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Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico

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Abstract

Jatropha curcas L. is a multipurpose shrub of significant economic importance because of its several potential industrial and medicinal uses. Four provenances of *J. curcas* from different agro-climatic regions of Mexico (1. Castillo de Teayo, 2. Pueblillo 3. Coatzacoalcos and 4. Yautepec), that differed in morphological characteristics, were studied. The seed kernels were rich in crude protein, CP (31–34.5%) and lipid (55–58%). The neutral detergent fibre contents of extracted *J. curcas* meals were between 3.9% and 4.5% of dry matter (DM). The gross energy of kernels ranged from 31.1 to 31.6 MJ/kg DM. The contents of starch and total soluble sugars were below 6%. The levels of essential amino acids, except lysine, were higher than that of the FAO/WHO reference protein for a five year old child in all the meal samples on a dry matter basis. The major fatty acids found in the oil samples were oleic (41.5–48.8%), linoleic (34.6–44.4%), palmitic (10.5–13.0%) and stearic (2.3–2.8%) acids. We also found previously unreported *cis*-11-eicosenoic acid (C20:1) and *cis*-11,14-eicosadienoic acid (C20:2) in the oil. Phorbolesters were present in high concentrations in the kernels of Coatzacoalcos (3.85 mg/g dry meal), but were not detected in the samples from Castillo de Teayo, Pueblillo and Yautepec. Trypsin inhibitors (33.1–36.4 mg trypsin inhibited g^{-1} dry meal), phytates (8.5–9.3% of dry meal as phytic acid equivalent), saponins (2.1–2.9% of dry meal) and lectins (0.35–1.46 mg/ml of the minimum amount of the sample required to show the agglutination) were the other major antinutrients present in all the seed meals. Different treatments were attempted on the seed meal samples to neutralize the antinutrients present in them. Trypsin inhibitors were easily inactivated with moist heating at 121 °C for 25 min. Phytate levels were slightly decreased by irradiation at 10 kGy. Measured saponin contents were reduced by ethanol extraction and irradiation. Extraction with ethanol, followed by treatment with 0.07% $NaHCO_3$ considerably decreased lectin activity. The same treatment also decreased the phorbolester content by 97.9% in seeds from Coatzacoalcos. The in vitro digestibility of defatted meal (DM) was between 78.6% and 80.6%. It increased to about 86% on heat treatment.
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Keywords: *Jatropha curcas*; Composition; Antinutrients; Detoxification

1. Introduction

Jatropha curcas (physic nut or purging nut) is a drought-resistant shrub or tree belonging to the Family *Euphorbiaceae*, which is cultivated in Central and South America, south-east Asia, India and Africa (Schmook & Seralta-Peraza, 1997). The *J. curcas* plant, which can easily be propagated by cuttings, is widely planted as a hedge to protect fields, as it is not browsed by cattle or other animals. Like many other *Jatropha* species, *J. curcas* is a succulent that sheds its leaves during the

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dry season. It is well adapted to arid and semi-arid conditions and often used for prevention of soil erosion (Heller, 1996). The seeds of physic nut are a good source of oil, which can be used as a diesel substitute. They are used also in medicines, and soap and cosmetics manufacture in various tropical countries. Although the seed meal, after extraction of oil, is rich in protein, it is toxic to rats, mice and ruminants and therefore cannot be used as an animal feed. Several cases of *J. curcas* nut poisoning in humans after accidental consumption of the seeds have been reported with symptoms of giddiness, vomiting and diarrhoea and in the extreme condition even death has been recorded (Becker & Makkar, 1998). The meal has high trypsin inhibitor and lectin activities, which could be inactivated by heat treatment. In addition, high concentration of the antimetabolic, metal-chelating and heat-stable factor, phytic acid, has been reported in *J. curcas* meal (Makkar, Aderibigbe, & Becker, 1998). Apart from these, phorbolesters that are present at high levels in the kernels have been identified as the main toxic agent responsible for toxicity (Adolf, Opferkuch, & Hecker, 1984; Makkar, Becker, Sporer, & Wink, 1997). After removing the toxic and heat-stable factors through solvent extraction, using 92% methanol, the extracted meal was found to be non-toxic to rats (Makkar & Becker, 1997). The defatted meal has been found to contain a high amount of protein, which ranged between 50% and 62%. Except for lysine, all other essential amino acids in *J. curcas* meal protein have been reported to be in higher concentrations than those of the FAO reference pattern suggested for pre-school children (Makkar, Becker, & Schmook, 1998). In addition to the more common toxic varieties, a non-toxic variety of *J. curcas* seeds, that contained negligible amounts of phorbolesters, but similar levels of trypsin inhibitors, lectin activity and phytic acid compared to the toxic variety, has been reported from the Papantla region of Veracruz state in Mexico (Makkar & Becker, 1999). The non-toxic seed kernels are consumed by local people after roasting. The hydrothermally processed defatted meal of the non-toxic variety did not show any toxicity to rats (Makkar & Becker, 1999). However, the growth rates of fish fed diets containing heated *Jatropha* meal were found to be lower than the unheated *Jatropha* meal group (Makkar & Becker, 1999). The decrease of growth rate was also related to increase in the time of heat treatment. Though various processing techniques have been attempted, no treatment has been successful in completely eliminating the antimetabolic factors and toxic principles of defatted *Jatropha* kernel meal of non-toxic and toxic varieties.

