Research Article

Study of the Proteins in Flour of Two Food Legumes: Peanut (*Arachis hypogea L*) and Cowpea (*Vigna unguiculata L).*

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Received: 30-10-2016; **Revised:** 08-01-2017; **Accepted:** 18-01-2017.

ABSTRACT

The rate of total protein and excerpts of each protein fraction are determined by multiplying the nitrogen content by a factor 6.25 according to the method of determination of nitrogen from kejdhal. Carbohydrates are evaluated by spectrophotometer at 625nm. The isoelectric-points are specified by pH precipitation. The temperatures at which proteins are denatured are obtained by heating. The amino acid composition is determined by high performance liquid chromatography (HPLC) after acid hydrolysis by HCL 6N to 110 °C for 18 hours, and basic hydrolysis with NaOH4N at 100 °C for 24 hours. Mw is estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). Results revealed that the pHi of albumins, globulins, prolamins and glutelins of peanut as well as those of albumins and globulins of Cowpea is acidic. Protein Cowpea highlighted groups are more thermo resistant. Cowpea proteins have high concentrations of polar and apolar amino acids (aspartic and glutami acids, tyrosine, lysine, arginine, valine, isoleucine, leucine, alanine and glycine). For peanut, this fraction rich in aspartic and glutamic acids, tyrosine, phenylalanine, proline, Isoleucine, alanine and glycine is deficient in sulfur amino acids and histidine Amounts of polar and apolar amino acids and the glyganic part of the peanut albumins are lower than those of Cowpea albumins. The abundance of polar and apolar amino acids and cysteine, as well as the important glyganic part are responsible of the thermo rsistance of Cowpea albumins. With the exception of cysteine, concentration of polar and apolar amino acids (aspartic and glutamic, arginine, serine, histidine, isoleucine, leucine, alanine, glycine, and valine) of Cowpea globulins are superior to those of peanut globulins. The presence of cysteine, wealth in polar and apolar amino acids and hight glycosylation explains the thermo resistance of Cowpea globulins. Comparison between water and salt soluble proteins of two food legumes studied highlights that cowpea albumins have the highest number of components compared to peanut proteins.

Keywords: Peanut -Cowpea – fractionation - amino acids -legumes. Glycans, molecular weights, thermo resistance.

INTRODUCTION

Legumes are among the most studied plants[1-3]. They are the plant family that provides the greatest number of useful species to man, be they food or industrial or medicinal[4-6]. They are considered one of the solutions to malnutrition affecting nearly 70% of the world population whose majority is located in developing countries[7]. Several legumes are a major source of protein and oils plant [8]. They are widely grown in the world for their economic and food importance. The bean (*Phaseolus vulgaris*), soybean (*Glycine max*), pea (*Pissum sativum)*, chickpea (*Cicer arietinum*), Cowpea (*Vigna unguiculata*) and peanut (*Arachis hypogea*) are widely consumed [9]. Peanut (Arachis hypogea) belongs to the family of the papilionaceae[10-12]. It is a source of proteins, lipids, vitamins and mineral salts[13]; the seeds contain 25% to 30% protein. These are classified into three groups, albumins (15%), the globulins (70%) and the glutelins (10%) [14-18]. The conarachine is made up of two components, I and II, of respective molecular masses of 142 and 295 kDa. It is found mainly proteins .of globulins 7S and 11S (viciline and glycinine) and the 2S albumin [19.20]. The Cowpea (*Vigna unguiculata L*) is an annual autogamous[21]. The Green pods and seeds are consumed in various form [22]. The seed contains 20.42-34.60% of proteins[23-25]. Protein fractions made up of globulins, albumins, prolamins and glutelins[26.27]. The globulins fall up into two groups: vicilines (fraction 7 S) and the legumines (fraction 11S). These two classes of proteins consist of several polypeptides, which are described in detail.[28-30] With the exception of sulfur amino acids which are deficient (methionine and cysteine), it is encountered (find) in the seeds of these two food legumes essential amino acids required by man [28.31]. Physicochemical and functional properties of protein fractions are in relate with amino acids composition and three-dimensional structure [32]. In this context of food legumes, that fits this work devoted to the fractionation, determination of the amino acids composition and the behavior of protein groups at temperature indefatted peanuts and Cowpea flours.

