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CHARACTERIZATION OF *JATROPHA CURCAS* L. SEED AND ITS OIL, FROM ARGENTINA AND PARAGUAY

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Abstract

Jatropha curcas L. is being analyzed in Argentina as a source of oil for biodiesel production. That is why the physical properties of its seed are important in designing and manufacturing equipment and structures for handling, transporting, processing and storage, and also for assessing its quality. Two different sources were taken into account: one coming from Paraguay and the other from Argentina, this last originally intended for sowing. Seeds sown in Argentina came from plants grown from Paraguayan seeds. Several physical properties as well as the proximal analysis were measured. High levels in protein (26,2%) and carbohydrates (56,5%) for press extraction cake were found. Possible uses are discussed.

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The fatty acid composition of the oil from both types of seed has been characterized by gas chromatography (GC). The oil is highly unsaturated, being linoleic acid the most important (42,6% for Paraguayan and 53,3% for Argentinian oil). The main feature for both types of oil is their high acidity (26.8 mg KOH / g of oil) which prevents direct transesterification.

Key words: biodiesel production; *Jatropha curcas* L.; press extraction; lipid composition

Resumen

El cultivo de *Jatropha curcas* L. se está analizando en la Argentina como fuente de aceite para la producción de biodiesel. Es por eso que las propiedades físicas de sus semillas son importantes en el diseño y fabricación de equipos y estructuras para el manejo, transporte, tratamiento y almacenamiento, así como para evaluar su calidad. Dos fuentes diferentes de semillas de jatropha se han tenido en cuenta: una procedente de Paraguay y la otra de Argentina, esta última originalmente destinada a la siembra. Las semillas sembradas en la Argentina se obtuvieron de plantas originadas de semillas del Paraguay. Se determinaron varias propiedades físicas, así como el análisis proximal de estas semillas. Se encontraron altos niveles de proteínas (26,2%) y carbohidratos (56,5%) para la torta de extracción por prensado. Se discuten sus posibles usos. La composición de ácidos grasos del aceite para ambos tipos de semillas se ha caracterizado por cromatografía de gases (GC). El aceite es altamente insaturado, siendo el ácido linoleico el más importante (42,6% en el caso de Paraguay y 53,3% para el aceite de argentina). La característica principal de ambos aceites es su alta acidez (26,8 mg KOH / g de aceite), lo que impide la transesterificación directa.

Palabras clave: producción de biodiesel; *Jatropha curcas* L.; extracción por prensado; composición lipídica

Introduction

As the future lack of petroleum is a current concern, biodiesel seems to be "part of the solution", by replacing partial or totally petro-diesel fuel in diesel engines. This reason, added to an increasing environmental concern, creates a scenario in which biodiesel production is expected to have a big development over the next few years.

Biodiesel is an alternative fuel produced from different types of renewable vegetable oil, animal fats or different types of recycled cooking oil, by transesterification reaction.

In conventional processes, biodiesel is manufactured by alkaline catalyzed transesterification of oil, in methanol [1]. The alkalis frequently used are KOH, NaOH or their corresponding alkoxides. Some solid catalysts were also assayed [2].

Nowadays petro-diesel is more used because it is less expensive to produce than the renewable fuel, but in the future this will probably change. Nevertheless, since 2010 the new Argentinian Regulations impose that the total amount of diesel sold in the country must contain at least 5% of biodiesel [3].

The oil to produce biodiesel can be obtained from different crops such as soy, rapeseed, *Jatropha curcas* L. and others. Nowadays, Argentinian biodiesel is largely made out of soy oil, but the industry is considering the possibility of using jatropha oil [4]. The main differences are: 1- the jatropha seeds, due to its toxicity, are not edible [5], 2- jatropha crop can tolerate harder climate conditions than soy and rapeseed [6], 3- the jatropha seeds can give four times more oil than soy [7] and 4- the aptitude of the jatropha oil extraction residue to recover infertile land [8].

An investigation has shown the good conditions existing for jatropha growth in Argentina [4]. As jatropha oil is not edible, it does not compete in the food market, resulting in a low-cost feedstock. Besides, jatropha does not compete for land use with rapeseed or soybean. These last two characteristics turn jatropha in a "second generation crop", which means that obtaining biofuels from it requires less resources than the "first generation (edible) crops"[9].