The *J. curcas* plant has, however, high agro-industrial potential in Mexico because of its various potentially beneficial products. The oil extracted from the seeds can be used as a substitute of diesel after transesterification. Biodiesel has currently high demand in the United States and Europe and is being promoted in a big way in countries such as India. The residual protein-rich seed cake, remaining after extraction of the oil, could form a protein-rich ingredient in feeds for poultry, pigs, cattle and even fish if it could be detoxified. The plant itself is very sturdy and can be an excellent candidate for re-greening of eroded zones, and for those lands that are not suitable for culture of more sensitive and demanding crops.

In view of these, the present research was designed to study the nutritional quality and the effects of various treatments (hydrothermal processing techniques, solvent extraction, solvent extraction plus treatment with $NaHCO_3$ and ionising radiation) to inactivate the antinutrional factors in defatted *Jatropha* kernel meal of both non-toxic and toxic varieties from different regions of Mexico.

2. Materials and methods

2.1. Collection of seeds and agroclimatic conditions

Mature and sun-dried seeds of *J. curcas* were collected from four different regions of Mexico in the month of August, 2003. The agroclimatic details of the different regions in Mexico, from where the *Jatropha curcas* seeds were collected, are as follows: (1) Castillo de Teayo, Veracruz state (hot sub-humid region with rains in summer), localization LN 20°45′, LO 97°38′, 80 m altitude, 1200 mm annual rainfall; soil type: calcaric regosol, (2) Pueblillo, Papantla, Veracruz state; climate similar to (1), LN 20°15′, LO 97°15′, 80 m altitude, 1500 mm annual rainfall; soil type: calcaric regosol (3) Coatzacoalcos, Veracruz state (hot humid climate with abundant rains in summer), LN 18°09′, LO 94°26′, 10 m altitude, 2500 mm annual rainfall; soil type: eutric regosol (4) Yautepec, Morelos state (semi-hot, sub-humid climate with rains in summer), LN 18°57′ LO 98°56′, 1210 m altitude, 902 mm annual rainfall; soil type: calcaric phaeozem + pellic vertisol (INEGI, 2001). Soon after the collection, the seeds were cleaned by hand using a paper towel, cracked individually without damage, and the kernels stored in a plastic container at room temperature prior to further analysis.

2.2. Physical properties of seeds

Thirty seeds were randomly taken from each provenance. The average weight, length and width of the seeds were estimated. These seeds were cracked using a mechanical cracker, the shells were carefully removed, and the weight, length and width of the kernels were recorded. Further, the average shell weight was calculated from the total seed weight minus kernel weight of the respective seeds.

2.3. Sample preparation

The seed kernels were ground, using a mechanical grinder, and defatted in a Soxhlet apparatus, using petroleum ether (boiling point of 40–60 °C), for 16 h. The defatted kernel meal was air-dried at room temperature and stored in a separate plastic container at 4 °C.

2.4. Treatments

The defatted kernel meal from seeds of each region (1–4) was divided into five equal portions and the first portion (a) was not treated further. The second portion was treated with 0.07 % $NaHCO_3$ solution in the ratio of 1:5 (w/v) and immediately autoclaved at 121 °C for 20 min. The autoclaved sample was freeze-dried as it was (without removing any supernatant) and it was designated as (b). The third portion of the defatted meal was extracted with 90 % ethanol for 2 h at room temperature with constant stirring. The meal to solvent ratio was 1:10 (w/v). The solvent was removed by filtration and the residue was freeze-dried and designated as (c). The fourth portion of the defatted meal, after treatment similar to (c), was air-dried, mixed with 0.07% $NaHCO_3$ solution in the ratio of 1:5 (w/v) and subjected to autoclaving at 121 °C for 20 min. After removing the residual water by freeze-drying, this was designated as (d). The fifth portion of the defatted meal was soaked in 0.07% $NaHCO_3$ in a ratio of 1:5 (w/v) for 30 min and filtered using a filter paper. The obtained residue was freeze-dried and subsequently irradiated at 10 kGy and this treatment was designated (e). The irradiation was from a cobalt source, and was done using GAMMA CELL 220 (Atomic Energy of Canada Ltd., Activity 14190 curie, Canada, Ontario, Model CG 220, Type B (U)). The dose rate was 19.70534 Gy/min.

2.5. Proximate composition

The moisture content of the samples was determined by oven-drying to a constant weight at 105 °C. Crude protein, lipid, neutral detergent fibre (NDF) and ash content were determined in accordance with the standard methods of AOAC (1990). Carbohydrates (nitrogen free extract) were determined by difference. Gross energy was estimated by an adiabatic bomb calorimeter (IKA C7000) using benzoic acid as a standard.