MATERIALS AND METHODS

Defatting and samples preparation

Fifty grams of raw peanut and Cowpea seeds are ground finely a mill Analytical IKa Mill 11 CA (staufen, Germany) in order to have meals. Flours (50g) are after treated with 500 ml of hot hexane in a Soxhlet Extractor for 8 hours [33]. Defatted flours are dried in the open air and then stored at 4 °C. The fractionation of proteins is accomplished

according to the diagram presented by Chen and Bushuk, 1970[34].

Determination of physicochemical parameters

The rate of total protein and excerpts of each protein fraction are determined by multiplying the nitrogen content by a factor 6.25 following the method of the (A.O.A.C) as described by Kjeldahl[35]. Carbohydrates are carried out by the anthrone method [36]. For I.p, acetic acid 0.1N or NaOH 0.1N are added progressively and carefully to protein solutions until the precipitation of the protein demand. The values are indicated on pH meter. Concerning the denaturing temperature, the test tubes containing the proteins are introduced in a water bath adjusted at 30 °C, temperature is increased by 1 °C each 3 minutes until to the precipitation of proteins. All parameters were measured in triplicates. All results were analyzed based on a comparison of the mean and an analysis of variance (ANOVA). Significance was considered at $p< 0.05$ using MiniTab software [Minitab, Ltd, United Kingdom (Version 16)].

Determination of the amino acids composition of the protein fractions

According to the method of Ozol[37], amino acid composition was determined after acid hydrolysis by HCL 6N to 110 °C for 18 hours with 4 M NaOH at 100 °C for 24 hours. The alkaline hydrolysis is mainly applied to calculate the tryptophan content, the separation by high-performance liquid chromatography (HPLC) using detection by cyanides of O-phtadialdehyde derivatives (OPA), is performed using two columns in series of C18 water guy of length equal to 10 cm. Particles diameter is 5 um. A pre column is placed at the beginning of each column. The amount loaded is 10µl and the flow rate of 0.5 ml/min.

Determination of the relative molecular masses by SDS-PAGE

The technique used is that of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) using discontinuous buffer described by lammeli [38, 39]. The gel dimensions are 100 x 100 x 10mm. 0.1 mg of protein to analyze are solubilized in an adequate volume of sample buffer (0.0625M tris HCL, pH=6.8, 2%SDS, 10% glycérol, 0.002% bleu de bromophénol and 5% B mercaptoéthanol). After homogenization, the tubes are placed in a boiling water bath for 5 minutes. After centrifugation at 10000t / 5 min, the supernatants are applied (loaded) in the gel[40]. The electrophoresis is carried out at 29.2mA and 180V for 8 hours. She is arrested one hour after the release of methylene blue;at the end of the migration, the revelation of the protein bands is taken. The stainig is done by placing the gel in a tray containing a 1.5 ml of blue of Coomassie R250, 125ml of methanol 50%, 50ml of glacial acetic acid 10% adjusted to 500ml with distilled water. The revelation of the tapes (bands) is complete after 24 hours. Discoloration of the gel is performed after a night by contact gel stained with a solution made up of 125ml of methanol 50% and 50ml of acetic acid 10% adjusted to 250ml with distilled water. The evaluation of molecular weights is carried out by using standard protein marker.

