GRASAS Y ACEITES, 63 (3),
JULIO-SEPTIEMBRE, 253-259, 2012,
ISSN: 0017-3495
DOI: 10.3989/gya.072511

INVESTIGACIÓN

Biochemical evaluation of protein fractions from physic nut (*Jatropha curcas* L.)

By **L. Peralta-Flores**[a], **S. Gallegos-Tintoré**[a], **J. Solorza-Feria**[a], **G. Dávila-Ortíz**[b], **L. Chel-Guerrero**[c] and **A. Martínez-Ayala**[a, *]

[a] CEPROBI-IPN Carretera Yautepec – Jojutla Km 6, Calle Ceprobi 8, Apdo. Postal No 24. Yautepec, Morelos, México C.P. 62731
[b] Departamento de Graduados e Investigación en Alimentos de la Escuela Nacional de Ciencias Biológicas (IPN). Prolongación de Carpio y Plan de Ayala 11340 México, D.F
[c] Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Av. Juárez # 421 Cd. Industrial, Apdo. Postal. 1226-A, 97288 Mérida, Yucatán, México
*Corresponding author: alayala@ipn.mx; almarayala@hotmail.com

RESUMEN

Evaluación bioquímica de fracciones proteínicas de piñón Mexicano (*Jatropha curcas* L.).

Las proteínas de semillas de *J. curcas* L. se fraccionaron empleando el método de Osborne y posteriormente se determinaron algunas de sus propiedades bioquímicas y nutricionales. Las fracciones mayoritarias resultaron ser glutelinas (378 g kg^{-1} de proteína) y globulinas (201 g kg^{-1} de proteína) mientras que las albúminas y prolaminas fueron las fracciones minoritarias. La digestibilidad de la proteína resultó ser más alta en las glutelinas y globulinas, con valores de 81 y 80% respectivamente. El análisis por electroforesis mostró que las globulinas y glutelinas presentaron perfiles similares, los resultados sugieren que podría existir una relación con proteínas de almacenamiento 2S, 7S y 11S de origen vegetal. Asimismo, de acuerdo con la FAO/WHO, las fracciones proteínicas tuvieron niveles aceptables para la mayoría de los aminoácidos esenciales, sin embargo, las globulinas y glutelinas fueron deficientes en Lys y Trp.

PALABRAS CLAVE: Albúminas – Globulinas – Glutelinas – Jatropha curcas L. – Prolaminas – Proteínas de almacenamiento.

SUMMARY

Biochemical evaluation of protein fractions from physic nut (*Jatropha curcas* L.).

J. curcas seed proteins were fractioned according to the Osborne method and some biochemical properties were determined for these fractions. Glutelins (378 g kg^{-1} protein) and globulins (201 g kg^{-1} protein) were the main components. Albumins and prolamins were the minor components. Protein digestibility was highest in glutelins and globulins with values of 81 and 80% respectively. Electrophoresis analysis showed that globulins and glutelins exhibited similar polypeptide profiles. Electrophoresis patterns suggested that there could be a structural relationship among 2S, 7S and 11S storage proteins from plant sources. According to the FAO/WHO reference, the protein fractions had acceptable levels of most of the essential amino acids, but its globulins and glutelins were low in lysine and tryptophan.

KEY-WORDS: Albumins – Globulins – Glutelins – Jatropha curcas L. – Prolamins – Storage proteins.

1. INTRODUCTION

J. curcas, a member of the *Euphorbiaceae* family, is a drought resistant multipurpose small tree of significant economic importance because of its various industrial and medical uses (Makkar *et al.,* 2008). The seeds of the physic nut are a good source of oil (60% content), which could be used as a diesel substitute. They are also used in medicine, soap and in cosmetic manufacturing in various tropical countries. However, the seed meal after the extraction of oils is rich in protein, but might be toxic to rats, mice and ruminants and therefore, it may not be particularly suitable to be used as animal feed (Martínez *et al.*, 2006). The toxicity of *J. curcas* seed meal is ascribed to phorbol esters, however, non toxic genotypes which do not contain phorbol esters have been reported solely in Mexico, where kernels are consumed by humans after roasting (Makkar *et al.*, 2008). Plant proteins can be divided according to their solubility in water-soluble albumins, saline solutions-soluble globulins; acid and alkaline solutions-soluble glutelins and finally, the alcohol-soluble prolamins (Osborne, 1924). Globulins are the main storage proteins in legume seeds, accounting for 35-72% of the total protein content, and even 90% in some cases, such as soya bean (Utsumi, 1992). The remaining protein fraction consists of albumins, usually from the 2S fraction, which frequently has a physiological role (Machuca, 2000). The amino acid composition and digestibility, among other nutritional properties,

could change from one fraction to another. Thus, *J. curcas* seems to be a promising source of nutrients and medical compounds; hence, the aim of this investigation was to study the chemical composition and nutritional properties of the protein fractions which remain largely unexplored.