In this research, the quality of *Jatropha curcas* L. oil obtained from Paraguayan and Argentinian seed was evaluated. The proximal analysis of seed shown in this work can as a guide to determine how to manage the seed and the possible uses of the oil extraction residue. Also, the physical characteristics of seed are useful in designing storage facilities, transportation and drying processes related. Finally, the characterization of jatropha oil is useful to define all the operational units needed to produce a biofuel according to 2010 Argentinian Regulations.

Materials and Methods

The seeds used in this research came from two sources: the light fraction from Horqueta town, department of Concepción, Paraguay; and seeds from Colonia Sudamérica, department of Pilcomayo, province of Formosa – Argentina. These last seed came from plants grown from Paraguayan seeds. They all were stored in well closed bags in a dark and cold place until milling.

Physical analyses of the seeds

Six groups of 100 seeds each were weighed with an analytical scale (OHAUS Adventurer). Its average weight and standard deviation were calculated from these values (W100S). The weight of one grain was calculated as W1S = W100S / 100.

The dimensions of the seed were chosen as shown in Figure 1, and measured with a 0,05mm precision caliber. Each sample was compound by thirty seeds. The mean and standard deviation value were obtained for each dimension.

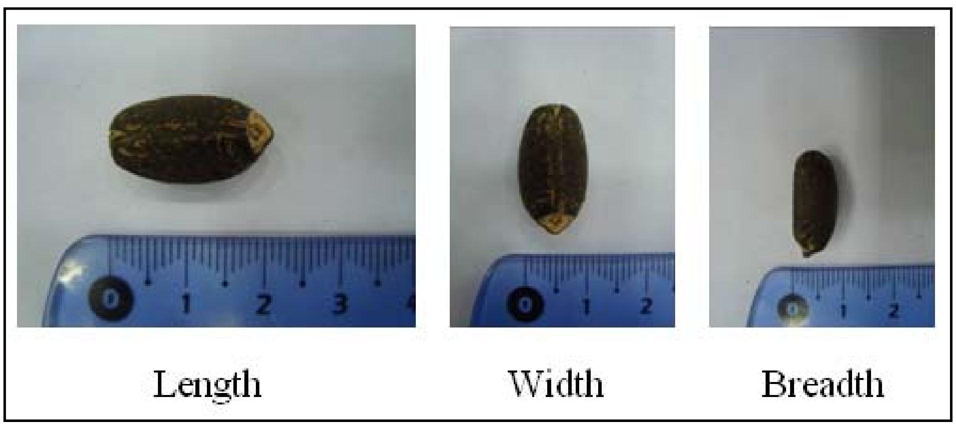

Figure 1. Dimensions of a sample of a typical seed of *Jatropha curcas* L.

For the relative density measure, the usage of the pycnometer method [10] was modified because the size and shape of the grain prevent us from using conventional pycnometers. Procedure: twenty entire grains were introduced in a 100 mL volumetric flask, filled with benzene up to the mark level and weighed in the analytical scale. The following expression was used to calculate the relative density:

$RD = (m_B – m_A) / [100 – (m_C – m_B) / \delta_{BENZ.}]$

Where: RD = relative density.

m_A = mass of the empty flask with the top on.

m_B = mass of the flask containing 20 seeds (with the top on).

m_C = mass of the flask containing 20 seeds with benzene to the mark level (with the top on).

$\delta_{BENZ.}$ = benzene density.

For the apparent density measure, a 250 mL graduated cylinder was filled with seeds and weighted. Apparent density was calculated as the ratio: mass of the seeds (g) / 250 mL. Every measure of apparent and relative density was done three times and the mean and standard deviation values were obtained.

For the Student t test, Excel Microsoft Office Professional Edition 2003 software was used. A Two-tailed test was performed and different variances for each group of data were assumed. A 95% Confidence limit (α=0.05) was used in all cases.

Proximal analysis

Ashes (A) were measured by total calcination until constant weight, in a programmable muffle (program: from 25ºC to 100ºC rate of 12ºC/min, 100 ºC for one hour, 100ºC to 300ºC rate of 12ºC/min, 300ºC for 3 hours, 300ºC to 600ºC rate of 12ºC/min, 600ºC for 3 hours, 600ºC to 900ºC rate of 12ºC/min, 900ºC for 10 hours). The fat contained (FC) in seeds was measured by solvent extraction using Soxhlet apparatus. The commercial hexane used was previously distillate (bp: 64 – 67 ºC). The seed's humidity (H) was obtained by oven drying at 100 ºC until constant weight. The protein content (PC) was obtained by Kjedahl method (BÜCHI K 424 digester, BÜCHI K 350 automatic distiller). A 6.25 factor was used to transform the percentage of organic nitrogen, into percentage of protein [11]. The Carbohydrates content (CH) was obtained using the following equation:

