ELECTROPHORESIS ON CELLULOSE ACETATE BAND FOR A COMPARATIVE STUDY OF COWPEA (VIGNA UNGUICULATA) AND SPIRULINA (SPIRULINA PLATENSIS) PROTEINS.

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Abstract — Electrophoresis is usually applied in serum protein characterization, we performed a study using this technic in the characterization of proteins in cowpea and spirulina to see the proteins content of the two species in a comparative purpose. Proteins are essential ingredients for good health. Their deficiency results in diseases which the must known is the kwashiorkor or marasmus which treatment consists essentially of a protein intake enabling to fill this observed deficit, malnutrition being a real public health problem especially in the least developed countries.

For each species we used three samples. After extraction, proteins were characterized by the biuret reacting and by electrophoresis on cellulose acetate band. With the latter method we found two protein fractions N1 and N2 for cowpea and S1 and S2 for spirulina. The dosage of proteins was carried out by the spectrophotometric method using the Biuret reagent and by densimetry after electrophoretic migration on a cellulose acetate band. These methods allowed to find a most important protein level for Spirulina (SD 0.54 g). The results obtained allow to confirm the applicability of the technic of electrophoresis in the characterization and dosage of vegetable proteins. It is also worth noting the sensitivity of the electrophoretic method which makes it possible to distinguish two identical molecular weight fractions for the two species while the biuret method gives the total protein level.

Index Terms — Electrophoresis, Protein, *Vignia unguiculata* (cowpea), *spirulina platensis* (spirulina).

1 INTRODUCTION

Electrophoresis is usually applied in protein characterization specially in serum protein [1, 2], we performed a study using this technic in the characterization of proteins in cowpea and spirulina to see the proteins content of the two species in a comparative purpose. Proteins are essential ingredients for good health in an individual. Their deficiency results in diseases of which the must known is the kwashiorkor or marasmus whose treatment consists essentially of a protein intake enabling to fill this observed deficit. Crisis in developing countries where animal resources are increasingly scarce, protein deficiency is often observed in young children. To remedy this situation, a policy of introducing into the diet of the latter a species of algae, the spirulina known to be rich in proteins, has been implemented [3, 4].

In this context we lead this study to verify the protein composition of the two plant in order to confirm the possibility to apply electrophoresis technic in protein quantification in various domains as food. in parallel we used the colorimetric technic to confirm the results

obtained with electrophoresis technics.

The awareness campaign on the use of spirulina as a food supplement has aroused the interest we have focused on the algae; We were interested in the proteins contained in it [5, 6, 7]. However, the problem is that the latter is not available locally, so we have considered it necessary to study at the same time the proteins of cowpea, grown in large quantities, accessible to all social strata, and deemed rich In protein [8].

2 MATERIAL

2.1 Apparatus

For the study, these equipment were used

- Electrophoresis tank,
- centrifuge
- visible UV spectrophotometer,
- densimeter.

2.2 Reagents

The reagent used are:

- Phosphate buffer solution pH 7,
- Sodium chloride solution 1M,
- Veronal buffer solution pH 8.8,
- Biuret reagent,
- Albumin solution (25 g/L)

3 METHODS

3.1 Extraction

- Extraction of proteins from cowpea flour

We used three samples of the same mass. To do this, we introduced 5 g of cowpea flour into a plastic container with a screw cap with a nominal capacity of 50 ml, to which we added 20 ml of phosphate buffer pH 7 and 10 ml of NaCl 1M.

After stirring for 60 min, the mixture obtained is filtered under vacuum and the filtrate collected.

- Extraction of spirulina proteins

We proceeded in the same way as for cowpea by introducing 5 g of spirulina, 20 ml of phosphate buffer pH 7 and 10 ml of 1M NaCl into a plastic container equipped with a screw cap with a nominal capacity of 50 ml. These ingredients were stirred with a magnetic stirrer for 60 min. The mixture thus obtained is of viscous texture. Separation of the two soluble and insoluble phases will be carried out by centrifugation at 3000 rpm for 30 min. The supernatant, containing the protein fractions of spirulina, was collected in a plastic container.

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3.2 Characterization

For the characterization, we used two methods:

- a colorimetric method using the Biuret reaction and,
- electrophoresis on cellulose acetate band (ddp = 200 V, 35 min).

4 RESULTS

4.1 Protein characterization by the Biuret réaction

For characterization by the Biuret method we obtained a blue violet complex characteristic of the peptide chain of the proteins

4.2 Calibration curve

The absorbance measurements of the calibration range, carried out using a spectrophotometer at 550 nm, gave the results indicated in Table 1. The volumes of the albumin solution taken will range from 0.20 ml to 0.45 ml for a total volume of 2 ml.

