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Quantification of inositols in *Jatropha curcas* L. of different provenances from Mexico

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The plant, *jatropha* has attracted worldwide attention for its high oil content. The use of high performance liquid chromatography (HPLC) to separate and quantify, for the first time, the phytic acid (inositol hexaphosphate) and lower inositol phosphates (tri-, tetra- and penta-phosphates; IP6, IP5, IP4 and IP3) in toxic and non-toxic (NT) *Jatropha curcas* seeds from different locations in Mexico was proposed. There are reports on the total phytic acids but the method of precipitation used was not specific to distinguish between the phytic acid (IP6) and its hydrolysis products; therefore, this technique underestimates the IP6 content. It was observed that the total inositol concentration is independent on the presence or absence of phorbolesters (PE). The analysis showed that the toxic seeds from Villaflores and Chiapa de Corzo had high concentrations of total IP (46.2 and 42.5 mg/g, respectively) but the NT seeds from Huitzilan is the highest (56.88 mg/g) followed by Pueblillo (41.427 mg/g), Cuautla (37.832 mg/g) and Xochitlan (35.868 mg/g) showed higher values of IP. Finally, the toxic seeds from Coatzacoalcos (22.5 mg/g) showed lower value. This is the first work showing the different inositol phosphates present in jatropha seed samples, highlighting the presence of hexaphosphate acid as the major component.

Key words: Phytic acid, high performance liquid chromatography (HPLC), anti-nutrients, phytates, IP5, IP6, hexaphosphate.

INTRODUCTION

The *Jatropha curcas* L. is a plant which belongs to the family, Euphorbiaceae; it is native to Mexico and Central America, but also cultivated throughout Central America, Africa and Asia (Francis et al., 2005). In Mexico, this

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plant is extensively found in several states such as Hidalgo, Morelos, Puebla, Sinaloa, Sonora, Veracruz, Tamaulipas, Michoacán, Chiapas, Oaxaca, Guerrero, San Luis Potosí, Jalisco, Nayarit, Sonora, Yucatan and Quintana Roo (Martínez et al., 2010). The non-toxic varieties have been reported in the states of Veracruz, Puebla and Hidalgo mainly in the region called Totonacapan while toxic varieties exist in Chiapas, Guerrero, Oaxaca and state south of Veracruz (Martínez et al., 2006, 2010). The seed has 25 to 30% of protein and 52 to 60% of oil (Martínez et al., 2006, 2010). The authors reported an excellent protein and lipids content, as well as the amino acid and fatty acid profiles in the seeds from Veracruz and Morelos. In one of them, phorbol esters were identified, which characterizes mainly the toxic variety; moreover, a high content of trypsin inhibitors, lectins and phytates were found.

Some important physiological roles for phytate in plants are: (1) phosphate reserve; (2) energy store; (3) a competitor for ATP during its biosynthesis near maturity, when metabolism is inhibited and dormancy is induced; (4) an immobiliser of divalent cations needed for the control of cellular processes and released after germination; and (5) a regulator of inorganic phosphate (Pi) in seeds (Cosgrove and Irving, 1980).

Phytate removal is desirable because it forms complexes with minerals and dietary proteins which decrease their bioavailability. Due to the heat stability of phytates, they are not easily removed by cooking, autoclaving, roasting, or any of the conventional heat processing methods (Zhou and Erdman, 1995). The solubility of phytates in aqueous solvents can be used to reduce or eliminate them from food when it would be convenient. The uses of acid hydrolysis as well as the ability of endogenous and/or added enzymes to affect phytate hydrolysis are additional techniques to reduce or eliminate phytates from food. The election of a method for phytate reduction is largely dependent on the type of food and the final product formed (La Frano et al., 2014).
Phytates can chelate minerals such as calcium, zinc and iron, resulting in insoluble complexes. Certain minerals such as iron and copper catalyze oxidative enzymes that generate free radicals, resulting in undesirable oxidative damage such as cell membrane damage (La Frano et al., 2014). The ability of phytates to chelate the divalent minerals makes them a natural antioxidant. For this reason, the phytate reduction or the elimination of it from food may not always be desirable. The role of the phytic acid in health and disease has been recently reviewed (Zhou and Erdman, 1995).