2.6. Estimation of total soluble sugars and starch

About 100–200 mg of defatted and processed kernel meal was taken in a 15-ml screw-capped test tube and, to this, 10 ml of 80% ethanol was added. The tubes were transferred to a boiling water bath and cooled under tap water to reach normal room temperature after 90 min. Then, the contents were centrifuged at 3000*g* for 10 min and the supernatant was collected. The remaining pellet was washed once again with 3 ml of hot ethanol (80%) and, after centrifugation, both the supernatants were pooled and the total soluble sugars were estimated according to the phenol–sulphuric acid method, using glucose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The remaining pellet, after air-drying, was extracted with 52% perchloric acid and the total starch content was estimated by the anthrone–sulphuric acid method using a spectrophotometer at 630 nm (Hodge & Hofreiter, 1962).

2.7. Amino acid analysis

The amino acid composition of the defatted kernel, was determined using an amino acid analyser (Bassler & Buchholz, 1993) after hydrolysing the samples with 6 M HCl at 100 °C for 22 h. The sulphur-containing amino acids were oxidised using performic acid before the acid hydrolysis. The contents of different amino acids recovered were presented in g 16 g^{-1} of nitrogen. The amino acid contents of the total seed proteins were compared with the FAO/WHO (1990) reference pattern and soybean (Vasconcelos et al., 1997) proteins.

2.8. Analysis of fatty acid composition

The kernel oil was extracted using petroleum ether (40–60 °C) in the ratio of 1:20 (w/v) for 8h at room temperature with stirring. After centrifugation, the solvent was removed under a nitrogen atmosphere. Methyl esters were prepared from the total lipids by the method of AOAC (1990). These fatty acid methyl esters were analysed by gas chromatography (GC-14A, Shimadzu, Japan) using an instrument equipped with an auto sampler (AOC-20s, Shimadzu, Japan), auto injector (AOC-20i) and a CB52 Varian Carbowax Capillary Column (50 m × 0.25 mm ID) and a flame ionisation detector (FID). The column temperature gradient ranged from 160 to 225 °C and nitrogen, at a flow rate of 1 ml per min, was used as a carrier gas. A standard fatty acid methyl ester mixture was run and retention times were used in identifying the sample peaks. Fatty acid levels were estimated as area percent of total peak area of methyl esters.

2.9. Determination of total phenolics

Total phenolics and tannins were extracted and determined by spectrophotometric methods described by Makkar et al. (1997). Total phenolics were quantified by the Folin–Ciocalteu reagent and results were expressed as tannic acid equivalents.

2.10. Determination of trypsin inhibitor activity, phytic acid and saponin contents

Trypsin inhibitor activity was determined essentially according to Smith, Van Megen, Twaalfhoven, and

Hitchcook (1980) except that the enzyme was added last, as suggested by Liu and Markakis (1989). The phytic acid content of the sample was determined by a colorimetric procedure described by Vaintraub and Lapteva (1988). Suitable aliquots were diluted with distilled water to make 3 ml and then used for the assay. Results are expressed as g 100 g^{-1} phytic acid, using standard phytic acid (sodium salt; Sigma, St. Louis, MO, USA). Total saponin content was determined using a spectrophotometric method, described by Hiai, Oura, and Nakajima (1976). The concentration of saponins was read off from a standard curve of different concentrations of diosgenin in 80% aqueous methanol and expressed as diosgenin equivalents.

2.11. Determination of phytohemagglutinating activity

Analysis of the lectin content was conducted by hemagglutination assay in round-bottomed wells of microtitre plates using 1% (v/v) trypsinised cattle blood erythrocytes suspension in saline phosphate buffer, pH 7.0 (Makkar et al., 1997). The hemagglutination activity was defined as the minimum amount of the kernel material (in mg per ml of the assay medium), which produced agglutination. The minimum amount was the material per ml of the assay medium in the highest dilution that was positive for agglutination. One hemagglutinating unit (HU) was defined as the least amount of material per ml in the last dilution giving positive agglutination (Grant, More, McKenzie, Stewart, & Pusztai, 1983).

2.12. Extraction and estimation of phorbolesters by HPLC

About 2 g of each variety of non-defatted, defatted and treated samples were weighed and subsequently extracted with methanol as described by Makkar, Becker et al. (1998). The analytical column was a reverse phase C18 (LiChrospher 100, endcapped 5 μm) 250 × 4 mm I.D. column protected with a guard column containing the same material as the main column according to the procedure outlined by Makkar, Aderibigbe et al. (1998). The separation was performed at room temperature (23 °C) and the flow rate was 1.3 ml min^{-1}. The four phorbolester compound peaks that appeared between 26 and 31 min were identified and integrated at 280 nm. The results are expressed as equivalent to a standard, phorbol-12-myristate 13-acetate, which appeared between 34 and 36 min.

