Effect of thermal treatment on the extraction efficiency, physicochemical quality of *Jatropha curcas* oil, and biological quality of its proteins

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Abstract *Jatropha curcas* seeds are an important source of oil (5–60%), used to obtain biodiesel. The generated residual paste has a high concentration of proteins (50–55%); however, the seeds contain non-nutritional factors that limit their use. The objective of this work was to analyze the effect on the physicochemical properties of the oil obtained from *J. curcas* seeds subjected to different thermal treatments and to evaluate the biological quality of the proteins contained in the residual cake. The best extraction of oil (95%) was obtained after 10 h from roasted or boiled seeds. In the oil from roasted samples, the acid index increased significantly ($p \leq 0.05$) with respect to the untreated sample, whereas the iodine index increased significantly ($p \leq 0.05$) in the oil extracted from the boiled samples. With respect to the proximal chemical

composition of the flour, roasting and boiling treatments allowed for greater oil extraction (97 and 92%), achieving, in turn, a higher content of proteins (59.56 and 58.5 g/100 g) and fiber (6.67 and 6.67 g/100 g), and lower activity of trypsin inhibitors (45 and 38%) and phytates (63 and 72%), respectively. According to the in vivo biological quality test, conducted on Wistar rats, the thermal treatments applied to the seeds improved digestibility (> 70%) and the protein efficiency index (PER). The thermal treatment allowed extracting more efficiently the oil and improved the quality of the proteins present in the residual paste.

Keywords *Jatropha* · Protein digestibility · Oil characterization · Microwave · Roast · Boil

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Introduction

Among the potential uses of *Jatropha curcas* seeds are the oil, which is used to elaborate biodiesel, hence, it is important to optimize its extraction procedure at the industrial level. Oil extraction implies diverse preliminary actions, such as cleaning, peeling, drying, and milling; but the total amount of extracted oil depends mainly on the time of extraction, temperature, moisture content, and size of the oil particle (Gutiérrez et al. 2008). Félix-Bernal et al. (2016) physically characterized the seed oil of *J. curcas* of non-toxic varieties obtained by mechanical press and reported the following fatty acids: linoleic (50.32%), oleic (26%), palmitic (13.96%), stearic (8.28%), palmitoleic (0.49), with an iodine value of 82.77 g of iodine per 100 g of oil and 0.62% non-saponifiable material. Thi et al. (2018) reported that, in addition to the fatty acids, palmitic

acid, oleic acid, linoleic acid, and stearic acid were identified with a total content of 12% in the seed kernel.

After obtaining the oil, a residual paste with high concentration of protein (50–55%) is generated. Boudjeko et al. (2013) reported that glutelins are the major proteins in this seed, followed by globulins, of which nine bands could be detected (7.3, 8.43, 14.79, 25.47, 27.18, 29.85, 47.8, 54.3, and 64 kDa) by means of SDS-PAGE electrophoresis. On the other hand, these proteins have an in vitro digestibility greater than 78% and, under the effect of heat, they exceed 88%; besides, their amino acid profile is higher than the reference standard used by FAO/WHO, except in the concentration of lysine (Martínez-Herrera et al. 2006), which could be exploited for animal or human diets. In fact, flours obtained from nontoxic *J. curcas* seeds have been used to improve the nutritional or rheological quality of some foods, such as tortillas (Arguello García et al. 2016) and hot cakes (Martínez-Herrera et al. 2016). Some other varieties present disadvantages like the presence of non-nutritional factors, including trypsin inhibitors, lectins, saponins and phytates, which can be eliminated through chemical-thermal treatments, such as boiling (Martínez-Herrera et al. 2006; Xiao et al. 2015). Treatments such as roasting, microwaving or boiling allow improving the nutritional quality of the press cake to achieve the extraction of more oil in less time (Chemat et al. 2011; Popov et al. 2016). However, it is important to determine if thermal treatments modify the physical, chemical, and biological characteristics of proteins and oil. The objectives were to analyze the effect on the physicochemical properties of the oil obtained from *J. curcas* seeds subjected to different thermal treatments and to evaluate the biological quality of the protein of the press cake.

Materials and methods

Material

Seeds of non-toxic *J. curcas*, from the Centro de Productos Bioticos (CEPROBI-IPN) in Yautepec, state of Morelos, Mexico, were used. For the biological test, the experimental animals were obtained from the bioterium of the National School of Biological Sciences (ENCB of IPN); the protocols accepted by the institutional bioethics committee were followed.

