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Fortification of hotcakes from edible flour of non-toxic Mexican *Jatropha curcas* L.

Jorge Martinez Herrera[1]*, Elizabeth Arguello García[2], Odilón Sanchez Sanchez[3] and Ofelia Andrea Valdés-Rodríguez[4]

[1]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Huimanguillo, Km 1 Carretera Huimanguillo-Cárdenas. Huimanguillo, Tabasco C.P. 86400, Mexico.
[2]Universidad Popular de la Chontalpa. Km 2 Carretera Cárdenas-Huimanguillo Ranchería Paso y Playa. Cárdenas, Tabasco. C.P. 86500, Mexico.
[3]Centro de Investigaciones Tropicales (CITRO-UV) Ex-Hacienda Lucas Martín Priv. Araucarias s/n, Col. Periodistas. Xalapa, Veracruz. C.P. 91019, Mexico.
[4]El Colegio de Veracruz. Carrillo Puerto No. 26, Col Centro. Xalapa, Veracruz C.P. 91000, Mexico.

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The *Jatropha* flour is a promising source of protein in the fortification of various foodstuffs made from wheat and corn. The objective was to evaluate the use of non-toxic or edible *J. curcas* flour at different percentages (5, 10, 15 and 20%) in combination with wheat flour to make hotcakes. Whole and defatted *J. curcas* flour presented values of moisture between 4.5 and 6.8%, nutritional protein 23.5 and 54.0%, lipids 52.52 and 1.56%, ashes 5.29 and 11.4%. The protein content of a control sample of hotcakes was 8.4%, increasing to 16.5% when 20% *Jatropha* flour was added to wheat flour. The microbiological analysis of defatted flour recorded a count of aerobic mesophilic of 7100 UFC/g, fungus 200 UFC/g and yeasts 20 UFC/g. At last, it was determined that lead and cadmium were not detected in flour. Thus, the edible *Jatropha* flour has a potential for fortification in processed foods, but is very important to ensure the safety or low toxicity level of *Jatropha* seeds previously by chemical analysis (HPLC) to avoid problems of severe intoxication; therefore non-toxic plantations of *Jatropha* must be certified to avoid mixtures with other toxic seeds.

Key words: Physic nut, *Xuta,* flour, meals, fortification, microbiology, protein

INTRODUCTION

Jatropha curcas is a perennial plant belonging to the Euphorbiaceae family and considered to be originating in Mexico and Central America, growing in altitudes from 0 to 1700 msnm, and annual precipitations averages of

*Corresponding author. E-mail: martinez.jorge@inifap.gob.mx

300-2500 mm (Martinez et al., 2010). Traditionally it has been used as a hedge plant and for medicinal uses. In recent years, tropical countries like India, China, Malaysia, Indonesia, Mozambique, Brazil, and Mexico, among others, have explored the potential of the *Jatropha* plant as a feedstock for biofuel production, mainly biodiesel. The *Jatropha* tree has been proposed as a potential second-generation biofuel due to its many toxic components (phorbol esters mainly). These compounds have been reported in seeds from Central America, South America, African, and Asian continents (Makkar et al., 1997; Contran et al., 2013).

In Mexico, *J. curcas* is found in more than 17 states; among them are Veracruz, Morelos, Puebla, Hidalgo, Tamaulipas, Tabasco, Chiapas, Yucatán, Oaxaca, Guerrero, Michoacán, Colima, Jalisco, Nayarit, Sinaloa, Campeche and Sonora (Martinez et al., 2010). The plant is known by different names according to the region of Mexico as: pinion, hill pinion, piñoncillo, Aixte (Náhuatl language) Xuta or chuta (Totonaco language), Sikil Té (Maya language), Scu-Lu´u. The diversity in Mexico is such that there are ecotypes with different levels of toxicity (Martinez et al., 2010). The main toxicity is due to the higher phorbol esters contents, which are co-carcinogenic compounds that attack the central nervous system, being capable of killing animals and humans (Wink et al., 1997). However, some provenances of *J. curcas* in Mexico have very low or zero phorbol ester contents. These provenances are considered edible or non-toxic because they are eaten by the natives of their regions (Martínez et al., 2006).