2. MATERIALS AND METHODS

2.1. Proximate analysis of *J. curcas* L. flour

The seeds, collected from the experimental field of CEPROBI, were dehulled manually using pliers, and the kernels were milled subsequently in a Cyclotec 1093 mill (Tecator, Sweden) to obtain a whole flour of small particle size (80 mesh). This flour was defatted twice using hexane in a ratio of 1:3 (w/v) with continuous stirring for 24h at 4 °C. The defatted flour was dried at room temperature.

The proximate composition of the whole and defatted flours was determined following the AOAC methods: nitrogen (954.01), fat (920.39), ash (923.03), crude fiber (962.09) and moisture (925.09) respectively. The Carbohydrate content was estimated as Nitrogen-free extract (NFE).

2.2. *J. curcas* L. proteins fractionation

The method used for extraction was adapted from Osborne (1924). Technical details of the extraction procedure are described in Figure 1.

Albumins: Defatted *J. curcas* meal was first extracted with distilled water by one stirring step of 2 h at 4 °C and then, centrifuged at 10 000 × g for 30 min at 4 °C. The supernatant was freeze-dried.

Globulins: The residue from albumins extraction was extracted under magnetic stirring for 2 h with NaCl (10 g/100 g) at 4 °C and centrifuged at 10 000 × g for 30 min at 4 °C. The supernatant was dialyzed against distilled water for one day to eliminate salt, using spectrapore membranes (6-8 kDa molecular weight cut off), changing water dialysis 4 times and then it was freeze dried.

Prolamins: The residue resulting from globulin extraction was extracted under magnetic stirring for 2 h at 4 °C with 70% (v/v) 2-propanol and centrifuged at 10 000 × g for 30 min at 4 °C. The supernatant was freeze-dried after the evaporation of alcohol by a rotary evaporator.

Glutelins: The glutelins fraction (highest content) were extracted with NaOH 0.1M, the sample was suspended in the solution with magnetic stirring for 2 h and centrifuged at 10 000 × g for 30 min at 4 °C. The supernatant was then freeze dried.

The protein content in the fractions (N × 6.25) was used to calculate the proportion of each fraction:

$$\text{Proportion of protein fraction} = \\ = (\text{g of protein in fraction / g total protein in flour}) \times 1\,000$$