RESULTS AND DISCUSSION

Table 1 concern the comparison of the physicochemical properties of proteins extracted from the defatted flours of peanut and Cowpea. It is shown that P-values calculated by the analysis of the variance in criterion of fixed model classification are null ($P< 0.0001$).There are differences very highly significant differences between each variable data. According the results of figures 01 and 02, which shown that the rate of total proteins in the defatted peanut and Cowpea flours are successively 22.3% and 21.8%. This last percentage is comparable to those of *Cicer arietinum* (23.9%,) and *Phaseolus vulgaris* (20%) and differ from those of *Vicia faba. L* (27.7%) and *Lens culinaris* (27.6%) obtained by Comai *and al*; 2007, Tresina and al2011, Cai *and al* (2002) and Bravo et al 1999[41-44]. For peanut flour, globulins, albumins, prolamins and glutelins rates are : 72.73%, 16.59%, 1.65% and 1.32% compared to the total protein respectively. Concerning the Cowpea Flour, the globulins and proteins represent consecutively 65.59% and 21.56% compared to the total protein. Values revealed by Mahjan *and al*, 1988[45]for Vigna mungo globulins differ from those of the studied variety. They are close to the data presented for albumins. The fractionation of proteins allowed highlighting different protein groups. It is interesting to note that for DD. M Ragab 2004[24], there are albumins (71%), the globulins (11.1%), prolamins (2.20%) and the glutelins (11.1%), for Shoshima *and al*, 2005[46], there are albumins (10.11%), the globulins (41.90%), and for Sathe Venkatachalan, 2007[47], in *Vigna aconififolia,* it is find albumins (5.06%) and glutelins (27.83%). The differences between the results are due to genotypes and the agro-climatic conditions. The Figures 3, 4 and 5 do seem the iso electriques points, denaturing temperatures and amounts of carbohydrates of protein extracts. The pHi of albumins, the globulins, prolamins and glutelins of peanut as well as those of cowpea albumins and globulins are consecutively 5.20, 4.43, 4.87, 4.71, 4.6 and 4.5.IT is shown that the Ip are acidic. It is revealed the concordance of findings of peanut and Cowpea with those,published by Mondoulet 2005, Allesaandra and al2003. and CY Arem.1989 [48.49.50]. Denaturing temperatures of albumins, of globulins, prolamins and glutelins of peanut as well as albumins and the globulins of Cowpea, and the amounts of carbohydrates of albumins, the globulins, prolamins and glutelins of peanuts such as cowpea albumins and the globulins are successively: 59 °C, 64 °C, 50 °C, 52 °C, 78 °C and 82 °C and 6.1mg/ml, 7.741mg/ml, 4.263mg/ml, 5.741mg/ml, 8.656mg/ml and 12.967mg/ml. Cowpea Protein highlighted are the most thermoresistant groups they have the most important glycanic part. The glycan moiety increase thermal resistance by contracting hydrogen bonds with some amino acids residue of the peptide chain, which has the effect of rigidify the protein