All the ecotypes have anti-nutritional compounds such as trypsin inhibitors, lectins, phytates, saponins and tannins. When defatted flour of *J. curcas* is cooked, non-nutritional compounds like trypsin inhibitors and lectins are partially or fully eliminated avoiding damage by ingestion (Martínez et al., 2006; Makkar and Becker, 2009; Martínez et al., 2010). Additionally, due to their physicochemical properties, *J. curcas* seeds provide a good source of oil that can be used as diesel substitute.

The chemical composition of the seeds, flour and nutritional quality of edible *J. curcas* have been reported and compared to toxic seeds. The oil content of the seeds and their fatty acid composition are similar in both edible and inedible ones. However, based on the agro-climatic conditions of the region they come from, the genotypes may have a major percentage of oleic acid than linoleic acid. The amino acid composition is not different in both, toxic and non- toxic seeds; their essential amino acid levels plus lysine are higher, according to the reference standard of FAO/WHO, and these are comparable to soybean flour (Martínez et al., 2006; Martínez et al., 2010; Azevedo et al., 2016). The edible Mexican *J. curcas* has high lipid (45-48%) and protein contents (18-30%). The defatted flour, also known as “press cake” or residual paste, has up to 55 to 60% of protein.

The press cake is characterized by its high protein content. A whole seed press has 38-48% of protein with 10% of residual oil, and when it is extracted with organic dissolvent like hexane, the kernel has values higher than 55% of protein or more. Also, there are reports on protein efficiency (REP) of *Jatropha* flour supplemented with lysine of 1.77 in Wistar rats, which was higher than that reported for soybean (1.4). Therefore, it can be considered that non- toxic flour has high quality ingredients for animal nutrition (Makkar and Becker, 2009; Martinez et al., 2012; Richter, 2012).

Jatropha edible seed is used by the people of Papantla and the mountains of Puebla to prepare traditional dishes. These authors have used defatted flour to produce various industrial products to fortifying foods devoid of protein from wheat and corn. Thus, defatted flour of *Jatropha* with wheat flour was used to make breads, cookies, cakes, pancakes, pizza, etc. Also, it is permissible to make tortillas with corn flour (Arguello et al., 2016). Food fortification is an alternative for improving nutritional contents, through the addition of nutrients, such as vitamins, minerals, and amino acids (Figueroa et al., 2001). The aim of this study was to determine the fortification, quality flour and protein content in hot cakes added with *Jatropha* flour.

MATERIALS AND METHODS

Collection and conditioning of material

The *Jatropha* edible seed was recollected in August 2013 in Papantla, Veracruz, Mexico and were manually husked. The resulting kernel was ground in a mill Cyclotec™ Model 1093 (Höganás, Sweden), for 16 h. The seeds were defatted with hexane P.A. with Soxhlet equipment at 68°C. Excess of hexane was eliminated in room temperature in exhaust hood for 12 h. The content of phorbol esters was determined in the flour to confirm its non-toxicity. Later, four mixtures of 0, 5, 10, 15 and 20% of *Jatropha*/wheat flour were prepared to cook the hotcakes as traditionally.

Extraction and estimation of phorbol esters by HPLC

The phorbol esters were determined as described by Makkar et al. (2007) based on the method of Makkar et al. (1997). Ground seed kernels/defatted meal (2 g) was extracted with1 mL solvent (99 percent methanol/1percent THF) and centrifuged (Allegra Model X-15 R, USA) (10,000 × g) to collect methanolic supernatant. The residue was re-extracted (three times), centrifuged and supernatant was collected. The supernatant was concentrate together and condensed using pressurized air to get fraction. This fraction was re-dissolved in methanol (1 mL). A suitable aliquot was injected into a high-performance liquid chromatography (HPLC) fixed with a reverse-phase C1 8 LiChrospher100, 5 mm (250 mm × 4 mm id, from Merck (Darmstadt, Germany)) column. The column was protected with a head column containing the same material. The separation was performed at room temperature (23°C) using gradient elution (1.3 mL/min flow rate) (Makkar et al., 2007). The