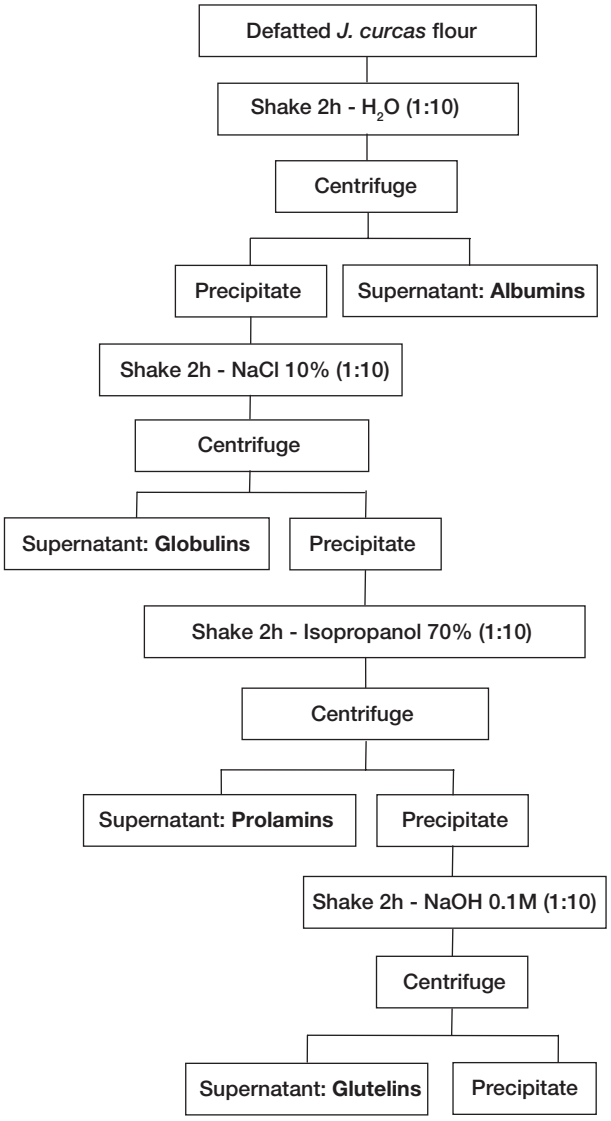

Figure 1
Scheme for extraction of protein fractions from *J. curcas.*

2.3. Biochemical evaluation

2.3.1. In vitro digestibility of protein fractions from J. curcas defatted flour

It was determined following the method of Hsu *et al.* (1977), using a multi-enzymatic solution containing 1.6 mg trypsin (Sigma T-0134, Type IX, 14190 units BAEE mg^{-1} protein), 3.1 mg chymotrypsin (Sigma C-4129, Type II, 60 units mg^{-1} dust) and 1.3 mg peptidase (Sigma P-7500, Grade III, 40 units g^{-1} dust) per ml. The decrease in pH was measured after 10 min, and the apparent *in vitro* digestibility (Y) was calculated with the following equation:

$$Y = 210.464 - 18.103X$$

Where X = pH of the protein suspension, immediately after digestion for 10 min in the multienzymatic solution.

2.3.2. *Amino acid content of protein fractions from J. curcas defatted flour*

Amino acid analysis was carried out by acid hydrolysis and high performance liquid chromatography (HPLC), after derivatization with diethyl ethoxymethylenemalonate according to the method described by Alaiz *et al.* (1992). Tryptophan was determined after basic hydrolysis, according to Yust *et al.* (2004).

2.3.3. *Non denaturing polyacrylamide gel electrophoresis*

This analysis was done following Laemmli (1970), using 8% acrylamide gels. About 15 g protein were loaded into each well, and the running condition was 20 mA/1.5 h. Thyroglobulin (669 kDa), apoferritin (443 kDa), amylase (232 kDa), alcohol dehydrogenase (140 kDa) and bovine serum albumin (66 kDa) were used as standards.

2.3.4. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein fractions from J. curcas defatted flour*

This analysis was done following Schagger and Jagow (1987), using 11% acrylamide gels and a 4% stacking gel. Runs were done at 40 mA for 1.5 h. The wells were loaded with 10 g of protein from one fraction type, and 8 l of a low molecular weight standard (BIORAD, USA), containing Phosphorylase b (113 kDa); bovine serum albumin (93.0 kDa); ovoalbumin (50.3 kDa); carbonic anhydrase (35.5 kDa); soybean trypsin inhibitor (28.8 kDa) and lysozyme (21.4 kDa).

2.3.5. *Isoelectric focusing (IEF)*

Each protein fraction (1 mg) was dissolved in 50 L of a sample buffer (10 L of ampholine SIGMA, USA, pH 3-10, and 90 L of a 25% (v/v) glycerol solution) and then heated at 95 °C / 120 s. Finally, the sample was centrifuged at 3 900 × g, 10 °C for 15 min.

The samples were loaded on top of a 5% polyacrylamide gel (2% w/v ampholine pH 3-10, 1% of glycerol). Isoelectric focusing standards (BIORAD, USA, pH 4.75-9.6) were used. Gels were run for 3 h at 200-400V (O'Farell, 1975) and then fixed and stained with Coomasie Brilliant Blue Stain.

3. RESULTS AND DISCUSSION

3.1. Proximate composition

Whole flour had a crude protein content of 243 g kg^{-1}, similar to that reported for legume seeds (190-440 g kg^{-1}), but higher than that reported for cereal seeds (70-170 g kg^{-1}) (Bernardino *et al.,* 2006). A crude fat content of 529 g kg^{-1} was detected in *J. curcas* whole flour (Table 1). The proximate composition is similar to the one reported by Makkar *et al.* (2008) in *J. curcas* kernels from Mexico. The slight discrepancies between the presented results and those reported by Martínez *et al.* (2006) are probably due to differences in the soil type, climate and seasonality, as well as the fat extraction method employed, among other factors (Ramulu and Udayasekhara, 2003).

3.2. *J. curcas* protein fractionation

The major fractions were the glutelins and globulins, followed by the albumins and prolamins (Table 2). This trend is similar to that found by Selje *et al.* (2007) using as raw material heat-treated defatted flour from both toxic and non-toxic varieties of *J. curcas* from Mexico; they reported as the major fractions, globulins and glutelins (27.4 and 56.9% respectively), and as the minor fractions; albumins and prolamins (10.8 and 0.6% respectively), obtaining a higher protein recovery (893 g kg^{-1}) than that reported in this study (556 g kg^{-1}). This could possibly be because the fat extraction method employed by these authors was more efficient.