The seed coat was removed to release the kernel, four batches were formed: untreated (U), roasted (R) (180 °C, 20 min), boiled (B) (121 °C, 15 min, 1.05 kgf/cm²), microwaved (M), (2 min/490 W, three cycles).

Extraction and evaluation of physicochemical properties of *J. curcas* oil

After treatments, seeds were pulverized to obtain flour (60 mesh). Oil was extracted with hexane by the Soxhlet method (70 °C, 10 h). A kinetic study was performed to evaluate the effect of the heat treatment on the oil extraction performance as a function of time; the solvent was removed at room temperature under an extraction hood, and the oil was recovered in a rotary evaporator (270 mbar/50 °C at 50 rpm for 30 min).

Odor, color, density and refractive index, saponification, acidity, iodine, peroxide, and esters were determined according to the methods reported by the AOAC (2005).

Proximal chemical analysis of defatted *J. curcas* seed meal

Chemical composition of the samples was determined according to AOAC (2005) guidelines, which comprise the following analyses: (a) moisture (method 925.09), (b) crude protein (method 954.01, NX6.25), (c) crude fat (method 920.39), (d) crude fiber (method 962.09), (e) ashes (method 923.03), carbohydrates content by difference to 100%.

Determination of non-nutritional compounds in *J. curcas* flour

Trypsin inhibitor activity was determined according to the method of Smith et al. (1980). Phytic acid content was determined by a colorimetric procedure (Vaintraub and Lapteva 1988). Total saponin content was determined using a spectrophotometric method (Hiai et al. 1976). Total phenolic compounds content was estimated by the Folin-Ciocalteu colorimetric method (Singleton et al. 1999).

Determination of the biological quality of the protein

The press cake (rich in proteins) of *J. curcas* from the three thermal treatments was evaluated, casein was used as reference. Five groups were formed of eight Wistar rats (20–23-day-old), with 10 animals each, a weight difference not greater than 5 g; animals were housed in individual cages under controlled conditions (20 °C; 55% relative humidity; 12 h light/12 h dark cycle). The diet composition was of 10 g protein, 10 g corn oil, 4 g mineral mixture, 1 g vitamin mix, 5 g cellulose, and 70 of corn starch per 100 g. Vitamin (AIN-93-VX) and mineral (AIN-936-MX) mixes were obtained from Harland Tekland Laboratory Animal Diets (Madison, WI). Food and water were provided ad libitum. The protein efficiency ratio (PER) was calculated by feeding the rats with the test diets for 28 days and

measuring food intake daily and weight gain weekly (Eq. 1). The net protein ratio (NPR) was evaluated over a 14-day period by feeding a separate group of 10 animals with a protein-free diet for 14 days, measuring endogenous nitrogen uptake and average weight loss (Eq. 2). In the animal group fed with the protein-free diet, true digestibility (TD) was corrected for endogenous nitrogen feces excretion (Eq. 3). Protein digestibility was calculated by collecting feces between days 14 and 21 of the assay period, and, then, drying, weighing, milling, and storing until protein determination. Feed and fecal nitrogen contents were analyzed by the Kjeldahl method (955.04; AOAC 2005).

$$PER = \frac{\text{Weight gain (g)}}{\text{consumed protein (g)}} \quad (1)$$

$$PER(\text{corrected}) = \frac{PER * 2.5}{PER \text{ in rats that consumed casein}} \quad (2)$$

$$NPR = \frac{WTA - (WLP - WPF)}{\text{consumed protein (g)}} \quad (3)$$

where WTA weight gain of the test animals, WLP weight loss of the protein, WPF weight of protein in the protein-free diet group.

$$\text{True digestibility} = \frac{Nc - (Ne - Nef)}{Nc} * 100 \quad (4)$$

where Nc nitrogen consumed, Ne nitrogen excreted, Nef Nitrogen excreted in the protein-free diet group.

All determinations were performed at least in triplicate and the mean values were reported $\pm$ standard deviation (SD). Comparison of means was carried out according to Tukey's test ($p \leq 0.05$).

Results and discussion

Extraction and physicochemical analysis of seed oil from *J. curcas*

Figure 1 shows the kinetics of oil extraction from *J. curcas* seeds without treatment (U), R, B, and M, maximal oil extraction was obtained after 10 h (95%) with either the toasted or boiled seed. The heat treatment makes the extraction of lipids more efficient, because the applied heat increases cells' porosity, by weakening the cell membrane and making it more accessible for the solvent (Ramos et al. 2017).