structure. In table 2 are mentioned the relationships between physicochemical parameters of peanuts and Cowpea proteins. The correlation coefficient between temperature denaturation and the amount of carbohydrate is r=+ 0.934. Resistant protein has an important and considerable glycanic part. Thanks to glycan-protein binding, there is preservation and maintenance of spatial structure of the proteins. Table 3 gives the amino acids composition of the proteins studied. Cowpea albumins have high concentrations in polar and apolar amino acid viz : (aspartic and glutamic acids, tyrosine, lysine, arginine, valine, isoleucine, leucine, alanine and glycine), For peanut, this fraction rich in aspartic and glutamic acids, tyrosine, phenylalanine, proline, Isoleucine, alanine, and glycine is poor and deficient in sulfur amino acids and histidine. Quantities of polar and apolar amino acids and the glycanic part of the peanut albumins are lower than those of cowpea albumins. The abundance of polar and apolar amino acids and cysteine, as well as the important part glycanic are responsible for the thermoresistance of cowpea albumins. With the exception of cysteine, concentrations in polar and apolar amino acids (aspartic and glutamic acids, arginine, serine, histidine, isoleucine, leucine, alanine, glycine, and valine) of cowpea globulins are superior to those of peanuts globulins. The richness of the globulins in polar amino acids established hydrogen bonds and hydrophobic interactions performed by hydrophobic groups, the presence of cysteine and high glycosylation explain the thermal resistance of cowpea globulins. It is specified when the protein is rich in hydrophilic and hydrophobic amino acids and cysteine, with a considerable rate of carbohydrates have high denaturing temperature. The S-S bridges, hydrogen bonds, hydrophobic interactions and glycosylation gives to the molecule a compact conformation which is resistant to opening and the bending of the space arrangement of peptide chains under heat. Figures N 7 and N 8 shows electrophoretic diagrams of proteins isolated from the defatted peanut and cowpea flours. The peanut albumins have three fractions. Mw are successively 14.61 KD, 24.35 KDa and 36.45KDa. For the cowpea seeds, it is observed six. bands of Mr 10KDa, 12KDa, 13.57KDa, 15.41KDa et39.8KDa 27KDa. It is revealed that the number of peanuts albuminic fraction is lower than those of Cowpea. Pedalino and al, 1990[51], note that albumins of the Brazilian varieties of *Vigna unguiculata* are made up of six strips which Mw are equals to 16KDa ,19KDa, 27KDa, 30KDa, 33KDa and 81KDa. Nwanga and al., 2000 [52] detected 4 components of Mw 27KDa 33KDa, 62KDa and 87KDa in Cameroon IT 81 D 985 and IT 87 D 1676 varieties. These differences are due to genotypes and agro climatic conditions. Mw of the peanuts globulins are 14.51KDa, 24.15KDa, 25.18 KDa, 37KDa, 41.66KDa, 47.35KDa and 75.65KDa, Cowpeas are 10.84KDa, 12.02KDa, KD, 35KDa, 36.5KDa 25 and 38.5 KDa. Pedalino and al., 1990 reported that the globulins are made up of compounds varying from 49KDa to 63KDa. Mw of the globulins of the two pulses are low and different. The comparison between water and salt soluble proteins of two food legumes studied brings out that cowpea albumins have the highest denaturing temperature, glycanic part and number of components compared to peanut albumins. They are richer in polar and apolar amino acids and cysteine. The cowpea globulins have denaturing temperature and the most important glycanic part compared to peanuts globulins. On the one hand, they are richer in polar and apolar amino acids and in other poor in cysteine.

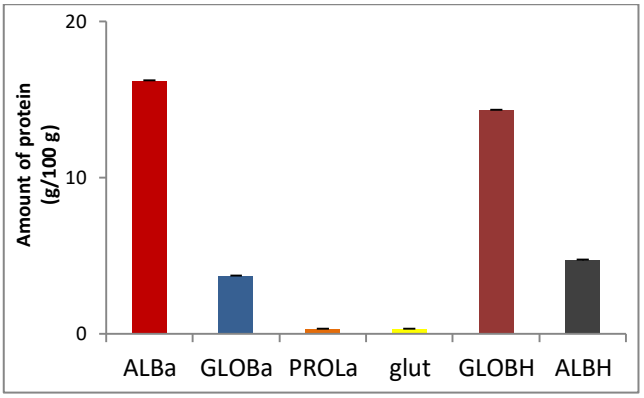
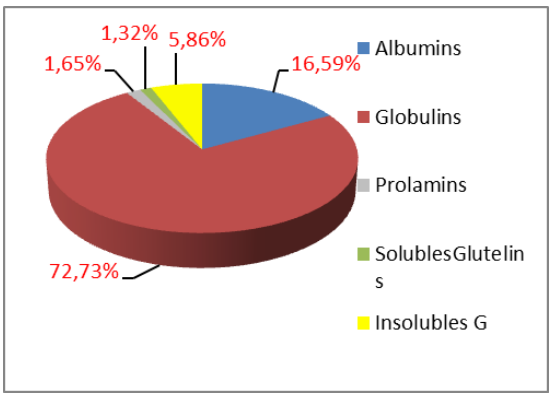