Table 1
Proximate composition of whole and defatted *J. curcas* flours (g kg^{-1} flour) *. Data represent the average ± standard deviation of two measurements

Component	Whole flour	Defatted flour
Protein	243 ± 0.5	453 ± 1.0
Fat	529 ± 1.0	29 ± 0.5
Ash	47 ± 0.5	86 ± 0.4
Fiber	48 ± 0.5	81 ± 0.7
NFE	133	350

* Dry basis. NFE, nitrogen free extract.

Table 2
Protein proportion and *in vitro* digestibility of *J. curcas* fractions and defatted flour. Data represent the average ± standard deviation of two measurements

Sample	Proportion (g kg^{-1} total protein)	Digestibility (%)
Defatted Flour	–	75.3 ± 1.0
Albumins	151	78.1 ± 0.8
Globulins	201	80.0 ± 2.0
Prolamins	64	75.6 ± 1.8
Glutelins	378	81.0 ± 2.1
Insoluble residue	206	Nd

Nd: not determined.

Thus, the lipids could interfere during the protein extraction by interacting with some of the flour proteins. Furthermore, it is known that some factors such as the flour particle size, the applied shear stress, the ratio flour: solvent, the number of extractions by steps, as well as the seed physiological stage affect the different fraction proportions (Shewry and Miflin, 1985).

3.3. Biochemical evaluation of protein fractions from *J. curcas* defatted flour:

3.3.1. In vitro *Digestibility*

The *in vitro* digestibility value (75.3%) of the flour from *J. curcas* defatted at room temperature (Table 2) was lower than those reported by Martínez *et al.* (2006) in flours from *J. curcas* grown in Papantla, Veracruz (80.6%) and Yautepec (79.6%), Mexico; which were defatted using thermal treatment. Differences in protein digestibility may arise from inherent differences in the nature of food protein constituents, which may be modified by the presence of antiphysiological factors (Adebowale *et al.,* 2007). Improvement in dry bean globulin proteolysis as a consequence of heating is well documented (Sathe and Venkatachalam, 2007). Also, the Soxhlet defatted system could improve the digestibility value in both *J. curcas* flour and protein fractions derived from it.

The higher *in vitro* digestibilities for the protein fractions of *J. curcas* were those of the glutelins (81%) and globulins (80%) (Table 2). In a similar work, Selje *et al.* (2007), reported that the gastric digestion by pepsin and pancreatin of protein fractions from the toxic defatted flour of Mexican *J. curcas* were in albumins and globulins, with 64% and 61% respectively, while a higher value (95%) was obtained for glutelins.

3.3.2. Amino acid content

Like most plant proteins, the highest amino acid content in fractions from defatted *J. curcas* was found in the acid components (Fukushima, 1991) and the basic Arg, followed by aromatic (Phe + Tyr) and nonpolar (Leu, Ile, Ala, Gly, Val, Pro) amino acids (Table 3).

Based on the FAO/WHO (1991) reference pattern for infants, data from Table 3 indicate that the protein fractions of *J. curcas*, which, with the exception of Lys (with low content solely in globulin and glutelin), Trp (with low content in all fractions) and sulfur amino acids (with low content solely in prolamin), provide all essential amino acids in sufficient amounts. Selje *et al.* (2007) mentioned a Lys content of 79 g kg^{-1} protein in the albumin

Table 3
Aminoacid composition of protein fractions and defatted flour of *J. curcas* (g kg^{-1} protein). Data represent the average ± standard deviation of two measurements