The level of phytates (7 to 11%) in *J. curcas* is relatively high when compared with other sources (Lott et al., 2002). The method of precipitation used was not specific to distinguish between the phytic acid (IP6) and its hydrolysis products; therefore, this technique underestimates the IP6 content in food. For this reason, the use of high performance liquid chromatography (**HPLC**) is proposed in the present work as a reproducible technique to quantify for the first time IP6, IP5, IP4 and IP3 contents in the different *J. curcas* seeds.

MATERIALS AND METHODS

Sample materials

The seeds were collected in 1: Yautepec; 2: Cuautla, Morelos; 3: Coatzacoalcos; 4: Pueblillo, Veracruz; 5: Huitzilan; 6: Xochitlan, Puebla; 7: Chiapa de Corzo; 8: Villaflores, Chiapas, in July, 2014. The edaphoclimatic conditions of the different regions in Mexico, from where the *J. curcas* seeds were collected, are as follows: (1) Yautepec, (Aw° semi-hot, sub-humid climate with rains in summer), localization LN 18°49´45" N, LO 99°05´35", 1210 m altitude, 902 mm annual rainfall; soil, 902 mm average annual rainfall, 22.7°C average temperature, soil type: calcaric phaeozem + pellic vertisol; (2) Cuautla, (Awº = semihot, sub-humid climate with rains in summer), localization LN 18°50´22", LO 98°56´56"; 1300 m altitude, 856 mm average annual rainfall, 22.6°C average temperature, soil type: calcaric phaeozem + pellic vertisol; (3) Coatzacoalcos, (Am = hot humid climate with abundant rains in summer), LN 18°08´06", LO 94°28´10", 10 m altitude, 2500 mm average annual rainfall, 25.6 average temperature, soil cambisol; (4) Pueblillo, (hot sub-humid region with rains in summer), LN 20°15´20", LO 97°15´20", 80 m altitude, 1500 mm annual rainfall; soil type: calcaric regosol; (5) Huitzilan, (Acf= semi-hot humid climate with rains all year), LN 19°58´10", LO 97°41´30", 900 m altitude, 2021 mm annual rainfall; 18.0 average temperature, soil luvisol; (6) Xochitlán, (Acf= semi-hot humid climate with rains all year), LN 18°42´91", LO 97°46´22", 1040 m altitude, 1400 mm annual rainfall; 24.0 average temperature, soil pellic vertisol; (7) Chiapa de Corzo, (A(w) hot sub-humid region with rains in summer), LN 16°44´26", LO 93°01´50", 450 m altitude, 990 mm annual rainfall; 26.0°C average temperature, soil regosol; (8) Villaflores, (A(wl) = hot sub-humid region with rains in summer), LN 15°45´26", LO 92°16´13", 560 m altitude, 1209 mm annual rainfall; 24.3°C average temperature, soil regosol.

Sample preparation

The individual inositol phosphates were extracted according to Burbano et al. (1995) with some modifications and determined according to the method of Lehrfeld (1994). A sample (0.5 g) was extracted with 5 mL of 0.5 M HCl by homogenization for 1 min at room temperature using an Ultraturrax homogenizer. The extract (2.5 mL) was diluted with 25 mL of water and placed into a SAX column (Varian). The column was washed with 2 mL of water, and then the inositol phosphates were eluted with 2 mL of 2 M of HCl. The eluted product was evaporated until dry and the residue was dissolved in 0.5 mL of a vacuum filtered buffer solution prepared by adding 1.6 mL of tetrabutylammonium hidroxide (TBNOH, 40% w/w solution in water), 0.2 mL of 5 M sulfuric acid and 0.1 mL of formic acid (ACS reagent, 91%) into 100 mL of methanol-water solution (51.5%). The solution was centrifuged at 12100 xg for 6 min to remove any suspended material before injecting it into the HPLC.