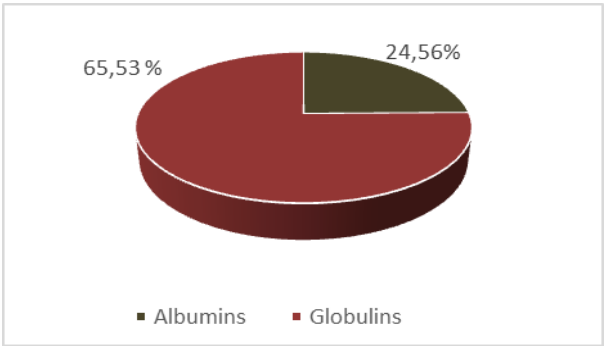
Figure 1: Amounts of proteins in protein extracts from defatted peanut and cowpea flours.

Proteins extracted from peanut flours: Albumins (ALBa), globulins (GLOBa), prolamins (PROLa), soluble Glutelins (glut).

Proteins extracted from cowpea flours: Globulins **(globH**), Albumins **(albH)**



(A)



(B)

Figure 2: Rate of proteins extracted from defatted peanut (A) and cowpea (B) flours.

Table 1: Comparison of the physicochemical parameters of extracted proteins from defatted peanuts and cowpea flours.

Parameters	Factorial differences			Residual differences			Statistics
	ddl	SCEf	CMf	ddl	CMr	CMr	F obs
Iso-electric point	**5**	1.068	0.214	12	0.067	0,006	37,982***
Denaturation Temperature (°C)	**5**	2032,000	406,400	12	18.00	1,5	270 ,933***
Amount of proteins (g/100g)	**5**	730,576	146,115	12	0,001	0,000	2619594,801***
Amount of carbohydrate (mg/ml)	**5**	140,360	28,072	12	0,000	0,000	3609260,157***

NS: no significant differences; **Highly significant differences; *Significant differences; ***Very highly significant differences.

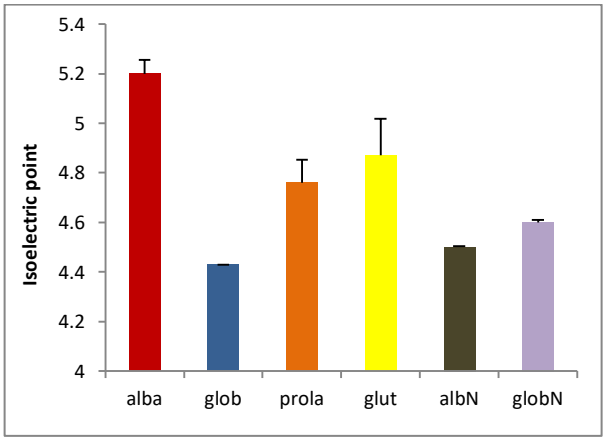


Figure 3: Isoelectric points of protein extracts from defatted peanut and cowpea flours.

Proteins extracted from peanut flours
Albumins (alba), globulins (glob), prolamins (prola), soluble Glutelins (glut).
Proteins extracted from cowpea flours
Globulins **(globN)**, Albumins **(albN)**

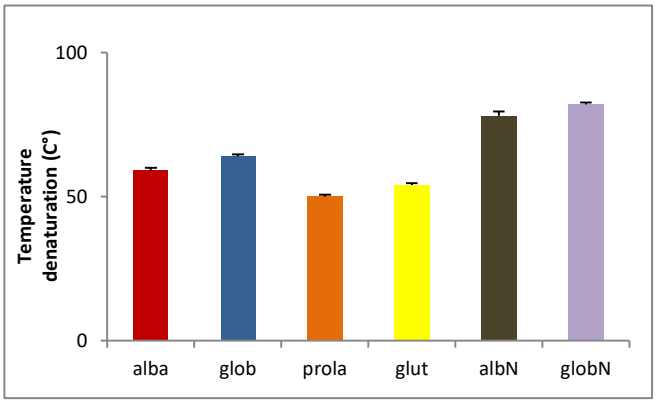


Figure 4: Temperatures denaturation of protein extracts from defatted peanut and cowpea flours.