Amino acid	Flour	Albumins	Globulins	Glutelins	Prolamins	FAO/WHO (1991)
Essential						
Lys	38.9 ± 0.0	66.2 ± 0.1	45.6 ± 0.3	26.4 ± 0.0	69.8 ± 1.5	58
Trp	7.9 ± 0.2	10.5 ± 1.5	5.6 ± 0.2	7.8 ± 0.0	ND	11
Phe + Tyr	70.9 ± 0.2	67.2 ± 1.0	74.2 ± 0.5	76.8 ± 0.5	79.2 ± 2.5	63
Met + Cys	13.5 ± 1.2	28.7 ± 0.2	40.0 ± 1.4	26.6 ± 1.0	10.0 ± 1.9	25
Thr	41.3 ± 0.1	43.7 ± 0.4	34.3 ± 0.0	39.1 ± 0.3	60.0 ± 0.5	34
Leu	76.9 ± 0.1	71.8 ± 0.0	74.0 ± 0.6	78.5 ± 0.2	84.1 ± 0.1	66
Ile	41.6 ± 0.3	30.1 ± 0.0	33.0 ± 0.0	31.5 ± 0.0	43.8 ± 0.2	28
Val	53.0 ± 0.0	40.5 ± 0.2	39.4 ± 0.2	40.6 ± 0.1	51.6 ± 6.6	38
Non essential						
Asp	110.5 ± 0.1	118.3 ± 0.1	99.1 ± 2.0	116.5 ± 1.7	91.6 ± 0.6	
Glu	185.0 ± 2.2	193.2 ± 0.3	186.4 ± 0.0	182.7 ± 0.3	155.5 ± 1.1	
Ser	58.4 ± 0.2	59.6 ± 0.1	67.8 ± 0.2	62.5 ± 0.3	61.6 ± 2.1	
His	27.4 ± 0.0	21.6 ± 0.2	25.4 ± 0.2	29.6 ± 0.1	22.2 ± 1.1	
Gly	53.0 ± 0.1	49.9 ± 0.0	46.3 ± 0.1	54.9 ± 0.2	76.3 ± 0.9	
Arg	134.9 ± 0.2	97.8 ± 0.5	144.8 ± 0.6	137.8 ± 0.8	90.3 ± 0.8	
Ala	52.5 ± 0.0	62.4 ± 0.3	50.2 ± 0.0	54.2 ± 0.3	67.5 ± 0.2	
Prol	34.4 ± 3.3	38.4 ± 2.6	33.8 ± 1.4	34.4 ± 4.4	36.4 ± 1.4	

ND: not detected.

fraction from the heat-treated defatted meal of *J. curcas*, which is consistent with that reported in this study (Table 3). The amino acid composition of *J. curcas* meal was similar and comparable to the values reported previously for the heat-treated defatted flours of *J. curcas* grown in Mexico (Martínez *et al.,* 2006). Overall, the *J. curcas* flours from this work contained adequate levels of Leu, Ile, Thr, Val and Phe + Tyr, as well as minor levels of Lys, Met + Cys and Trp.

3.3.3. Electrophoresis of protein fractions from J. curcas *defatted flour*

In native conditions, a major predominant component with apparent molecular weight of 298 ± 8 kDa (Figure 2A) was observed for the albumins fraction. Moreover, a certain number of polypeptidic chains was detected in a wide molecular weight (MW) range (113 – 26.4 kDa) under denaturing conditions. In native conditions, the globulins presented a main component with apparent molecular weight of 322 ± 8 kDa. In denaturing conditions however, bands with MW between ~ 107.3 - 25 kDa were observed. Both in the albumins and the globulins, proteins with MW higher than 113 kDa and lower than 21.4 kDa, as well as with similar MW were detected (Figure 2B, Table 4).

In the electrophoretic pattern, it can be observed that the globulins are constituted by nine strips, which could correspond to the globulins type 11S (37.5, 31, 25 and < 21 kDa) and 7S (64 and 59 kDa). Moreover, strips with MW lower than 21.4 kDa were detected, which could correspond to 2S globulins, since these are low MW proteins, usually in the order between 10 and 14 kDa (Fukushima, 1991). The albumin 2S (~ 12 kDa) was identified as the first known allergen from *J. curcas* (Menezes *et al.,* 2009). *J. curcas* also contains a toxic protein, named curcin, with a molecular weight of ~ 28.2 kDa (Juan Lin *et al.,* 2010) similar to that found in albumin and prolamin fractions.

Table 4
Apparent molecular weight (SDS-PAGE) of polypeptides from *J. curcas* protein fractions

Molecular weight (kDa)			
Albumins	**Globulins**	**Prolamins**	**Glutelins**
> 113.0	> 113.0	–	> 113.0
113.0 ± 0.0	–	109.4 ± 1.4	–
107.0 ± 1.2	107.3 ± 1.3	–	–
–	89.0 ± 1.2	–	89.1 ± 1.2
–	–	–	71.3 ± 0.9
60.9 ± 0.0	63.8 ± 0.8	62.6 ± 0.8	–
–	58.7 ± 0.0	–	56.0 ± 0.7
51.5 ± 0.0	–	52.0 ± 0.7	–
–	49.0 ± 0.0	–	46.0 ± 1.2
–	43.0 ± 0.0	–	41.0 ± 0.0
37.5 ± 0.0	37.6 ± 0.0	37.8 ± 0.5	–
32.4 ± 0.0	31.2 ± 0.0	32.4 ± 0.0	35.8 ± 0.5
28.0 ± 0.0	–	27.6 ± 0.3	–
26.4 ± 0.6	25.0 ± 0.0	–	23.8 ± 0.3
< 21.4	< 21.4	< 21.4	< 21.4

Data represent the average ± standard deviation of two measurements.