2.13. In vitro protein digestibility

The in vitro protein digestibility (IVPD) of non-defatted, defatted and treated seed kernel meal of different varieties was measured using a multienzyme technique and protein digestibility of the sample was calculated using the following regression equation: $Y = 234.84 - 22.56(X)$, where $Y = \%$ protein digestibility and $X =$ pH of protein suspension after 20 min of digestion with the enzyme solution (Satterlee, Marshall, & Tennyson, 1979).

3. Results and discussion

3.1. Jatropha samples

The physical characteristics of the four varieties of *J. curcas* are shown in Table 1. Samples 2 (0.723 g) and 3 (0.714 g) had the highest average whole seed mass. Samples 1 (0.642 g) and 4 (0.448 g) had lower mass. The percentage kernel mass was highest in sample 4 (70.1%). All the values of whole seed weight (samples 1, 2, 3, 4) are very similar to those reported by Makkar et al. (1997) in 18 different seed provenances' *J. curcas*. But, the values of the percentage of kernel weight found are larger than that found by Makkar et al. (1997) and Makkar, Aderibigbe et al. (1998) and Makkar, Becker et al. (1998).

3.2. Chemical composition

The proximate composition, total soluble sugars and starch content of defatted and processed seed kernel meal of *Jatropha curcas* seeds from different agroclimatic regions in Mexico are shown in Table 2. There was some variation in the contents of crude protein, CP, (31–35%) and lipid (55–58%). The CP contents of defatted kernels varied from 62.0% in Coatzacoalcos to 65.0% in Castillo de Teayo. In some treatments, the CP content in the samples was higher (mainly treatment's c and d). The fibre content of *J. curcas* meals in the current study (between 3.9% and 4.5% NDF on dry matter basis) was lower than that in soybean meal (17.2% NDF, Makkar, Aderibigbe et al., 1998), but similar to those of other different seed provenances collected from Cape Verde (4.7%), Senegal (5.6%), Burkina Faso (5.3%), India (4.5%) and Nicaragua (3.8%), described in a previous publication (Makkar et al., 1997). The gross energy of whole kernels was relatively similar (31.1–31.6 MJ/kg). The contents of starch and total soluble sugars were below 6%.

3.3. Amino acid composition

The amino acid compositions of meals from Castillo de Teayo (1), Pueblillo (2), Coatzacoalcos (3) and Yautepec (4) were similar and comparable to the values reported for different provenances of *Jatropha curcas* (Cape Verde and Nicaragua) (Makkar et al., 1997). These results demonstrated that the amino acid compositions were not affected by agroclimatic conditions and

Table 1
Physical characteristics of different provenances of *Jatropha curcas* seeds (on dry matter basis)

Variety	Whole seed			Kernel			Shell weight (g)	% Kernel wt of whole seed wt	% Shell of whole seed wt
	Length (mm)	Width (mm)	Weight (g)	Length (mm)	Width (mm)	Weight (g)			
1	17.0c ± 0.96	8.4b ± 0.38	0.64b ± 0.07	15.1c ± 0.84	7.2c ± 0.38	0.43b ± 0.05	0.20a ± 0.08	68.06	31.94
2	18.0b ± 0.95	9.5a ± 0.73	0.72a ± 0.07	16.0b ± 0.58	7.7a ± 0.45	0.49a ± 0.04	0.23a ± 0.08	68.19	31.81
3	18.7a ± 0.56	9.3a ± 0.35	0.71a ± 0.04	16.4a ± 0.04	7.4b ± 0.39	0.48a ± 0.04	0.22a ± 0.06	68.34	31.66
4	15.0d ± 0.79	7.6c ± 0.45	0.448c ± 0.06	13.1d ± 0.71	6.1d ± 0.33	0.31c ± 0.03	0.13 ± 0.06	70.08	29.92

1. Castillo de Teayo (1200 mm of rainfall), Ver.; 2. Pueblillo (1500 mm of rainfall) (Papantla, Ver.); 3. Coatzacoalcos (2500 mm of rainfall), Ver.; 4. Yautepec (902 mm of rainfall), Mor. Values are means of 30 separate seeds of each sample.

Table 2
Proximate compositions, total soluble sugars and starch contents of defatted and processed seed kernel meals of different agroclimatic origins of *Jatropha curcas*