Proteins extracted from peanut flours
Albumins (alba), globulins (glob), prolamins (prola), soluble Glutelins (glut).
Proteins extracted from cowpea flours: Globulins **(GLOBH)**, Albumins **(ALBH)**

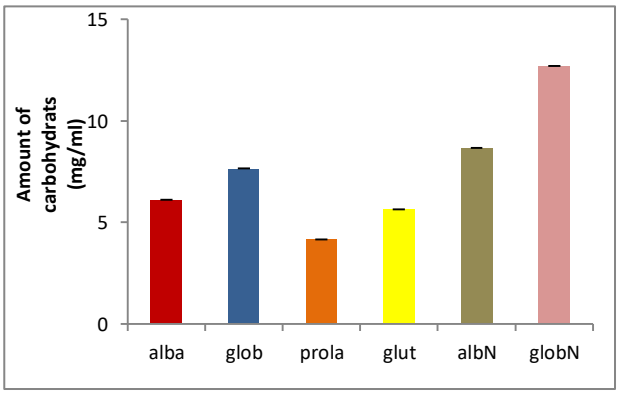


Figure 5: Amount of carbohydrates of protein extracts from defatted peanut and cowpea flours.

Proteins extracted from peanut flours

Albumins (alba), globulins (glob), prolamins (prola), soluble Glutelins (glut).

Proteins extracted from cowpea flours

Globulins **(globN)**, Albumins **(albN).**

Table 2: Relationships between physicochemical parameters of proteins extracts from defatted peanut and cowpea flours.

variables		Probability (P)	Correlation (r)	Coefficient of determination R %	Equation liner regression	Statistics
X	Y					Fobs
Amount of carbohydrates (mg/ml)	Temperature denaturation (C^0)	0,006**	+ 0,934	87,3%	y= 3,615x+38,61	28,356**
Amount of proteins (g/100g)	Temperature denaturation (C^0)	0,503NS	0,345	/	/	/
Isoelectric points	Temperature denaturation (C^0)	0,289NS	-0,521	/	/	/
Amount of carbohydrates (mg/ml).	isoelectric points	0,348NS	-0,469	/	/	/
Amount of proteins (g/100g)	isoelectric points	0,418NS	0,411	/	/	/
Amount of carbohydrates (mg/ml).	Amount of proteins (g/100g)	0,342NS	0,474	/	/	/

NS: no significant differences; **Highly significant differences; *** Very highly significant differences; *Significant differences

Table 3: Amino acids composition of the different proteins extracts from defatted peanut and cowpea flours.

Foods	Peanut (*Arachis hypogea L*)				Cowpea (*Vigna unguiculata L*)	
Pf / Aa (mg/g)	Albumins	Globulins	Glutelins	Prolamins	Albumins	Globulins
Aspartic acid	9.2	63.4	41.5	6.1	44.06	107.95
Glutamic acid	20.3	107.3	65.8	4.3	94.86	209.20
serine	5.1	36.2	20.2	0.5	3.63	36.33
proline	17.3	11.2	5.3	1.1	17.96	13.28
glycine	11.2	31.8	13.7	1.4	22.68	30.20
Histidine	0	11.8	7.4	0.2	4.77	20.80
Threonin	1.9	10.2	6.8	0.5	1.44	0.65
Arginine	1.8	74.6	38.5	0.4	40.20	74.80
Alanine	6.2	20.2	17.4	1.7	17.40	35.40
Cystein	0	10.7	3.8	1.0	0.23	1.65
Tyrosin	5.1	22.3	16.2	0.8	14.98	22.23
Valine	3.1	21.7	16.2	0.5	16.40	60.76
Methionine	0	7.3	4.1	0.2	-	-
Tryptophane	0.8	6.3	4.8	0.5	-	-
Phenylalanine	36.8	9.5	5.9	0.1	1.06	2.63
Isoleucine	4.5	26.3	16.3	2.2	15.65	49.61
Leucine	1.2	37.7	25.5	0.2	17.75	100.92
lysine	16.5	18.1	11.3	0.3	11.3	18.1

Pf: Protein fraction; **Aa:** Amino acids.