The prolamins in native conditions presented a component with apparent molecular weight of 322 ± 8 kDa (Fig. 2A), while in denaturing conditions, strips with apparent molecular weights between 109.4 – 27.6 kDa were observed (Table 4). The glutelins presented a main component with ~

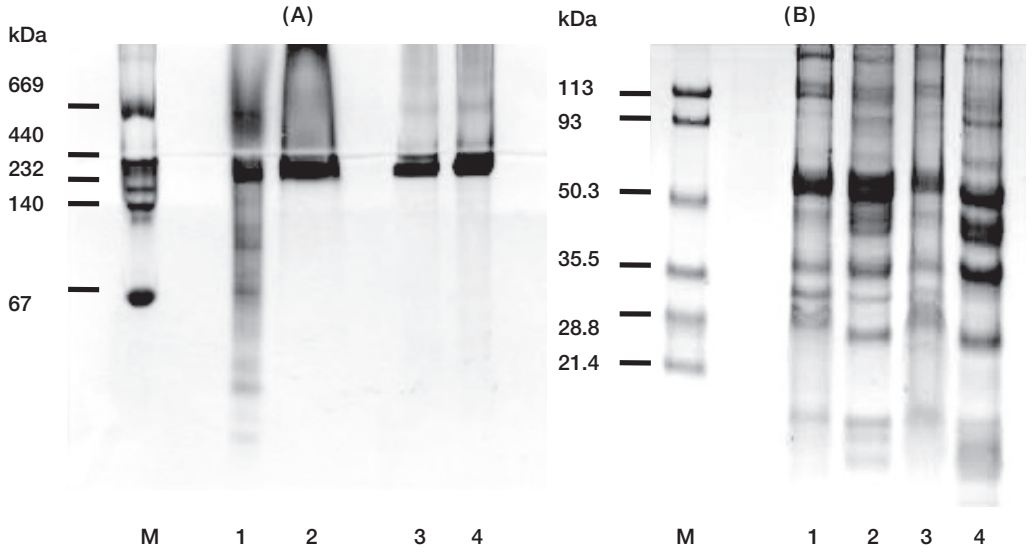

Figure 2
(A) PAGE, (B) SDS-PAGE of protein fractions from *J. curcas.* M, standard; 1, albumins; 2, globulins; 3, prolamins; 4, glutelins.

335 ± 9 kDa in native conditions, but under denaturing conditions, components in the range of ~ 113 and 23.8 kDa were observed. The electrophoretic profile of the globulin fractions was similar to the one obtained with the glutelin fractions (Figure 2B). Fukushima (1991) has suggested that some glutelins separated by sequential extraction could be classified either as globulins or as prolamins. Furthermore, the apparent molecular weights of these polypeptides are similar to those found in the protein isolate of *J. curcas* (48.7, 41.5, 27, 17, 13, 7 kDa).

3.3.4. IEF of protein fractions from J. curcas seeds

Figure 3 shows the isoelectric focusing patterns of protein fractions obtained with different extraction solvents. The native IEF pattern corresponding to the albumins (Fig. 3A, Line 1) included polypeptides with apparent isoelectric points (IP) of 9.6, 4.7, 4.6 and 4.4; globulins had six polypeptides with apparent isoelectric points of ~ 7.5, 7.1, 7.0, 6.5, 6.0 and 4.7; prolamins included four components with isoelectric points of ~ 9.6, 6.5, 6.0 and 4.4 and glutelins IP of ~ 8.0, 7.8, 7.5, 7.1, 6.5 and 6.0.

The Isoelectric focusing in denaturing conditions included complex banding patterns for each *J. curcas* protein fraction (Figure 3B). The IEF pattern for the albumins fraction was different from those observed in the patterns of the other three fractions; likewise, similar IPs were detected between this and the other fractions (acidic and alkaline isoelectric pH were detected in all fractions). Albumins exhibited polypeptides within an IP range of ~ 8.0 to 4.65, for globulins from ~ 9.6 to 4.65, from 9.6 to 4.25 for prolamins, and for glutelins, an isoelectric point range of ~ 8.0 to 4.25 was observed (Fig. 3B). Purified hemagglutinin from *J. curcas* seeds had a pI value of 5.75 (Cano-Asseleih *et al.,* 1989), while in curcin, the pI value was 8.54 (Juan Lin *et al.,* 2010).