Table 1: Absorbances etalon range

N°	1	2	3	4	5	6	7
$C_{Albumin}$ (g/l)	0	2.5	3.12	3.75	4.37	5	5.62
Absorbance	0	0.14	0.19	0.23	0.26	0.29	0.34

The calibration curve (fig. 1) is obtained by expressing the absorbance as a function of the albumin concentration.

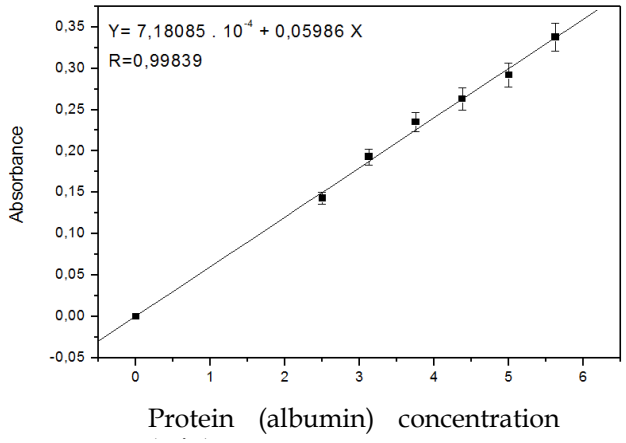

Fig. 1: Calibration curve for the determination of protein concentration.

4.3 Colorimetric assay of cowpea protein

After stabilization of the coloring, the assay using the spectrocolorimeter gave the results contained in Table 2.

Table 2: Absorbance of the three extracts of "cowpea"

Samples	1	2	3
Absorbance	0.269	0.272	0.241

The calibration curve was used to determine the protein concentrations of the three samples, this allowed to find an average concentration of 43.42 ± 0.222 g/l. The average extraction yield is 26.05%.

4.4 Colorimetric assay of spirulina protein

After stabilization of the coloring, the assay by spectrocolorimetric method gave the results contained in Table 3.

Table 3: Absorbances of the three spirulina extracts

Samples	1	2	3
Absorbance	0.345	0.342	0.335

The calibration curve allowed to calculate the protein concentrations of the three spirulina samples, which permited to find an average protein concentration equal to 56.76 ± 0.058 g/L. For spirulina, the extraction yield is 34.05% on average.

4.5 Electrophoresis of cowpea protein

After migration, it can be seen in fig. 2 that there are two fractions for each deposit, a fraction whose migration distance is on average 0.9 cm; And another that did not migrate (0 cm).

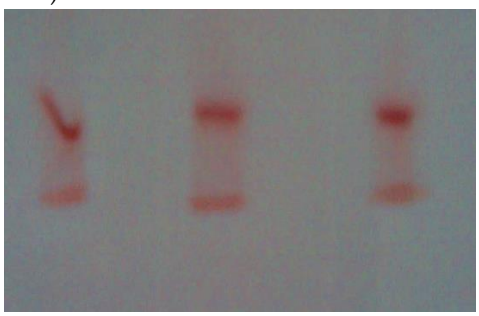

Fig. 2 Electrophoregram of the cowpea extract.

- Electrophoretic mobility

The electrophoretic mobility of the fraction that migrated was calculated using the relationship: Up = v / E = vd / U, where v = displacement velocity, d = distance between the two electrodes and U = difference of applied potential [7, 8].

$v = 4.28.10^{-6}$ m/s and $1 / E = d / U = 0.07 / 200 = 3.5 \times 10^{-4}$ m/V

The average electrophoretic mobility of this fraction is equal to:

$Up = 1.498.10^{-9}$ $m^2/s/V$.

- Quantification of proteins at the densitometer

In fig. 3 we have the photometric curve of the electrophoregram of the cowpea extract obtained after reading at the densitometer.

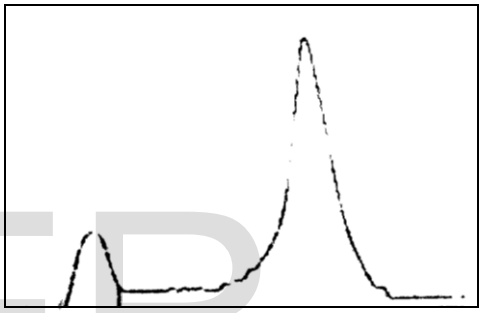

Fig. 3 Photometric curve of the cowpea extract electrophoregram

4.6 Electrophoresis of spirulina proteins

After migration, we also observed two protein fractions whose migration distances are 1 cm and 0 cm on average for the deposits (fig. 4).