Physical and chemical characteristics of *J. curcas* oil are shown in Table 1. In the obtained oils, there were no differences between the refractive index and the density. In a study where cumin oil was extracted, with a thermal

Fig. 1 Oil extraction kinetics from *Jatropha curcas* seeds, after different thermal treatments: Untreated (U), Roasted (R), Microwaved (M), Boiled (B)

treatment by microwaves and conventional methods, no refractive index differences were found, suggesting that these methods do not affect the quality of the oil (Behara et al. 2004). In the samples that received M or R treatment, the acid index increased with respect to the U sample; this increase agrees with that reported by Tan et al. (2001) for corn and soybean oil, where the acid number increased due to the effect of the treatment temperature. In fact, it is known that free fatty acids are formed during oxidation, hydrolysis, and pyrolysis, as a result of the breakdown of triglycerides (Ulusoy et al. 2004). The change was lower compared to that obtained by Sirisomboon and Kitchaiya (2009) in seeds of *J. curcas*, when subjected to thermal treatments (40–80 °C), their acidity index was 0.175 (40 °C) and 0.661 (80 °C). In the B sample, the acidity index was lower. Other authors have reported a decrease in the level of free fatty acids by increasing the treatment temperature, an effect that has been attributed to the reduction of the activity of hydrolytic enzymes, such as lipases and peroxidases (Özdemir et al. 2001).

Regarding the saponification index obtained from each of the treatments (Table 1), the values found are similar to those found in olive oil (184–196), colza (168–181), sunflower seed (188–194), and pumpkin seed (174–197) (Nichols and Sanderson 2002); in R seeds, this value was lower, which indicates that roasting decreases the amount of ester bonds.

Jatropha curcas oil samples showed an average iodine index of 97.65 g I_2 /100 g; it is important that this value is not greater, because heating of the oils and their unsaturated fatty acids will result in polymerization of glycerides. In the oil obtained from the M and R samples, a slight increase in the iodine index was observed compared to the

Table 1 Physicochemical characterization of *J. curcas* seed oil subjected to different treatments

	Untreated*	Microwaved	Boiled	Roasted	NMX-F-590-SCFI-2009
Refractive index	1.459 ± 0.001 ^a	1.454 ± 0.000 ^b	1.458 ± 0.000 ^a	1.457 ± 0.001 ^a	1.471 max.
Specific gravity	0.866 ± 0.002 ^a	0.819 ± 0.002 ^b	0.823 ± 0.005 ^b	0.859 ± 0.015 ^a	0.916 max.
Saponification index (mgKOH/g)	186.90 ± 0.59 ^a	185.22 ± 0.51 ^{ab}	184.59 ± 1.08 ^b	158.43 ± 0.31 ^c	185–210
Acid index (%)	5.06 ± 0.34 ^b	5.38 ± 0.02 ^b	4.39 ± 0.07 ^c	6.09 ± 0.25 ^a	8 max.
Iodine index (gI ₂ /100 g)	96.22 ± 0.45 ^c	98.44 ± 0.22 ^b	101.55 ± 0.39 ^a	94.41 ± 0.98 ^c	95–110
Peroxide index (mEq O ₂ /kg oil)	0.54 ± 0.05 ^{ab}	0.50 ± 0.09 ^b	0.54 ± 0.06 ^{ab}	0.70 ± 0.11 ^a	–
Index of esters	181.85 ± 0.25 ^a	181.58 ± 0.06 ^a	180.21 ± 1.02 ^a	156.02 ± 0.28 ^b	–

Different letters in the same row indicate a significant difference ($p \leq 0.05$)

*Without heat treatment

U sample, which may be due to the high temperatures of the material in a short period of time, which causes changes in fatty acids and triacylglycerols. because the iodine value of a mixture of fatty compounds depends on the amounts of the different unsaturated fatty components in the mixture (Knothe 2002). The effect of roasting can be attributed to the reduction in the number of unsaturated sites as a result of the oxidation, polymerization, or rupture of long-chain fatty acids (Anjum et al. 2006).

Peroxide index of untreated *J. curcas* seed oil and in the M sample had values of 0.54 and 0.5 mEq/kg, respectively, demonstrating the relative oxidative stability of the oil. These values were lower than those found by Anjum et al. (2006) in oil extracted from *J. curcas* from Malaysia. The low value of the peroxide index indicates the slow oxidation of these oils. The formation of peroxide is slow at the beginning during an induction period that can vary from a few weeks to several months, according to the oil and the temperature in particular; similar results have been reported in crude peanut oil, sun-dried, and roasted, observing that the oil extracted from the latter can resist better lipolytic hydrolysis and oxidative deterioration (Ayoola and Adeyeye 2010).