Sample	Dry matter	Crude protein	Crude Lipid	Crude ash	Crude fibre	Gross energy	NDF	Different carbohydrate fractions	
								Total soluble sugars	Starch
1	95.3	34.5	57.2	3.8	2.8	31.5	3.9	4.4	5.9
2	95.4	31.1	57.7	4.7	3.2	31.1	4.1	3.7	4.9
3	95.3	33.6	56.3	3.9	3.4	31.5	4.5	4.4	5.7
4	94.5	32.1	55.3	5.1	3.1	31.4	4.4	3.4	5.6
1a	93.6	64.9	0.4	10.4	4.9	18.2	9.2	10.3	11.2
1b	97.8	66.1	0.8	11.4	6.1	19.2	9.9	6.7	7.9
1c	96.8	68.6	0.8	11.8	5.9	19.0	9.5	2.9	10.4
1d	97.7	68.1	0.5	12.2	6.2	18.7	11.2	3.2	10.7
1e	92.0	65.5	0.6	11.8	8.1	18.4	11.8	0.2	6.8
2a	90.3	63.1	0.5	9.8	5.5	19.0	9.7	8.8	9.4
2b	94.3	65.7	0.7	9.7	5.2	19.1	13.1	7.8	9.5
2c	97.1	67.5	0.4	11.7	6.2	18.8	10.5	3.3	10.3
2d	94.7	67.9	0.6	12.1	6.0	18.3	12.8	3.3	10.6
2e	92.6	70.7	0.8	8.8	7.5	19.0	10.9	0.2	6.8
3a	90.2	61.9	0.6	10.4	6.1	18.8	10.3	10.2	10.6
3b	92.9	63.9	0.7	10.1	6.2	18.5	13.0	8.4	10.4
3c	96.4	67.9	0.5	11.6	6.2	18.8	9.8	2.5	9.3
3d	92.9	68.9	0.4	11.6	5.7	18.6	11.7	3.6	11.1
3e	92.2	63.9	0.6	9.7	8.8	18.8	12.5	0.1	7.8
4a	93.2	62.5	0.5	10.8	5.7	18.0	10.0	7.7	10.2
4b	95.8	63.7	0.9	11.1	5.4	18.8	10.0	7.1	10.7
4c	97.0	68.3	0.5	12.4	6.2	18.6	9.3	2.5	12.0
4d	88.7	70.9	0.6	12.1	5.8	18.2	11.2	2.8	11.1
4e	91.6	66.9	0.3	11.2	7.7	18.0	11.3	0.2	7.6

1.Castillo de Teayo, Ver.; 2. Pueblillo (Papantla, Ver.); 3. Coatzacoalcos, Ver.;4. Yautepec, Mor.
1, 2, 3, 4 = sample raw; a = defatted sample; b = $NaHCO_3$ 121 °C/25 min; c = ethanol 90%; d = ethanol 90% + $NaHCO_3$ 121 °C/25 min; e = irradiation.

were similar in toxic or non-toxic seeds. The levels of essential amino acids, except lysine, were higher than that of the FAO/WHO reference pattern (Table 3). A comparison between the amino acid composition of *Jatropha* meal and soya beans (Vasconcelos et al., 1997) revealed an almost similar pattern for all essential amino acids, except lysine and sulphur amino acids. Levels of lysine and sulphur amino acids were lower and higher, respectively, in the *Jatropha* meals. The levels of essential amino acids, in the *Jatropha* meals were higher than or similar to those of castor bean meal (Makkar, Aderibigbe et al., 1998).

3.4. Fatty acid composition

The fatty acid composition of the oils in the different *J. curcas* seeds, compared with the reported values, is recorded in Table 4. The fatty acids found common in all the oil samples were oleic, linoleic, palmitic and stearic. The major fatty acid in the Veracruz samples was oleic acid, whereas in the Morelos sample it was linoleic acid. This variation is possibly due to soil and climatic conditions. The results showed that the oil is composed mainly of unsaturated fatty acids (oleic and linoleic acid). The results obtained are very similar to those

Table 3
Amino acid compositions of defatted seed kernel of *Jatropha curcas* from different agroclimatic regions in Mexico and soybean and essential amino acid pattern suggested by FAO/WHO (g 16 g^{-1} N) for children up to 3–5 years

Aminoacid	Samples					FAO/WHO reference[b]
	1	2	3	4	Soybean[a]	
Essential						
Cystine	1.60	1.81	1.77	1.80	1.64	2.5[c]
Methionine	1.38	1.42	1.56	1.58	1.39	
Valine	3.79	4.00	4.35	4.26	4.72	3.5
Isoleucine	3.08	3.35	3.93	3.66	3.98	2.8
Leucine	6.01	6.03	6.55	5.92	7.61	6.6
Tyrosine	2.64	3.37	2.45	2.63	4.94	6.3[d]
Phenylalanine	4.20	3.82	4.08	3.93	5.76	
Histidine	2.65	2.68	2.81	2.89	3.03	1.9
Lysine	3.49	3.51	3.63	3.57	6.84	5.8
Threonine	3.15	3.17	3.33	3.33	3.85	3.4
Trypthophan	ND	ND	ND	ND	0.88	1.1
Non-essential						
Aspartic acid	11.7	11.4	12.2	11.5	11.9	
Proline	3.86	4.21	4.13	3.93	5.10	
Serine	4.91	4.65	4.67	4.59	4.15	
Glutamic acid	16.5	14.7	16.7	16.5	18.6	
Glycine	4.18	4.16	4.40	4.22	3.93	
Alanine	4.51	4.32	4.36	4.26	4.19	
Arginine	10.4	11.0	11.8	11.3	7.47	