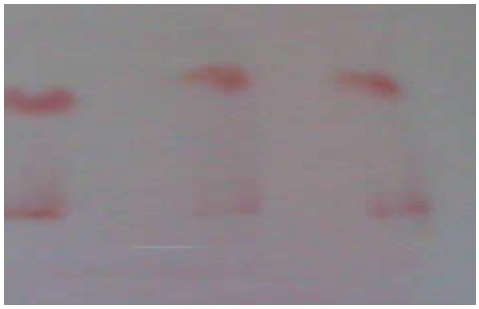

Fig. 4 spirulina extract electrophoregram

- Electrophoretic mobility

The average electrophoretic mobility of the protein fractions of the spirulina extract is: $Up = 2.16 .10^{-9}$ $m^2/s/V$.

- Quantification of proteins at the densitometer

The same principle used for cowpea is adopted for spirulina. The photometric curve obtained is shown in fig. 5.

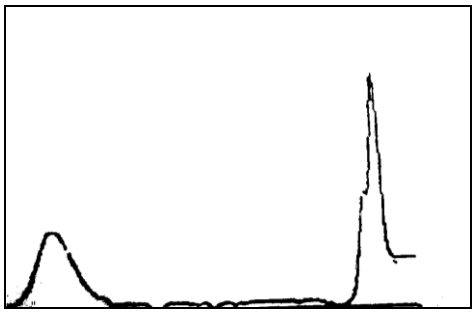

Fig. 5 Photometric curve of the spirulina extract electrophoregram

5 Discussion

This work, which consisted in the determination of proteins in two plant species, was based on the observation that in developing countries nutritional deficiency in proteins constitutes a real public health problem [9-11].

Food is a rare commodity and for some localities, food habits have made food unbalanced, as in Senegal where rice is the basis of almost all foods. The use of other cereals such as millet, sorghum, maize is not extensive and only a small proportion of the population uses them.

It was on this basis that a media campaign was launched on the properties of spirulina which can be used as a dietary supplement because of its high protein content [2, 3].

For our part, we thought of carrying out the extraction, characterization and dosage of the proteins contained in the spirulina that came to us from Burkina, and to associate with the study, the cowpea that is grown locally and whose production does not need Important means.

Extraction allowed to obtain yields that are 26.02% on average for cowpea and 34.03% on average for spirulina, a much higher yield for spirulina than for cowpea.

For the characterization of proteins, several methods are used, among which we have retained two, namely the so-called Biuret method and that using electrophoresis on cellulose acetate band.

For characterization by electrophoresis on cellulose acetate band, the potential difference applied between the two electrodes of the electrophoresis cell was set at 200 volts and the migration time at 35 minutes. Electrophoresis showed the existence of two proteins N1 and N2 for cowpea and S1 and S2 for spirulina. The calculated migration rates gave the following results: $6.19.10^{-6}$ m/s for spirulina and on average $4.76.10^{-6}$ m / s for cowpea. Since electrophoretic mobility is a function of velocity, we can say that the protein content of spirulina is higher than that of cowpea proteins, which suggests a lower molecular weight for the protein fraction of spirulina that has migrated.

For characterization by the Biuret method we obtained a blue violet complex characteristic of the presence of the peptide chain of the proteins.

Quantitatively, analysis of N1 and N2 fractions of the electrophoregram of cowpea proteins at densitometer revealed grades which averaged 15% for the first fraction not migrating N1 and 85% on average for the second fraction having migrated N2, whereas for spirulina, the contents are respectively 40% for the fraction S1 which has not migrated and 60% for the fraction S2 which has migrated. In this case, the protein density is slightly higher for the N2 fraction than for the S2 fraction and the opposite for the N1 and S1 fractions that have not migrated. This suggests that the N2 fraction of cowpea is much higher in protein than the S2 fraction of spirulina.

For quantification by the Biuret method, the wavelength of the absorption maximum of the complex formed varies as a function of the number of bonds, this wavelength becomes stable at 550 nm. Measurement of the optical density of the different samples and calculation of the concentrations from a calibration curve yielded the following results:

- for cowpea: 43.42 g /L on average, ie 1.16 g of protein per 5 g of cowpea flour,

For spirulina: 56.76 g /L on average, ie 1.70 g of protein per 5 g of spirulina.

In view of the results we can say that the protein content is slightly higher for spirulina than for cowpea, with a difference on the values found of 0.54 g in favor of spirulina.

6 Conclusion

This study of cowpea and spirulina has enabled us to confirm the presence of proteins in appreciable quantities in these extract, even though the levels are much higher for spirulina than for cowpea.

Thus, high protein leguminous cowpea, well assimilated in our dietary habits, available in large quantities in Senegal, can be a good source for a satisfactory amount of protein

intake due to its regular consumption.

In addition to the proteins studied, a much more extensive study on the identification of fractions, especially the N2 and S2 fractions and the search for other constituents present in cowpea may be of particular interest for infant nutrition, which poses many problems in countries developing.

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