and that r $<$ 0.960 cause the rejection of the hypothesis of identity between the samples.

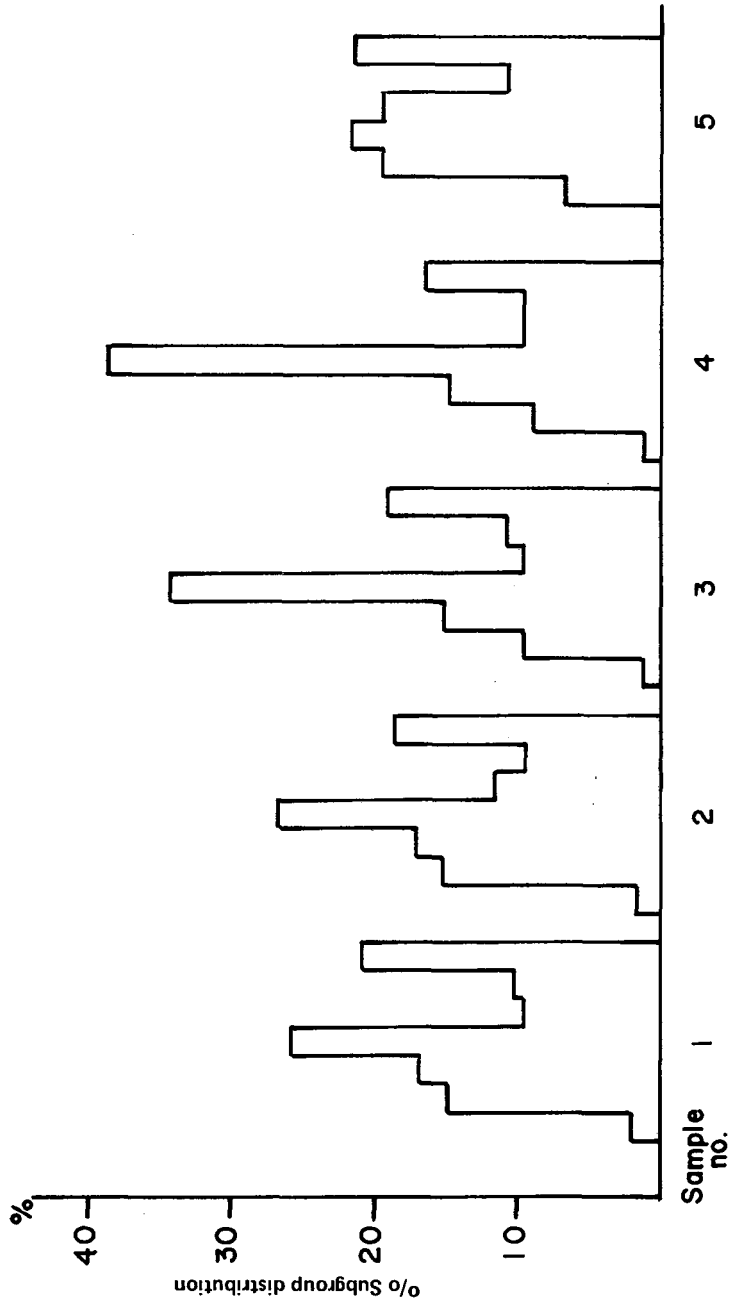
Samples were also compared by superposition of their graphic profiles. Profiles of all samples analyzed in which each subgroup was plotted as its mean value $\pm 2\sigma$ were superposed in such a way that every profile was checked against all others. Each comparison between samples was evaluated in relation to the number of subgroups with coincident values in the range of $\pm 2\sigma$ ; the number of overlapping subgroups may vary from 0 to 6. According to the 190 comparisons reported by Pinto *et al.* (1), if 4 or less subgroups overlap samples are considered to be different; superposition of 5 or 6 subgroups is highly suggestive of identity between samples.