Analytical methods

The HPLC analysis was performed using a Beckman System Gold equipped with a refractive index detector. 10 µL were injected into a Hamilton macro-porous polymer PRP-1 (150x4.1 mm, 5 µm) which was used at 45°C with a rate of 1.2 mL/min. A reverse phase Cl8 column (Spherisorb ODS 5 pm, 250 X 4~6 mm) heated to 45°C was

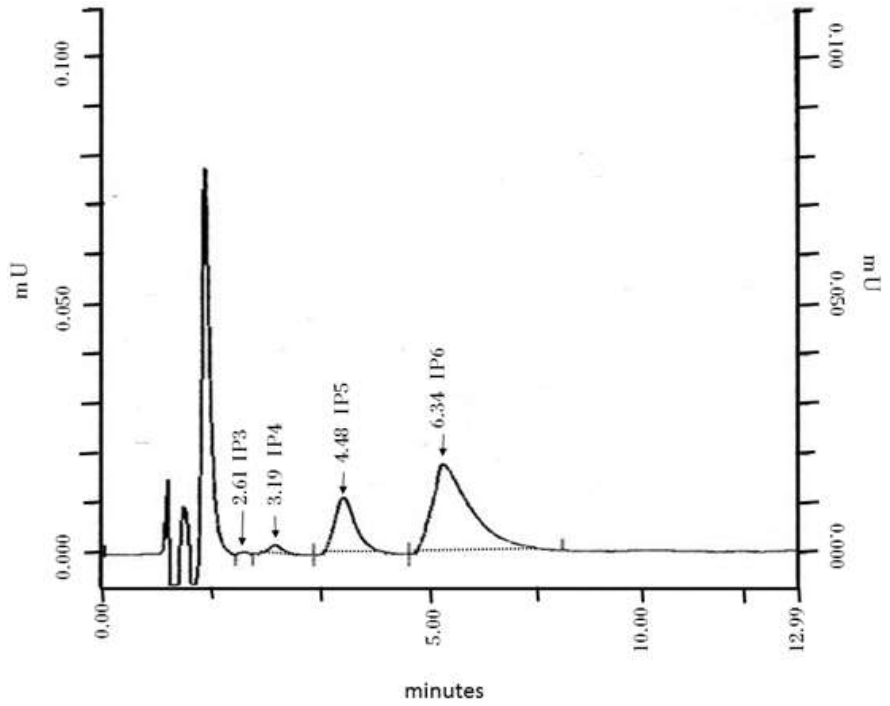

Figure 1. Characteristic peaks of inositol phosphates found in Jatropha seeds.

equilibrated with the mobile phase for 1 h. The mobile phase consisted of 515 mL of methanol added in 485 mL water. Afterwards, 8 mL of TBNOH, 1 mL of 5 M sulphuric acid, 0.5 mL of formic acid (91%) and 0.2 mL of phytic acid solution (6 mg/mL) were sequentially added. The pH registered was 4.1. The individual inositol phosphates were quantified by comparison with the external standards of phytic acid (Sigma). Chromatographic analysis was carried out three times on each sample.

Statistical analysis

Data were processed with Statistical Analysis System software (version 9.2.; SAS Institute Inc., Cary, NC, USA), under the completely randomized model, and the means were compared with the Tukey test (p = 0.05).

RESULTS

Quantification of phytates in seeds

There is scarce information on the phytates composition of *J. curcas* L. seeds from different provenances of Mexico. Figure 1 shows the characteristic peaks of inositol phosphates found in each seed sample. The retention time (minutes) detected was 6.34 for IP6, 4.48 for IP5, 3.19 for IP4 and 2.61 for IP3. As shown in Figure 1, IP6 is clearly the major component in all samples analyzed. Table 1 shows the values obtained for inositol phosphate content in each sample.