Figure 1. Whole flour (A) and defatted *J. curcas* (B)

four phorbol ester peaks (containing 6 PEs) which appeared between 25 and 30 min were detected at 280 nm. The spectrum of each peak was taken using Merck-Hitachi L-7450 photodiode array detector. Phorbol-12-myristate 13-acetate (PMA) was used as an external standard which appeared between 31 and 32 min. The area of the four phorbol ester peaks was summed and the concentration was expressed as equivalent to PMA. The PEs in the meal was analyzed in triplicates.

Elaboration of hotcakes

Ingredients: 4.0 g Spring Chantilly® unsalted margarine, 500 g of flour for hot cakes, 2 eggs (107 g approximately), 500 ml of whole milk, *J. curcas* flour at 5, 10, 15 and 20%. Procedure: 1) all the ingredients were mixed until lumps dissolved completely, 2) over a hot skillet, a little margarine Primavera® was lightly spread, 3) the mixture was gently poured over the cookie sheet at a temperature of 50-55 °C, forming the circle of the desired size). Bubbles were formed and it was turned gently to cook the other side.

Proximal chemical analysis

Percentages of protein, lipids, ash and moisture, were quantified by triplicate in *J. curcas* flour (whole and defatted), and the fresh hotcakes (5, 10, 15 and 20%), according to AOAC methods (1995). Protein was estimated by the Kjeldahl method, using a digester (Digestive System 61007 digester) and a distilled (Kjeltec 1002 Distilling unit system Tecator) by manual operation. Lipids were obtained by extraction with petroleum ether in a Goldfish extractor. Ash was obtained by carbonization and subsequent samples calcined at 550°C to constant weight. Moisture was estimated by circulation oven drying air at 90° C to constant weight (AOAC, 2010).

Microbiological analysis of the defatted flour

The total mesophilic count, fungi and yeast were determined according to the following rules:

Mesophilic aerobic count: This assay was made with agar for standard count; it was incubated for 48 h at 35°C, in an Incubator 132000 3M, according to NOM-092-SSA1-1994.

Fungi and yeasts: Trials of fungi and yeasts were performed over potato dextrose agar acidification, incubated at 25°C ± 1°C for 5 days, as indicated in NOM -111 - SSA1-1994.

Heavy metals analysis

Lead and cadmium content were determined using the spectrophotometric atomic absorption equipment Shimadzu Model AA-7000, according the method indicated by the NOM-247-SSA-1-2008.

Statistical analysis

Data were processed with Statistical Analysis System software (version 9.0.; SAS Institute Inc., Cary, NC, USA), under the completely randomized model, and the means were compared with the Tukey test ($p< 0.05$).

RESULTS AND DISCUSSION

Flour yield

An analysis of whole flour of *J. curcas* had 57.17% oil and defatted flour has 42.83%. It will required 1.64 kg of whole seed to obtain 1.0 kg of whole flour; it takes 2.33 kg of whole flour to obtain 1.0 kg of defatted flour, which can be used in the fortification process of different foodstuff (Figure 1). These values can oscillate doubtlessly depending on the seed origin, which has different percentages of shell and kernel. Papantla seed had 39.1% of shell and 60.9% of kernel; it has higher values when compared to other Mexican seeds of