These data are similar to those reported by Shridhar *et al.* (2007), in the protein fractions from the cold defatted flour of moth beans (*Vigna aconitifolia* L). These fractions were obtained employing a fractionation method similar to the one reported in the present study. These authors found proteins with an isoelectric point in albumin and globulin fractions within the range of 4.85 to 7.92, while in glutelins, four proteins with IPs lower than 4.3 were detected.

4. CONCLUSIONS

The protein fractionation of defatted *J. curcas* flour showed that glutelins and globulins are the main protein fractions in the seeds, followed by albumins and prolamins. Electrophoresis patterns suggest that storage proteins from *J. curcas* fractions could have a structural relationship with 2S, 7S and 11S plant proteins.

The higher *in vitro* digestibility for the protein fractions of *J. curcas* was that of the glutelins and globulins. According to the FAO/WHO reference, the protein fractions had acceptable levels of most of the essential amino acid patterns for pre-school children and adults, but glutelins and globulins were low in lysine and all fractions were low in tryptophan. The development of possible applications of protein

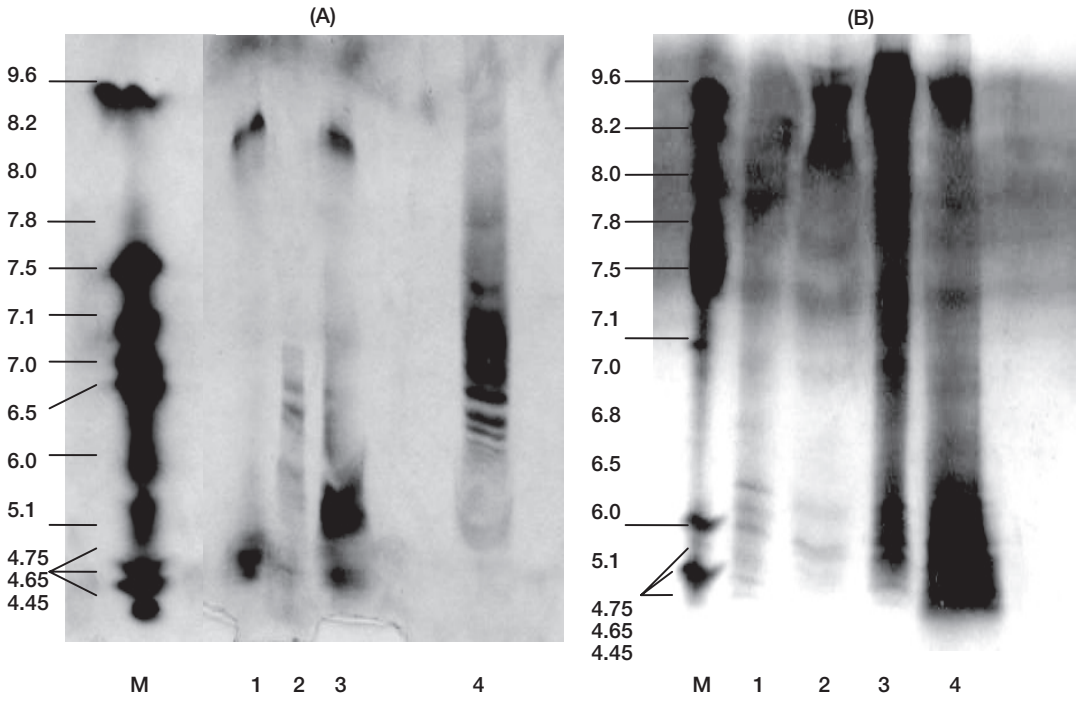

Figure 3
Isoelectric focusing PAGE (A) and SDS-PAGE (B) M, standard; 1, albumins; 2, globulins; 3, prolamins; 4, glutelins.

fractions from *J. curcas* will require further interdisciplinary research, such as the functional and toxicological evaluation of these proteins, but so far, a description of its different fractions has been achieved.

ACKNOWLEDGEMENT

This research was funded by the CONACyT-CONAFOR Project SIP 20090926. The authors are grateful to the Instituto Politécnico Nacional – México.