Proximal chemical analysis of defatted *J. curcas* seed meal

Table 2 shows the proximal composition of the *J. curcas* flour. Sample M presented a lower protein concentration (49.65 g/100 g), whereas sample B and R had a similar value to that of U samples. In the defatted meal, the protein content increased from 22.85 ± 0.51 to $59.64 \pm 0.74\%$. In a study of detoxifying *J. curcas* seeds with thermal treatment, Jarra Arroyo et al. (2014) reported a loss of nitrogen, indicating that this phenomenon could be due to the thermolability of some proteins; on the other side, no changes have been reported in the concentration of proteins after an autoclave treatment at 121 °C (Martínez-Herrera et al. 2006).

The thermal treatments applied to the seeds improved the oil extraction efficiency, observing that in the untreated sample, the residual oil content was of 7.35 g/100 g, whereas in the treated samples the values were 6.47, 4.6, and 2.02 g/100 g for samples M, B, and R, respectively. The ash content of the four samples did not show significant differences (10.88 to 12.2 g/100 g, whereas fiber content diminished from 7.76% (U) to 4.07, 5.84, and 6.67% for M, B, and R, respectively, this decrease was more marked in the M sample; this could have been due to a partial digestion of the fiber by the applied temperatures. The carbohydrates content had significant variations (13.5–28.9%), being higher than that reported by other authors (Martínez-Herrera et al. 2006).

Determination of non-nutritional compounds

Concentrations of the diverse non-nutritional factors in *J. curcas* with and without treatment are presented in Table 3. As observed, trypsin inhibitors diminished from 32% (M) to 45.3% (R) respect to U; obtained results were lower than those reported by Abou-Arab and Abu-Salem (2010), where a 100% inhibition was obtained, this difference could be due to the time and temperatures used (30 min/160 °C). The content of phytic acid in U was 4.70 g/100 g, with the thermal treatment the diminution was larger than that reported by Martínez-Herrera et al. (2012); the concentration of the latter compound in the treated samples varied from 0.128 g/100 g in B to 0.166 g/100 g in R, observing a higher reduction (72.65%) in B, followed by M (64.96%), and in R the reduction was of 64.53%. The loss of phytates can be due to the combination of heat and/or migration between the matrix and the boiling medium (Hurrell et al. 2002).

The content of phenolic compounds diminished in M (7.83%) and R (4.89%) with respect to U, whereas, in B, the total phenolic compounds increased (31.97%) (Table 3). The decrease of the phenolic compounds in M and R could be caused by degradative oxidation, which

Table 2 Proximal chemical composition of the flour from *J. curcas* seeds (g/100 g in dry base) subjected to different treatments

	Defatted seed meal				
	Raw seed*	Untreated**	Microwaved	Boiled	Roasted
Protein	22.85 ± 0.51	59.64 ± 0.74 ^a	49.65 ± 0.21 ^b	58.48 ± 0.51 ^a	59.56 ± 0.21 ^a
Lipids	61.52 ± 0.18	7.35 ± 0.3 ^a	6.47 ± 0.67 ^a	4.60 ± 0.19 ^b	2.02 ± 0.71 ^c
Ash	3.62 ± 0.03	11.72 ± 0.33 ^a	10.88 ± 0.45 ^b	11.75 ± 0.02 ^a	12.20 ± 0.53 ^a
Fiber	5.18 ± 0.05	7.76 ± 0.03 ^a	4.07 ± 0.03 ^d	5.84 ± 0.01 ^c	6.67 ± 0.05 ^b
Carbohydrates ¹	6.83	13.53	28.93	19.33	19.55

Different letters in the same row indicate a significant difference ($p \leq 0.05$)

*Without defatted treatment, **without heat treatment, ¹Calculated by the difference regarding the other components

Table 3 Analysis of non-nutritional compounds of *J. curcas* seed subjected to different treatments

	Untreated	Microwaved	Boiled	Roasted
Trypsin inhibitors*	85.81 ± 0.53 ^a	58.44 ± 0.29 ^b	53.25 ± 0.11 ^c	46.94 ± 0.46 ^d
Phytates**	4.70 ± 0.10 ^a	1.60 ± 0.10 ^b	1.30 ± 0.40 ^b	1.70 ± 0.30 ^b
Total phenolics**	6.10 ± 0.10 ^b	5.60 ± 0.10 ^c	8.10 ± 0.10 ^a	5.80 ± 0.10 ^c
Phenolic condensates**	1.80 ± 0.05 ^b	1.40 ± 0.01 ^c	1.00 ± 0.10 ^d	2.80 ± 0.01 ^a
Saponins**	0.17 ± 0.01 ^a	0.13 ± 0.01 ^b	0.03 ± 0.00 ^c	0.14 ± 0.01 ^b

Different letters in the same row indicate a significant difference ($p \leq 0.05$)

*mg of inhibited trypsin/g of flour, **mg/g of flour

seems to be accelerated with the increase in temperature (Nasar-Abbas et al. 2008). The content of condensed phenolic compounds in the flour of *J. curcas* diminished significantly in M and B (22% and 44% less than in U); however, in R, they increased 50% as compared to sample U. Although this increase is irregular, it has been observed in other seeds like in cocoa clones (Zapata Bustamante et al. 2014).