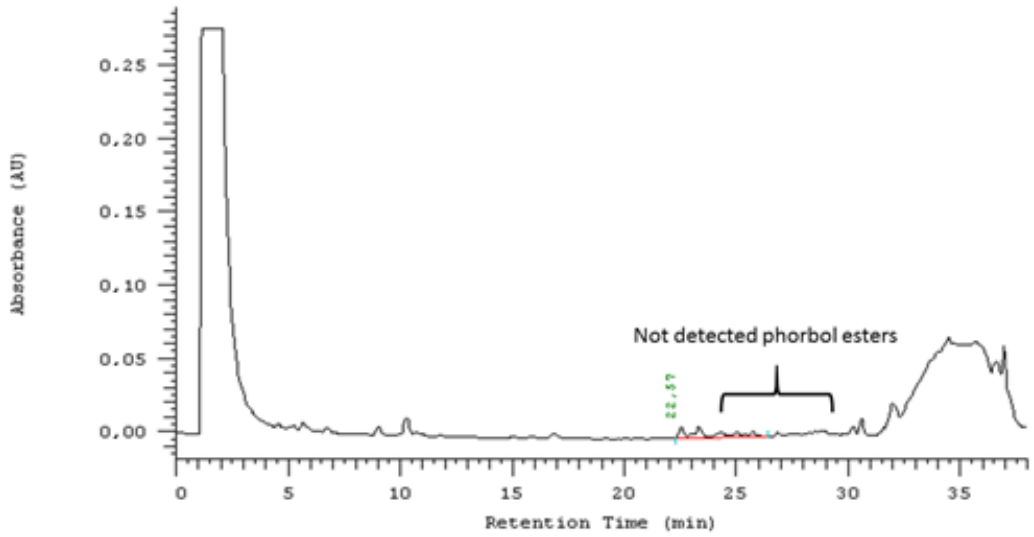

Figure 2. High resolution liquid chromatography of *J. curcas* deffated flour.

Table 1. Chemical composition of whole flour and edible defatted *J. curcas*

Chemical composition	Whole flour	Defatted flour
Moisture (%)	4.5±0.2*	6.8±0.1*
Ashes (%)	5.2±0.4*	11.4±0.2*
Protein (%)	23.5±0.1*	54.0±0.3*
Lipids (%)	52.5±0.4*	1.56±0.2*

*Average of three repetitions ± standard deviation.

different states (Makkar et al., 1997, 1998; Martinez et al., 2006, 2010).

Estimation of phorbol esters by HPLC

In defatted *J. curcas* flour used for fortification of hot cakes, there was no toxicity found. The presence of phorbol esters (PE) was not detected by HPLC, which ensure their safety for human consumption (Figure 2). Since PE are the main toxic components in *J. curcas*, Martínez et al. (2010) recommend to corroborate that the flour has not PE or that only non-toxic seeds are used to prepare the flour. Toxic seeds have never been used to make food for human consumption, only edible from Mexico that do not have PE.

Nutritional chemical composition

Table 1 shows the values of the whole and defatted *J. curcas* flour chemical composition, highlighting high protein content of flour without oils, surpassing even oilseeds such as soybeans.

Hotcakes were made by mixing defatted *J. curcas* flour at concentrations of 5, 10, 15 and 20%. Table 2 shows that the proximal composition of the different flour mixtures contains more *Jatropha* flour. It is observed that in the control, the initial protein content of 8.45% increased to 16.49%, when 20% *Jatropha* flour was added. It is noteworthy that the addition of 20% flour of *J. curcas* led to obtain fortified traditional hotcakes. In every *Jatropha* meal and wheat mixture, there were meaningful differences in protein increase. Increasing the *Jatropha* flour concentration in the mixture has a direct relation with the protein content, without affecting its organoleptic quality (flavour, color, texture) (Arguello et al., 2016). The inclusion of *Jatropha* flour is nutritionally recommendable because it improves the end product. The ash increases of 1.57% to 2.54% are likely due to the minerals that *J. curcas* seeds contain (calcium, magnesium, potassium, among others) (Heller, 1996; Toral, 2008).

Nowadays, there are rheological results of *J. curcas* used to produce bread making products, even tortillas, giving a broad perspective of use. However, it is necessary to certify that *Jatropha* flour does not have phorbol esters with the use of liquids chromatography coupled to mass. There is certain reluctance in the large-scale utilization of flour by humans due to the possible presence of phorbol esters. Nevertheless, there are no scientific conclusive studies that could demonstrate that an accumulation can exist in the human body, due to long consumption of the different food based on *J. curcas* kernel.