REFERENCES

Adebowale YA, Adeyemi IA, Oshodi AA, Niranjan K. 2007. Isolation, fractionation and characterisation of proteins from Mucuna bean. *Food Chem*. **104**, 287-299.

Alaiz M, Navarro JL, Girón J, Vioque J. 1992. Amino acid analysis by high performance liquid chromatography after derivatization with dietil ethoxymethylenemalonate. *J. Chromatogr. A*. **591**, 181-186.

AOAC. 1997. Official methods of analysis of AOAC International. *Association of Official Analytical Chemists*, 15th ed, USA, pp. 2–32.

Bernardino NA, Scilingo AM, Añón MC, Dávila OG. 2006. Guava seed storage protein: Fractionation and characterization. *Food Sci. Technol.* **39**, 902–910.

Cano-Asseleih LM, Plumbley RA, Hylands PJ. 1989. Purification and partial characterization of a hemagglutinin from seeds of *Jatropha curcas*. *J. Food Biochem.* 13, 1-20.

FAO/WHO Protein quality evaluation. 1991. Report of joint FAO/WHO expert consultation. In Food and Nutrition, Paper No. 51, *Food Agriculture Organizations and the World Health Organization*, Rome, Italy.

Fukushima D. 1991. Structures of plant storage proteins and their functions. *Food Rev. Int.* **7**, 356–381.

Hsu H, Vavak D, Satterlee L, Miller GA. 1977. Multienzyme technique for estimating protein digestibility. *J Food Sci.* **42**, 1269–1279.

Juan Lin, Xin Z, Jinya W, Peihong J, Kexuan T. 2010. Purification and characterization of curcin, a toxic lectin from the seed of *Jatropha curcas*. *Prep Biochem Biotechnol*. **40**, 107 – 118.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **217**, 680-685.

Machuca J. 2000. Characterization of the seed proteins of Velvet Bean (*Mucuna pruriens*) from Nigeria. *Food Chem.* **68**, 421-427.

Makkar HPS, Martínez HJ, Becker K. 2008. Variations in seed number per fruit, seed physical parameters and contents of oil, protein and phorbol ester in toxic and non toxic genotypes of *Jatropha curcas*. *J. Plant Sci.* **3**, 260-265.

Martínez HJ, Siddhuraju P, Francis G, Dávila OG, Becker K. 2006. Chemical composition, toxic / antimetabolic constituents and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L from Mexico. *Food Chem.* **96**, 80-89.

Menezes MF, Agra LM, Deus ON, Pinto FS, Martins dos Santos SA, Afonso VM, Tavares MOL. 2009. A new 2S albumin from *Jatropha curcas* L. seeds and assessment of its allergenic properties. *Peptides* **30**, 2103-2107.

Osborne TB. 1924. *The vegetable protein*. Longmans Green and Co, New York.

O'Farell PH. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem*. **250**, 4007-4012.

Ramulu P, Udayasekhara P. 2003. Total, insoluble and soluble dietary fibre contents of Indian fruits. *J. Food Comp. Anal*. **16**, 677-685.

Sathe SK, Venkatachalam M. 2007. Fractionation and biochemical characterization of moth bean (*Vigna aconitifolia* L.) proteins. *Food Sci. Technol.* **40**, 600-610.

Selje AN, Makkar HPS, Hoffmann EM, Francis G and Becker K. 2007. Quantitative and qualitative analyses of seed storage proteins from toxic and non-toxic varieties of *Jatropha curcas L,* EAAP Publication No. 124 *International Symposium on Energy and Protein Metabolism*. Vichy, France, pp. 625.

Schagger H, Von JG. 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* **166**, 368-379.

Shewry PR, Miflin BJ. 1985. Seed storage proteins of economically important, Pomeranz (Ed.) *Cereals advances in cereal science and technology*. American Association of Cereal Chemistry, USA.

Shridhar KS, Mahesh V. 2007. Fractionation and biochemical characterization of moth bean (*Vigna aconitifolia* L.) proteins. *Food Sci. Technol.* **40**, 600-610.

Utsumi S. 1992. Plant food protein engineering, Kinsella JE (Ed.) *Advances in food and nutrition research*. Academic Press, San Diego, CA, pp. 89-208.

Yust MM, Pedroche J, Girón CJ, Vioque J, Millán F, Alaiz M. 2004. Determination of tryptophan by high-performance liquid chromatography of alkaline hydrolysates with spectrophotometric detection. *Food Chem*. **85**, 317-320.

Recibido: 29/7/11
Aceptado: 14/2/12