Moreover, it was observed that the inositol concentration is independent of the presence or absence of phorbolesters (PE). The analysis showed that the toxic seeds from Villaflores and Chiapa de Corzo had high concentrations of IP (46.5 and 42.5 mg/g, respectively) and the non-toxic seeds from Pueblillo, Huitzilan and Cuautla showed higher values of IP (41.4, 56.8 and 37.8 mg/g, respectively). Finally, the toxic seeds from Coatzacoalcos (22.4 mg/g) and the non-toxic ones from Xochitlan (35.8 mg/g) and Yautepec (30.5 mg/g) showed lower values of IP. Table 1 shows the phorbol ester content previously reported by Martínez et al. (2006, 2010).

The concentration of total IP in Huitzilan seeds (non-toxic) found by HPLC is greater than that found by the method of precipitation of 9.2% (Martínez et al., 2010), similar results were observed in others seeds (Table 1).

DISCUSSION

The total content of phytates present in the raw seed of *J. curcas* were relatively high. These values differed greatly from those reported by Makkar et al. (1997) and Martinez et al. (2006, 2010) by using precipitation methods. The methodology used in the present work allowed the quantification of the individual inositol phosphates, which gave higher precision.

The seeds which reported a relatively high inositol phosphate content (IP) were from: Huitzilan, Puebla (Pue), Villaflores, Chiapas (Ch) and Chiapa de Corzo,

Table 1. Inositols content in *J. curcas* seeds from different provenances of Mexico.

Content (mg/g)	IP3	IP4	IP5	IP6	IP total	IP6 total (%)	**Phytic acid total (%)	**Phorbol esters (mg/g)
Yautepec, Morelos		0.598^{a}± 0.06	4.312^{a}± 0.13	25.659^{a}± 0.60	30.569 ± 0.58^{a}	83.93	9.27	ND
Cuautla, Morelos		0.414^{b}± 0.04	3.820^{b}± 0.07	33.599^{b}± 0.60	37.832 ± 0.60^{b}	88.81	8.76	ND
Pueblillo, Veracruz		0.708^{c}± 0.06	5.654^{c}± 0.11	35.066^{b}± 0.68	41.427 ± 0.62^{c}	84.64	8.54	ND
Coatzacoalcos, Veracruz		0.411^{b}± 0.04	3.815^{b}± 0.42	18.270^{c}± 0.74	22.496 ± 0.99^{d}	81.12	8.55	3.85
Huitzilan, Puebla	0.191 ± 0.020	0.833^{d}± 0.04	5.871^{c}± 0.19	50.040^{d}± 0.86	56.886 ± 0.94^{e}	87.96	9.2	ND
Xochitlán, Puebla		0.552^{a}± 0.02	4.577^{a}± 0.31	30.739^{b}± 0.97	35.868 ± 0.89^{b}	85.7	7.8	ND
Villaflores, Chiapas		0.706^{c}± 0.04	6.690^{d}± 0.14	38.858^{e}± 0.52	46.254 ± 0.66^{f}	84.01	7.7	0.60
Chiapa de Corzo, Chiapas		0.577^{a}± 0.04	6.749^{d}± 0.18	35.239^{b}± 0.31	42.565 ± 0.52^{c}	82.78	7.3	4.05

*Three replicate of each sample and analyzed in triplicate with HPLC (mean values with their standard deviations expressed on a dry weight basis). Means with the same letter in each column are not statistically different (Tukey, $p \leq 0.05$); ** Martínez et al. (2006, 2010).