The graphic profiles presented throughout this paper are averages of at least 3 independent hydrolysis operations, each of them analyzed via 8 aminograms developed in a single chromatographic run. According to our experience, valuable data may be obtained by averaging the values from 2 aminograms in the same paper with aliquots of the same hydrolysate. However, when routine used is intended, the parallel analysis of a reference sample is exceedingly useful.

Further details on methodology may be found elsewhere (1).

## RESULTS AND DISCUSSION

The graphic profiles for samples No. 1-9 are shown in Figure 2 (derived from Table 1). Table 2 depicts the coefficients of correlation calculated by comparing each of the samples with all others, and the number of overlapping subgroups after comparing 2 samples according to the "two standard deviation" criterion (superposition of graphic profiles).


%
40
30
20
10
% Subgroup distribution
Sample no.
1
2
3
4
5

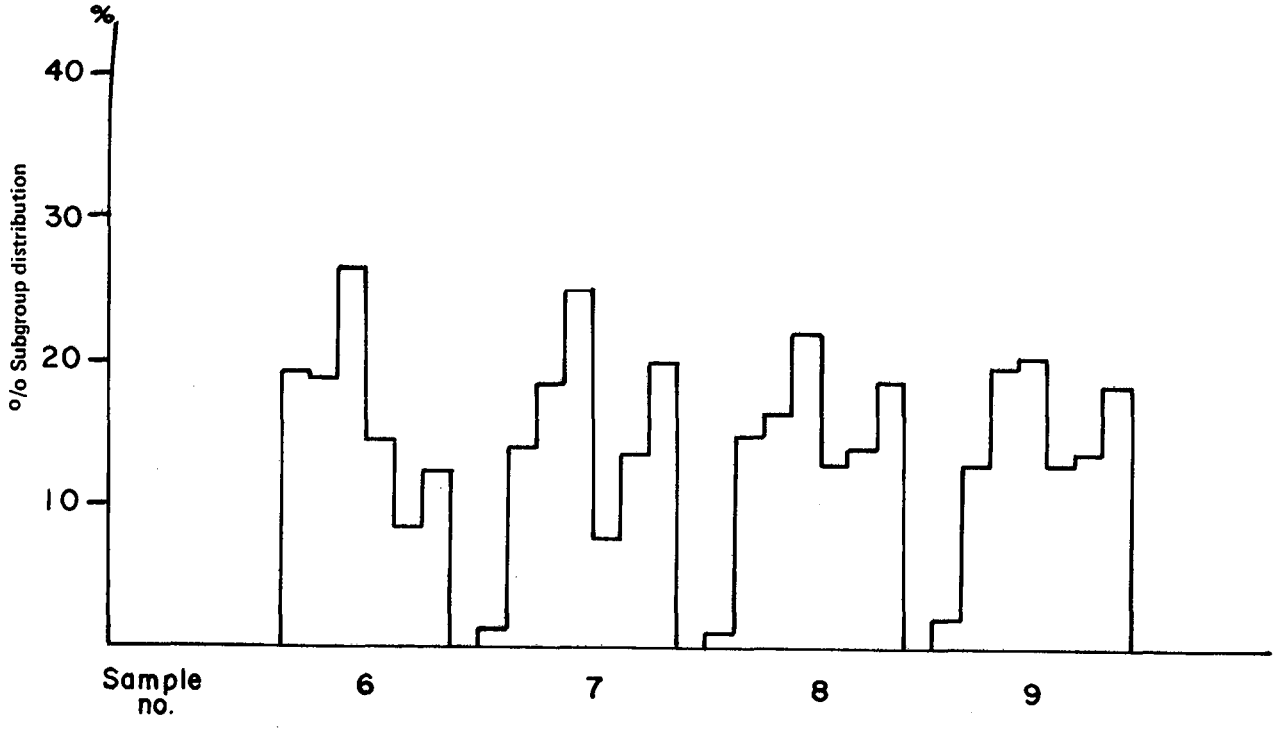



FIGURE 2

Graphic profiles for samples No. 1-9.