1. Castillo de Teayo, Ver.; 2. Pueblillo (Papantla, Ver.); 3. Coatzacoalcos, Ver.; 4. Yautepec, Mor. ND, not determined.
[a] Vasconcelos et al. (1997).
[b] FAO/WHO (1990) reference pattern suggested for pre-school children (2–5 years old).
[c] cystine + methionine.
[d] Tyrosine + phenylalanine.

reported for *J. curcas* seed provenances from different countries (Banerji et al., 1985; Foidl, Foidl, Sanchez, Mittelbach, & Hackel, 1996; Gübitz, Mittelbach, & Trabi, 1999; Heller, 1996; Nasir, Memon, Valhari, & Khatri, 1988).

The present analysis, however, shows the presence of both *cis*-11-eicosenoic acid (C20:1) and *cis*-11,14-eicosadienoic acid (C20:2), which have not been reported by earlier workers. It is very important to mention that the phorbolesters were found in high levels only in the sample from Coatzacoalcos (3.85 mg/g). In the oils from Castillo de Teayo, Pueblillo, and Yautepec seed samples, phorbolesters were not detected. These seeds could thus be recommended for utilization by the food/feed industry after treatment for removing the less toxic antinutrients. It is, however, neccessary to carry out in vivo studies to confirm their harmlessness.

Table 4
Fatty acid profiles of seed oils of different provenances of *Jatropha curcas* (area %)

Fatty acid	Samples			
	1	2	3	4
Myristic acid (C14:0)	0.18	0.15	1.18	0.3
Palmitic acid (C16:0)	11.4	12.3	13.0	10.5
Palmitoleic acid (C16:1)	0.44	0.55	0.52	0.32
Stearic acid (C18:0)	2.27	2.80	2.53	2.45
Oleic acid (C18:1)	45	47.1	48.8	41.5
Linoleic acid (C18:2)	40.3	36.7	34.6	44.4
Linolenic acid (C18:3)	0.11	0.18	0.12	0.21
cis-11-Eicosenoic acid (C20:1)	0.12	0.19	0.14	0.14
cis-11,14-Eicosadienoic acid (C20:2)	0.11	0.11	0.1	0.13

1. Castillo de Teayo, Ver.; 2. Pueblillo (Papantla, Ver.); 3. Coatzacoalcos, Ver.; 4. Yautepec, Mor. Values are mean of duplicate determinations.

3.5. Antinutritional factors

The quantities of different antinutritional factors detected in investigated samples are presented in Table 5. Trypsin inhibitor activities (TIA, mg trypsin inhibited per g sample) for the defatted kernels meals were 36 ± 1.4 (sample 1a), 36.4 ± 0.5 (2a), 34 ± 0.12 (3a), and 33.1 ± 2 (4a). Similar values were observed for treatments c and e in all samples of *J. curcas*. The trypsin inhibitor values of the treatments b and d indicate that trypsin inhibitors were easily inactivated by moist heating at 121 °C for 25 min. These values, obtained for treatments b and d, were slightly better than those reported by Aderibigbe, Johnson, Makkar, Becker, and Foidl (1997). Trypsin inhibitors interfere with the physiological process of digestion through interference with the normal functioning of pancreatic proteolytic enzymes in non-ruminants, leading to severe growth

depression (White, Campbell, & Combs, 1989). It is possible that the antinutrient effect of trypsin inhibitors is due to their direct interaction with pancreatic proteolytic enzymes and a corresponding reduction in the digestibility of the proteins of the diet (Hajos et al., 1995). Trypsin inhibitors are heat-labile and can be partially or completely denatured when exposed to elevated temperature. Our treatments b and d were successful in inactivating the trypsin inhibitors in the *Jatropha* meals.

Phytate level is not affected by the treatments b, c, and d and only slightly by e (see Table 5). The high level of phytate present in *Jatropha* meals might decrease the bioavailability of minerals (especially Ca^{2+} and Fe^{2+}). Phytates have also been implicated in decreasing protein digestibility by forming complexes and also by interacting with enzymes such as trypsin and pepsin (Reddy & Pierson, 1994). The phytate content of all samples from *J. curcas* was much higher than that of peanut presscake (Aderibigbe et al., 1997). The values obtained are very close to those reported by Makkar et al. (1997). Treatment with synthetic phytases might help mitigate the problem of high phytate contents.

Only negligible amounts of total phenol were found in all samples of *J. curcas* (0.2–0.4%, results shown in Table 5).