$$\%CH = 100\ \% - (\%H + \%A + \%FC + \%PC)$$

Oil analysis

The fatty acid characterization was done for both types of oil, Argentinian and Paraguayan, by gas chromatography. The sample for this assay was the oil extracted by Soxhlet. Both types of oil were derivatized through a two-step methylation procedure: 1-alkaline hydrolysis followed by unsaponified matter remotion by solvent extration and 2- fatty acid esterification in methanol / sulfuric acid [12]. The FAME obtained was injected onto a VARIAN 3700 chromatograph, using a PEGS 5% on SUPERGOPORT 80/100, as a stationary phase. 1 mL/min N_2 carrier gas was employed. Inyector and detector (FID) temperatures were 220 ºC and 200 ºC, respectively. Column temperature was programmed from 130 ºC (5 min) to 170 ºC, at 4º/min. Identification of FAME was carried out using proper reference compounds (SIGMA single component sol.).

Before screw pressing, seeds were first grinded twice in a rolling grinder (first path: 9.10 mm; second path: 5.40 mm), and then humidified up to 10%, by adding and mixing a pre-determined amount of distilled water. After moistening, the samples were well-sealed in polyethylene bags and stored in a refrigerator at 5 ºC for at least 48 hours and agitated frequently to allow uniform moisture distribution throughout the sample.

The oil was extracted in hot (70º C approximately) with a screw press, and then it was centrifuged (50mL plastic flask, 10 minutes, 22800g) and vacuum filtered. The oil aptitude for biodiesel production was evaluated through several parameters: refraction index (Misco PA202 digital refratometer) at 25 ºC, iodine index was calculated from fatty acid composition [13], phospholipid content was evaluated following the Stewart procedure, which is based on the colorimetric reaction with ammonium ferrothiocyanate [14], saponification index (SI) and acid number (AN) were measured following AOAC procedures (920.160 and 966.18 (adapted) respectively), the esther index was calculated substracting AN from SI, and unsaponifiable matter was analyzed following AOAC 933.08 method [15].

Calorific value for the residue (the cake) obtained by press has been calculated through the following equation [16]:

$$\Delta H_T = (\Delta H_{CH} \times \%CH + \Delta H_F \times \%FC + \Delta H_P \times \%PC)/100$$

CV[MJ]=-16.5268 MJ/Kg x CH% -42.4558 MJ/Kg x FC% -21.7884 MJ/Kg x PC%/100

Where: CV= Calorific value

ΔH_{CH} = Carbohydrates heat of combustion

ΔH_F = Fat heat of combustion

ΔH_P = Proteins heat of combustion

Results and discussion

The results of the physical properties and the proximal analysis are listed in Table 1.

Table 1. Physical and proximal analysis of seed.

Properties	Units	Paraguayan seed	Argentinian seed
100 seed weight	g	61.8 ± 1.7	60.1 ± 0.1
1 seed weight	g	0.62 ± 0.02	0.601 ± 0.001
Length	cm	1.8 ± 0.1	1.78 ± 0.08
Width	cm	1.10 ± 0.06	1.07 ± 0.04
Breadth	cm	0.86 ± 0.06	0.78 ± 0.03
Relative density	g/cm^3	0.71 ± 0.01	0.81 ± 0.08
Apparent density	g/cm^3	0.37 ± 0.01	0.46 ± 0.01
Humidity	%w/w	6.5 ± 0.1	7.2 ± 0.1
Total ashes (d.b.%)	%w/w	4.50 ± 0.05	4.55 ± 0.07
Fat content (d.b.%)	%w/w	28.6 ± 0.4	39.8 ± 0.2
Protein content (d.b.%)	%w/w	22.57 ± 0.13	19.59 ± 0.04
Carbohydrates (d.b.%)	%w/w	44.1 ± 0.4	38.0 ± 0.2
d.b.%: Dry basis percent. Data are expressed as mean ± standard deviation			

The differences between physical properties of Paraguayan and Argentinian seeds were evaluated by t tests of their means. Results are shown in table 2. It could be established that both the length and breadth of Paraguayan and Argentinian seeds were different but not their average weights, in a 95% confidence level. This could explain the differences in both relative and apparent densities between the two groups of seeds. It is important to mention that the relative density shows how seeds are randomly stacked in a silo and it is strongly related with the shape of the seeds.