TABLE 2

CORRELATION COEFFICIENTS ($r \times 10^3$) AMONG THE 9 SAMPLES KNOWN TO BE DIFFERENT AND NUMBER OF OVERLAPPING SUBGROUPS IN A RANGE OF $\bar{x} \pm 2\sigma$

| | S1* | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 |
|---|---|---|---|---|---|---|---|---|---|
| | $r \times 10^3$ | | | | | | | | |
| S1* | + | 973 | 909 | 868 | 508 | 692 | 944 | 985 | 837 |
| S2 | 6 | + | 929 | 918 | 544 | 825 | 886 | 959 | 810 |
| S3 | 4 | 4 | + | 990 | 606 | 684 | 876 | 958 | 808 |
| S4 | 3 | 4 | 5 | + | 571 | 747 | 827 | 921 | 756 |
| S5 | 3 | 2 | 2 | 1 | + | 277 | 424 | 547 | 718 |
| S6 | 3 | 3 | 1 | 1 | 2 | + | 574 | 656 | 514 |
| S7 | 4 | 4 | 2 | 0 | 3 | 2 | + | 960 | 892 |
| S8 | 3 | 4 | 3 | 2 | 2 | 2 | 4 | + | 861 |
| S9 | 3 | 4 | 2 | 1 | 3 | 2 | 4 | 5 | + |
| | Number of overlapping subgroups | | | | | | | | |

* S1. . . . 9 refer to samples No. 1-9 as listed in Table 1.

Table 3 summarizes the per cent of correct and false-identical results when previously published data (1) and those obtained in the present study were analyzed according to the correlation coefficient and the "two standard deviation" criteria.

According to the correlation coefficient criterion, 32 out of 36 comparisons between 9 truly different samples (88.9%) have depicted this difference. However, comparisons of 8x2 and 8x3 were judged on the basis of the 3rd decimal figure and the difference between the samples is very questionable. If the 3rd decimal figure in all coefficients of correlation is rounded off, two more comparisons would lead to false results, and among 36 comparisons only 30 (83.3%) would demonstrate the actual real difference. The sum of false results (11.1% or 16.7%) is somewhat higher than when we compared pure proteins, microorganism hydrolysates, protein concentrates, etc. (11.1% or 16.7% x 2.6%); this difference may have arisen from diverse sample sizes since in the first study (1) we compared 20 samples, performing

TABLE 3

DATA DERIVED FROM THE APPLICATION OF TWO DIFFERENT CRITERIA TO TWO SETS OF SAMPLES KNOWN TO BE DIFFERENT

| Sets of samples | Criterion | | | |
|---|---|---|---|---|
| | Correlation coefficient* | | Two standard deviations** | |
| | Results | | Results | |
| | Correct | False identical | Correct | False identical |
| According to Pinto *et al.* (1) | 97.4°/o | 2.6°/o | 90.5°/o | 9.5°/o |
| Present paper | 88.9°/o | 11.1°/o | 91.7°/o | 8.3°/o |

* $r < 0.960$, different samples.
$r \geq 0.960$, identical samples.

** Superposition of 4 or less subgroups, different samples.
Superposition of 5 or 6 subgroups, identical samples.

190 comparisons. Furthermore, the samples now analyzed show two strong reasons for being similar: (a) each protein-rich mixture is formulated based on aminograms close to human amino acid needs; (b) some raw materials frequently appear as the major component in different commercial products.

Samples 3 and 4, for example, have wheat meal as their major component. According to the manufacturer's description, both have more than 50°/o wheat meal in their formula. Since wheat meal protein as a whole is one of the richest sources of glutamic acid (3), graphic profiles are greatly characterized by subgroup 4, and comparison of samples No. 3 and No. 4 render a high correlation coefficient. This fact explains the false similarity observed when we compared 3x4 (r=0.990).