The saponin contents of deffated *J. curcas* seed meals (1a 2.1%, 2a 2.4%, 3a 2.9% and 4a 2.3%) were lower than that reported for soybean meal (4.7%; Makkar, Aderibigbe et al., 1998). It should, however, be noted that the method used for saponin determination was non-specific. This method was selected as the purpose is to get an estimate of total saponin content in the seed meal. There was a slight reduction in some treatments, but the initial values were lower in the treatments c, d and e (Table 5). Saponins from some plants produce adverse effects whereas those from others confer beneficial effects (Liener, 1994). The treatment d decreased the saponin content by about 50%. Reddy and Pierson (1994) have reported that saponins are not destroyed by cooking. The reduction of saponins, resulting from treatment d, was probably due to their extraction along with ethanol.

Lectin is generally considered to be another toxic factor in *J. curcas* seeds (Panigrahi, Francis, Cano, & Burbage, 1984). Lectin activity in the defatted meal was very close to that reported by Makkar et al. (1997), Aderibigbe et al. (1997) and Makkar, Aderibigbe et al. (1998). The Pueblillo meal had higher activity (0.35 mg/ml) than the other samples. Treatment d was the best as far as reduction of lectin activity was concerned, as it decreased the lectin content in all the samples tested. The lectin (curcin) of *J. curcas* seeds has been reported to be much less toxic than the well-known lectins, ricin and abrin (Makkar et al., 1997). But it is unclear which

Table 5
Effects of various treatments on different antinutrients in *Jatropha curcas* seeds of different provenances (on dry matter basis)

Sample	TI (mg/g) sample[a]	Phytic acid (%)	Total phenolics[b] (g/100 g)	Saponins[c] (g/100 g)	Lectin activity (mg/ml)[d]	Total phorbolesters (mg/g)[e]
1a	36.0 (1.43)	8.76 (0.39)	0.24 (0.00)	2.14 (0.03)	1.46	ND
1b	0.68 (0.01)	9.88 (0.31)	0.31 (0.04)	1.96 (0.09)	12.2	ND
1c	34.5 (1.73)	10.1 (0.33)	0.19 (0.00)	1.40 (0.05)	1.51	ND
1d	0.58 (0.01)	11.9 (0.42)	0.30 (0.12)	1.29 (0.07)	12.2	ND
1e	37.8 (1.46)	7.81 (0.22)	0.21 (0.01)	1.30 (0.11)	6.79	ND
2a	36.4 (0.49)	8.54 (0.06)	0.18 (0.07)	2.41 (0.06)	0.35	ND
2b	0.53 (0.01)	9.11 (0.31)	0.38 (0.01)	2.77 (0.06)	5.89	ND
2c	34.5 (0.23)	10.5 (0.46)	0.19 (0.04)	1.51 (0.079	0.38	ND
2d	0.58 (0.06)	11.4 (0.82)	0.32 (0.03)	1.18 (0.25)	11.8	ND
2e	33.2 (0.24)	7.07 (0.12)	0.17 (0.00)	1.37 (0.07)	6.75	ND
3a	34.0 (0.12)	8.55 (0.78)	0.176 (0.02)	2.85 (0.06)	1.41	3.85 (0.77)
3b	0.66 (0.01)	8.92 (0.28)	0.301 (0.03)	3.00 (0.14)	11.6	0.95 (0.07)
3c	30.6 (0.12)	10.7 (0.08)	0.142 (0.02)	1.42 (0.09)	0.75	0.16 (0.02)
3d	0.57 (0.03)	12.0 (0.90)	0.273 (0.01)	1.07 (0.17)	23.2	0.08 (0.01)
3e	34.3 (0.24)	6.04 (0.33)	0.18 (0.01)	1.72 (0.39)	6.78	3.16 (0.07)
4a	33.1 (2.04)	9.27 (0.61)	0.151 (0.05)	2.32 (0.04)	1.46	ND
4b	0.54 (0.00)	9.72 (0.06)	0.290 (0.01)	2.98 (0.10)	5.99	ND
4c	36.0 (1.84)	10.4 (0.08)	0.158 80.02)	1.06 (0.05)	1.52	ND
4d	0.61 (0.01)	11.3 (0.03)	0.306 (0.02)	1.14 (0.07)	22.2	ND
4e	36.5 (0.24)	8.12 (0.21)	0.227 (0.01)	1.76 (0.07)	6.82	ND

1.Castillo de Teayo, Ver.; 2. Pueblillo (Papantla, Ver.); 3. Coatzacoalcos, Ver.;4. Yautepec, Mor.
a = defatted sample; b = $NaHCO_3$ 121 °C/25 min; c = ethanol 90%; d = ethanol 90% + $NaHCO_3$ 121 °C/25 min; e = irradiation; ND, not detected.
[a] TI, mg of pure trypsin inhibited/g sample.
[b] Tannic acid equivalents.
[c] Diosgenin equivalents.
[d] Minimum amount of the sample required to show the agglutination after two fold dilution in 1 ml of final assay medium.
[e] Equivalent to phorbol 12-myristate,13 acetate.

curcin has biological and enzymatic activities (Lin, Yan, Tang, & Chen, 2003). Higher agglutination, observed on heating, may be due to the presence of certain factors in *Jatropha* meal that mimic the action of lectin (agglutination of erythrocytes). The saponins, e.g., have been shown to produce such effects (Fenwick, Price, Tsukamoto, & Okubo, 1991).