Ch. Only the seed from Huitzilan showed the IP3 (triphosphate inositol). The IP concentrations found in the *J. curcas* seeds from Mexico were higher when compared with other sources such as cereals (0.3-6%), legumes (0.5-8.0%), oilseeds (0.11-7%) and freshly fruits (0.1-2%) (Lott et al., 2002). It is important to mention that IP6 is found in higher percentage than IP5, IP4 and IP3; values between 81.12 and 88.81% of total phytate in seeds of Jatropha were quantified.

Recent studies on toxic and nontoxic *Jatropha* seed, have shown that phytate concentration is highest in the endosperm at 78.1 g kg^{-1}, constituting 96.5% of the total phytate present in the whole kernel, whereas the cotyledon, hypocotyl and kernel coat contained 1.7, 0.27 and 0.84 g kg^{-1}, accounting for 2.1, 0.33 and 1.04% of the total phytate respectively, suggesting that the major supply of phosphate during germination for metabolic activities is contributed by phytate present in the endosperm (Devappa et al., 2011).

The high phytate content found in protein concentrate prepared from jatropha seed cake indicates that phytate is strongly bound to protein in jatropha kernel and also has high affinity towards protein at low or high pH (Makkar et al., 2008). The calculated value of phytate for defatted jatropha kernel meal (89 g kg^{-1}) was within the range (72–101 g kg^{-1}) reported for various toxic and non-toxic varieties of *J. curcas*, but about 5.9 times higher than that for defatted soy (15 g kg^{-1}) (Makkar et al., 1998). High levels of antinutritional agents such oxalates, phytates and cyanates were more in the leaf than stem bark and root. Phytates were high in leafs (6.12%) but low in the stem bark (1.0%) and root (0.89%) (Agbor et al., 2015). The variation in the concentrations of inositol could be caused by different factors such as environmental fluctuations, culture site, irrigation conditions, type of soil, use of fertilizers and the year of crop.

Bassiri and Nahapetian (1977) observed that wheat varieties grown under dry land conditions had lower concentrations of phytate when compared with the ones grown under irrigated conditions. Also, the application of different fertilizers (nitrogen and phosphorus) had an effect on the crops during their growth; fertilizers are reported to increase phytate content in the seeds (Miller et al., 1980; Saastamoinen and Heinonen, 1985).

The *J. curcas* seeds studied were collected in wild areas, some of them were found close to some crops in the localities of Huitzilan, Pueblillo, Villaflores and Chiapa de Corzo; probably, they were influenced by the fertilizers or were irrigated during the culture period, these factors could have produced the presence of higher concentrations of IP in the seeds. In contrast, the seeds from the last four *J. curcas* plants (from Yautepec, Cuautla, Xochitlan and Coatzacoalcos) did not present high IP values.

Although, many chemical and physical methods have been reported to remove phytate from the meal, enzymatic (phytase) treatment could be beneficial owing to its high specific activity towards phytate. Phytase treatment could improve the nutritional value of jatropha meal as a feed for monogastrics and would also reduce phosphorus inclusion in their diets (Devappa et al., 2010), whereas ruminants are considered to utilise phytate through the action of phytase enzymes produced by ruminal microbes. The presence of phytate in the kernel coat is also found to inhibit aflatoxin B1 production by *Aspergillus flavus,* thus helping in postharvest storage of dry seeds (Chen et al., 1995).

Conclusion

The use of HPLC as a method to quantify the inositol phosphate is better in terms of precision than the spectrometric method. The use of non-toxic seeds of *J. curcas* can be proposed for human and animal nutrition; however, it will be necessary to reduce the IP content, maybe by the use of phytase enzyme, therefore, more

studies are necessary in order to understand better the human and animal physiology, considering an appropriate phytate concentration which can have a possible beneficial effect. In future studies, we will assess whether fertilization doses, soil type and environmental conditions affect the inositol phosphate concentration in *Jatropha* seeds from commercial plantations where agronomic crop management is carried.

Conflict of Interests

The authors have not declared any conflict of interests.

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