Samples No. 1 and No. 2 also present a high level of similarity as both have soybean proteins as their major component. Unfortunately, soy proteins and casein, raw materials widely used for protein-rich mixtures, show a great resemblance when analyzed by the present method (Figure 3). Indeed, these facts

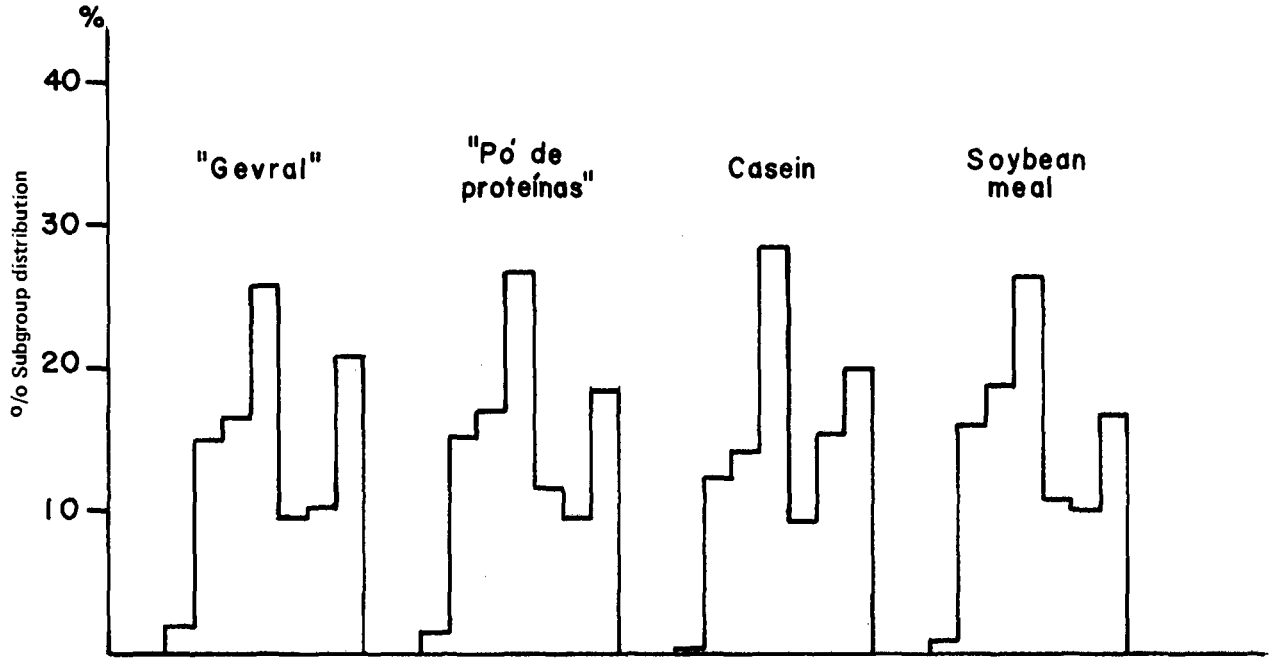



FIGURE 3

**Graphic profiles for soybean meal, casein, and protein-rich mixtures "Gevral" (major components: casein + soy proteins) and "Pó de Proteínas" (major component: pure soybean meal).**

are responsible for the increased percentage of false results in the present study but do not constitute general problems.

Interesting results have been derived from superposition tests. When comparing 20 samples (190 comparisons) we found 2.6°/o false results (according to the correlation coefficient). If 4 or less subgroups were superposed, samples always proved to be different by the coefficient of correlation criterion; 90.5°/o of the truly different samples presented 4 or less overlapping subgroups (Table 3). In the present study, 91.7°/o of the comparisons showed 4 or less overlapping subgroups, while correlation coefficients indicate that 83-89°/o of the comparisons lead to correct results (Table 3). These facts demonstrate that the correlation coefficient ($r \geqslant 0.96$ corresponding to identical samples) as well as superposition tests (identical samples presenting more than 4 overlapping subgroups) provide similar information. Therefore, both criteria provide valuable information.