Phorbolesters were present in high concentrations in the kernels of Coatzacoalcos (3.85 mg/g) whereas they were not detected in the samples from Castillo de Teayo, Pueblillo and Yautepec. The seeds of Pueblillo are consumed by humans and used as chicken feed, but Castillo Teayo and Yautepec are not consumed. According to the local people, consumption of seeds from Coatzacoalcos by humans causes diarrhea, giddiness and vomiting. It is not clear whether the high levels of phorbolesters in *Jatropha* seeds from Coatzacoalcos are caused by genetic or environmental factors.

It is very important to particularly mention the effect of treatments c and d on the toxic Coatzacoalcos seed meal. These treatments decreased the phorbolester content by 97.9% and 95.8%, respectively, whereas treatments b and e resulted in a reduction of only 75.3% and 17.9 %, respectively. The phorbolesters are moderately polar, and ethanol and methanol have major affinity for them. The use of ethanol has the advantage of it being a relatively non-toxic compared to methanol and the presence of any residues in the flour (even though not probable because of volatility) might not negatively affect animals consuming the treated flour.

Ionising radiation treatment (e) could serve as a possible additional processing method for inactivation or removal of certain antinutritional factors (Siddhuraju, Makkar, & Becker, 2002). However, the low irradiation dose levels of 10 kGy showed only a nominal effect on levels of phorbolesters, phytates, saponins and lectins in the samples of *J. curcas* of different provenances. An increase in the irradiation levels might have a more pronounced effect on the antinutrient levels.

The treatments b and d had similar effects to that reported by Aderibigbe et al. (1997) with regard to the decrease of trypsin inhibitors, lectins and saponins.

J. curcas plants from two regions of Mexico, i.e., from Veracruz and Yucatan states, have been previously reported to be non-toxic (Makkar, Aderibigbe et al., 1998; Makkar, Becker et al., 1998). In the current study, we found the *J. curcas* seeds from Castillo de Teayo and Pueblillo in Veracruz state and Yautepec in Morelos state to be non-toxic. Presence of high levels of antinutritional factors, such trypsin inhibitors, lectin and phytate, in the non-toxic samples is likely to provide resistance to the plant, and it is expected that it would survive and yield seeds under adverse conditions. Trypsin inhibitors and lectins are heat-labile, and their adverse effects could therefore be inactivated by heat treatment. The treatments that eliminate the phorbolesters are of great interest and show promise for completely detoxifying the seed cake and meal. This would pave the way for their use as protein-rich ingredients in livestock feeds or for human consumption. But it is necessary to conduct feeding trials to evaluate the toxicity and nutritive value of the processed *Jatropha* seed meals.

3.6. Protein digestibility

In vitro protein digestibility of the defatted meal and the different treatments are presented in Table 6. The lowest values were observed for the defatted samples 1a (80.6%), 2a (79.6%), 3a (78.6%) and 4a (79.6%). The values of the ethanol-treated and irradiated samples (see Table 6) were also similar. This low digestibility may be because of the high content of trypsin inhibitors present in *J. curcas* seeds undergoing these treatments. Similar observations were reported for other seeds, such as *Phaseolus vulgaris* (Carbonaro, Cappelloni, Nicoli, Lucarini, & Carvonale, 1997). All the samples that underwent the treatments b and d improved their digestibility by about 6–7%, probably because of the previous heating. The increase in protein digestibility observed after cooking has generally been attributed to protein denaturation and inactivation of protease inhibitors (Carbonaro et al., 1997). The values of digestibility of processed *Jatropha curcas* kernel meal from different provenancess were in some cases higher than those reported for faba beans (83.1%), lentils (82.5%),

Table 6
In vitro protein digestibilities of defatted and processed kernel meals of different provenances of *Jatropha curcas*

Sample	(%)[a]
1a	80.6
1b	86.4
1c	79.7
1d	87.2
1e	80.9
2a	79.6
2b	87.0
2c	80.3
2d	87.2
2e	80.4
3a	78.6
3b	87.1
3c	80.0
3d	87.0
3e	79.7
4a	79.6
4b	87.3
4c	80.5
4d	86.5
4e	79.5

1. Castillo de Teayo, Ver.; 2. Pueblillo (Papantla, Ver.); 3. Coatzacoalcos, Ver.; 4. Yautepec, Mor.
a = defatted sample; b = $NaHCO_3$ 121 °C/25 min; c = ethanol 90%; d = ethanol 90% + $NaHCO_3$ 121 °C/25 min; e = irradiation. Values are means of duplicate determinations.
[a] Based on multienzyme technique.

chickpea (78.3%) and dry beans (73.8%) (Carbonaro et al., 1997). To the best of our knowledge, our results are the first reports on the *in vitro* protein digestibility of defatted *J. curcas* seed meal determined in a system that simulated the digestive process in monogastric organisms.

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