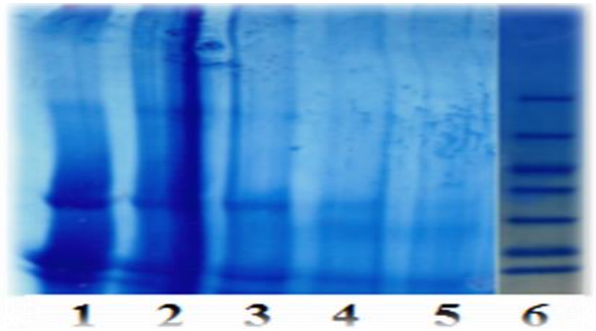


Figure 7: SDS PAGE of proteins extracted from defatted cowpea flours.

1et 2 : defatted crude extract, 3 :Albumins, 4 et 5 : Globulins, 6 : standard proteins marker (*116KDa, 66.2KDa 45KDa, 35KDa, 25KDa, 18.4KDa and 14.4KDa).*

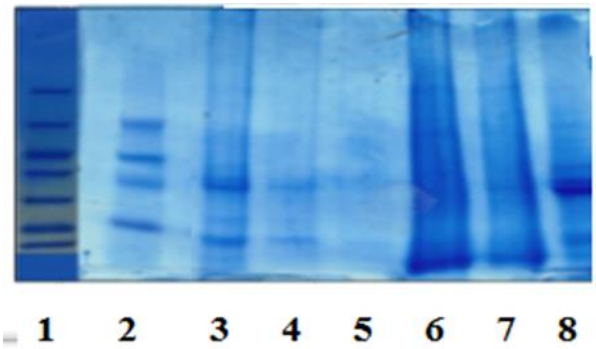


Figure 8: SDS PAGE of proteins extracted from defatted peanut flours.

1: standard proteins marker *116KDa, 66.2KDa 45KDa, 35KDa, 25KDa, 18.4KDa and 14.4KDa).*2 :deffated crude extract, 3: Globulins, 4: Albumins, 6: Prolamins, 8: Gluthelins soluble in CH3COOH 0.05N.,

CONCLUSION

The I.p of albumins, globulins, prolamins and the glutelins of peanut as well as those of cowpea glutelins and albumins are acidic, Protein groups highlighted of cowpea are most thermo resistant. Cowpea albumins have high concentrations in polar and apolar amino acid viz : (aspartic and glutamic acids, tyrosine, lysine, arginine, valine, isoleucine, leucine, alanine and glycine). For peanut, this fraction rich in aspartic and glutamic acids, tyrosine, phenylalanine, proline, Isoleucine, alanine, glycine is poor in sulfur amino acids and histidine. Quantities of polar and apolar amino acids and the carbohydrate part of the peanut proteins are lower than those of cowpea albumins. The abundance of polar and apolar amino acids and cysteine, as well as the important and considerable glycanic part are responsible for the thermoresistance of cowpea albumins. With the exception of cysteine, concentrations in polar and apolar amino acids (aspartic and glutamic acids, arginine, serine, histidine, isoleucine, leucine, alanine, glycine, and valine. The presence of cysteine, wealth in polar and apolar amino acids and high glycosylation explain the thermoresistance of cowpea globulins. The comparison

between the water and salt soluble proteins of the two food legumes studied, identified that cowpea albumins have the higher number of components compared to peanut albumins.

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Source of Support: Nil, **Conflict of Interest:** None.