These results seem very interesting and the number of successful comparisons suggests that the present method may be quite useful. In spite of some very similar formulae, as a rule, protein-rich mixtures and foodstuffs present different graphic profiles, correlation coefficients lower than 0.96 and 4 or less overlapping subgroups. Nevertheless, when formulae are very similar, false results may arise, but if a vehicle is changed, a protein concentrate is omitted or a strange meal is present, this method is largely suitable for revealing the alterations either if derived from error or from an intent to deceive.

It must be emphasized that the figure of 0.96 as a threshold value between identical and different samples is a general one. Manufacturers and authorities can develop other limits of acceptance or rejection through a number of analyses of several batches of the products under consideration or according to experiments in which the contents of the major raw materials are varied.

## CONCLUSIONS

The increasing importance of protein-rich mixtures in programs oriented towards a better nutrition will cause an increasing diversification in their formulae and an increasing need for more reliable methods of quality control. Indeed, developing countries lacking sophisticated systems for quality control will be most

involved with this problem and methods which do not depend on high-cost apparatus or skillful technicians may acquire great importance for government audit.

Protein-rich mixtures cannot be judged only by Kjeldahl analysis and sensorial examination. Thus, through error or intent to deceive, some manufacturers may omit raw materials or add foods of low biological value and, after some "corrections", obtain the same total nitrogen content and similar organoleptic characteristics. With the exception of expensive analytical techniques (such as the use of amino acid analyzers), this method appears to be a valuable tool for industrial quality control and government audit purposes. As shown by Pinto *et al.* (1) and confirmed in this study, there are few chances of taking different samples as identical or viceversa. Destruction of amino acids by hydrolysis procedures will not affect the reliability of the method. Indeed, the data used for constructing the graphic profile which is typical of each sample already takes into account the constant degree of amino acid destruction caused by standardized hydrolysis conditions.

If governmental authorities request from every industry that launches a new protein-rich mixture the graphic profile developed for the first batches as well as the corresponding statistical study, in the future many mistakes or adulterations would be easily detected by government audit. On the other hand, after developing their standard graphic profiles for every protein-rich mixture produced and their raw materials, manufacturers would have a rapid method for controlling not only the raw materials purchased but every new batch of each product as well.


## RESUMEN

### APLICACION DE UN METODO SENCILLO PARA LA CARACTERIZACION Y DIFERENCIACION DE ALIMENTOS PROTEINICOS

Con el objeto de caracterizar y diferenciar muestras proteínicas y mezclas aminoacídicas, se aplicó un método de separación a alimentos proteínicos (4 mezclas ricas en proteínas, un producto que se vende como suplemento dietético y 3 materias primas de frecuente uso en la elaboración de estos productos). Se obtuvieron perfiles gráficos (histogramas) que describen las cantidades relativas de grupos aminoacídicos en estas muestras, los que

luego fueron analizados estadísticamente. De acuerdo con criterios de identidad o diferencia establecidos previamente, los coeficientes de correlación mostraron que al menos 90% de las comparaciones se referían a diferentes muestras. Se propone el método como una herramienta valiosa para el control de calidad de alimentos ricos en proteínas y de sus materias primas.

## BIBLIOGRAPHY

1. Pinto, G. F., V. L. A. Costa-Carvalho, E. R. Souza & J. S. Araújo-Neto. A screening method for protein characterization and differentiation. **JAOAC, 59**: 584-590, 1976.
2. Araújo-Neto, J. S. Food staples as vechicles for protein concentrates. **Nutr. Reps. Internat., 9**: 85-90, 1974.
3. Orr, M. L. & B. K. Watt. **Amino Acid Content of Foods.** Washington, D. C., U. S. Department of Agriculture, 1957, 82 p.