The content of saponins in *J. curcas* was lower than that of the other non-nutritional compounds. In this study, the content was reduced 17.86% in R and 82.95% in B. Thermal treatment has a greater effect on the degradation of saponins because saponins B have a sugar chain linked to position C-3 of its aglycones (sapogenol B) with one hydrogen atom in position C-21 (Chen et al. 2007). This link between the sugar chain and sapogenol B breaks; aglycones can also be decomposed when high energy is applied, such as heating at high temperatures. Hence, the thermolabile saponins are degraded during boiling. The reduction in B could have been due to the water- and oil-soluble characteristics of saponins B, thus, there might be some in the eliminated oil (Shi et al. 2004).

Assessment of protein quality

As observed in Fig. 2, in general, rats fed with *J. curcas* seeds had lower weight gain, during the experiment, as compared to those that consumed the diet with casein as

protein source; however, rats that consumed seeds with some of the thermal treatments gained more weight than those consuming non-treated seeds ($p < 0.05$), obtaining better results in the groups fed samples M and B. In both cases, 3-times more weight gain was observed, suggesting that the seed is adequate to be consumed as food. Rakshit et al. (2008) reported a weight loss in male rats fed with a toxic variety of seeds of *J. curcas*, crude and detoxified with alkaline thermal treatment (− 11.2 to − 8.3 g), and mortality occurred between days 8 and 12. León-López et al. (2015) evaluated the protein intake isolated from *J. curcas* without any treatment, and reported that, in the short-term, its consumption increased serum glucose, insulin, triglycerides, and cholesterol levels, as well as the expression of transcription factors involved in lipogenesis and cholesterol synthesis, and increased insulin signaling; thus, it is important to determine the best way to eliminate any toxic compounds and to carefully select the seed.

Figure 2 shows the protein efficiency compared to the diet containing casein as reference protein; the protein of crude *J. curcas* underwent a 78% reduction; however, the efficiency improved with thermal treatments, particularly in M and B, where, although the efficiency value was below that observed for casein, it was 3-times higher than in the rats fed the U diet. This phenomenon can be related, on one side, to the inactivation of trypsin inhibitors and, on the other side, to the reduction in the concentration of saponins.

Fig. 2 In vivo assessment of the biological activity. **a** Weight gain. **b** Protein efficiency relationship (PER). **c** Ratio of net protein (RNP). **d** True digestibility in rats fed *Jatropha curcas* seeds subjected to microwave (M), roasting (R), and boiling (B) treatments, untreated (U), and casein (C)

Figure 2 depicts the proportion of net protein, which is 43% lower than for casein, this proportion increased 46% in M and 100% in B, with respect to the protein in U; it must be pointed out that for B, the RNP was similar to that of casein. The actual digestibility of the U protein was 48% lower than that of the reference protein and the digestibility improved 62% and 75% in R and B, respectively, whereas M showed no effect on digestibility. Aregheore et al. (2003) evaluated the quality and the consumption of the press cake of *J. curcas* seeds from Cabo Verde, Nicaragua, detoxified by chemical means and heating in microwave, and their results showed that the thermal treatment diminished the content of lectins and increased the digestibility of proteins.

Conclusion

Thermal treatments applied to *J. curcas* seeds allowed improving the oil extraction process, as well as diminishing most of the non-nutritional compounds contained in the seed. The latter modified the biological quality of the proteins contained in *J. curcas* flour, leading to a greater weight gain in the experimental animals. Of the thermal treatments, boiled led to attain the best protein efficiency

value, increasing it 5 times with respect to the untreated samples. Roasting led to a better digestibility value. In both conditions, the use of thermal treatments allowed diminishing the non-nutritional factors to levels at which they do not interfere with nutrients absorption and improved oil extraction without altering substantially its quality. Therefore, after extracting the oil for agroindustry use as biodiesel, the residual paste could be used as a protein source in the preparation of food for human and animal consumption.

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