In the results of the microbiological analyses, defatted flour of *J. curcas* was compared with other flours based

Table 2. Proximal chemical composition of mixtures of hotcakes flour with *J. curcas*

Chemical composition	Moisture (%)	Ashes (%)	Lipids (%)	Protein (%)
Control Hotcakes	0.580± 0.21[a]	1.57± 0.26[a]	0.3±0.28[a]	8.45±0.07[a]
5%	0.8 ± 0.13[b]	1.87± 0.03[b]	0.1±0.0.0[b]	9.95±0.14[b]
10%	1.10 ± 0.11[c]	1.96± 0.06[c]	0.13±0.03[b]	11.57±01[c]
15%	0.94 ± 0.03[d]	2.24± 0.03[d]	0.2±0.18[c]	13.25±0.03[d]
20%	1.25 ± 0.05[e]	2.54± 0.46[e]	0.1±0.01[b]	16.49±0.04[e]

Means with the same letter in each column are not statistically different (Tukey, $p < 0.05$); *Average of three repetitions ± standard deviation.

Table 3. Microbiologic analysis to defatted *J. curcas* flour compared to other flour source according to the official Mexican norm (NOM-247-SSA1-2008).

Flour source	Mesophilic aerobic (UFC/g)	Coliforms Totals (UFC/g)	Fungi (UFC/g)
Wheat flour, meal, or semolina	50,000	NA	300
Defatted Jatropha flour	**7,100**	**NA**	**200**
Corn flour	100,000	100	1000
Corn nixtmalizada flour	50,000	100	1000
Rye flour	100,000	100	200
Barley flour	100,000	100	200
Oats flour	50,000	50	100
Rice flour	100,000	100	200
Whole meal	500,000	500	500

UFC = colony forming units, NA = not applied

on the Official Mexican Norm for sanitary food regulations NOM-247-SSA1-2008 (Table 3). It clearly shows that the *Jatropha* flour has low aerobic Mesophilic bacteria compared to flours of wheat, maize, rice or oats. Even fungi are low in wheat and maize, similar to the values of rye, barley, and rice floors. These determinations are very important for the use of *Jatropha* flour in human consumption. In spite of its good properties, *J. curcas* flour is not a conventional food, and up to this moment it has not been considered for human consumption, as it lacks an official norm for its use. This is the first report showing the microbiological content of *J. curcas* defatted flour. Its moisture content is less than 6.8%, helping conservation and storage. Muhammad et al. (2003) concluded that wheat flour with less than 10% moisture content is suitable for extended shelf life and reduce the microorganism attack; in *Jatropha* flour moisture was 6.8%.

Contents of lead and cadmium

Finally, lead and cadmium contents were determined to verify that these heavy metals are not found in *Jatropha* flour. The type of fertilizers used, soil pH and plant irrigation with polluted water could be the cause for the presence of these metals (Kamran et al., 2014). Jatropha flour lacks these heavy metals. Some reports of Pb content in wheat found more than 1.627 mg/kg, while the Cd content in wheat was more than 0.344 mg/kg, most of times the bran will be exceeding feed safety standards, and cannot be used for feedstuff (Wei et al., 2016). For this reason, the *J. curcas* meal is an excellent nutritional source with good sanitary quality.

Conclusion

According to the results obtained and due to lack of food with high protein in many flour based foods, it is proposed to use edible seed and *J. curcas* flour to make hotcakes and different foods for human consumption. With the addition of *Jatropha* flour, fortification of foods made with wheat flour or corn with lower protein is achieved. Besides this, with up to 20% addition of Jatropha flour, the rheological properties are not altered and the sanitary quality of this flour is higher than other conventional flours already used by the food industry.

We consider that Jatropha edible seeds can be used in other countries such as Africa or Asia, including Central

and South America as a source for the development and fortification of various food products or traditional foods.

It is noteworthy that to ensure the safety of *Jatropha* flour, one should test for the presence of phorbol esters that normally exist in toxic seeds; since an error in mixing the toxic seeds could cause a serious toxicological problem.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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