Table 2. Results of T tests for Physical properties of *jatropha* seeds.

Weight of 100 seeds	Length of one seed	Width of one seed	Breadth of one seed	Relative density	Apparent density
0.053	0.034	0.054	1.3×10^{-7}	1.8×10^{-4}	2.5×10^{-3}
Results Show α value for each property					

The low moisture in both Paraguayan and Argentinian seeds would prevent them to be attacked by microorganisms.

Temperature control and light exposure on the seeds are very important, because of their high fat content. The seeds have a high level of protein and carbohydrates, also susceptible to microorganism's degradation. Paraguayan seeds have more of both components, while Argentinian ones have more oil. Comparing to soy, Argentinian jatropha has about 19 percent more oil [17], which is better for biodiesel production, being the oil the main raw material. Nevertheless, there are significant differences between seeds that make important the necessity of taking care about their quality in order to make profitable the oil extraction for biodiesel production. Comparing *jatropha* with other seeds (e.g. soy and rapeseed), there is a high protein content that could be used as animal feed but, taking into account the lack of knowledge of its characteristics and also, due to the seed's toxicity, further studies should be carried out.

The result of the fatty acid characterization of both types of oil is described below:

Table 3. Fatty acid characterization of oil from both types of seed.

	Percent fatty acids (FA)							
	16:0 Palmitic FA	18:0 Stearic FA	18:1 Oleic FA	18:2 Linoleic FA	Others	Sat. FA	MUFA	PUFA
Argentinian seed	10.0 ± 0.7	5.4 ± 0.3	30.2 ± 0.9	53.3 ± 2.1	1.1 ± 0.1	15.4 ± 1.0	30.2 ± 0.9	53.3 ± 2.1
Paraguayan seed	13.2 ± 0.9	3.0 ± 0.2	40.2 ± 1.6	42.6 ± 1.3	1.0 ± 0.1	16.2 ± 1.1	40.2 ± 1.6	42.6 ± 1.3

MUFA: Mono-unsaturated FA. PUFA: Poly-unsaturated FA. Data are expressed as mean ± standard deviation

While the level of saturated fatty acid in both types of oil is nearly the same, the Argentinian seed oil is more unsaturated. This means a less stability due to oxidative processes. The biodiesel obtained from the Argentinian oil would be less stable but with better performance in cold weather [18], although the difference in fatty acid composition between both types of oil is not as high as to be considered important.

The results of the tests made on the Paraguayan seeds oil, extracted by screw press, are listed in Table 4. Experiences with Argentinian seeds were not performed since the available amount of them was not enough to feed the screw press.

The refraction index is a characteristic of the oil and it helps to check its purity. According to the iodine index value, this oil is classified as semi-drying [23]. The phospholipids level in jatropha crude oil is markedly lower than those from soy (1.5 to 2.5%)[24] and rapeseed (2.1 to 2.8%)[25]. Nevertheless, phospholipids must be eliminated through degumming before producing the biofuel. If not, their amphipathic nature would make difficult the process of phases separation during biodiesel production and also, because the law prohibits phosphorus contents in biodiesel above 10 ppm [3]. The acid number of 26.75 for jatropha is higher than the typical values of soy and rapeseed oil (1.0 and 2.0, respectively [26]). This result is not surprising, since values as high as 30 mg KOH / g oil can be found in previous researches [27]. While the high acidity could be attributed to the action of lipases as a result of prior wetting of the seed before oil extraction, it is true that such a process is needed to obtain a high yield of oil. As high levels of acidity make uneconomic the traditional process of transesterification, new approaches are necessary. Some alternatives have been tried [9].

Table 4. Characteristics of Paraguayan seed oil extracted by crew press-

Assay	Results of Paraguayan oil tests	Bibliographic data [19,20,21,22]
Refractive index	1.4692 ± 0.0005	-
Iodine index (g de I_2 /100 g of Oil)	112.4	92 - 112
Phospholipids content (%)	0.46 ± 0.06	-
Saponification index (mg KOH/g of Oil)	180.6 ± 1.1	102.9 - 209.0
Acid number (mg KOH/g of Oil)	26.75 ± 0.06	0.92 - 28
Esther index (mg KOH/g of Oil)	153.9 ± 1.2	74.9 - 208.9
Unsaponified matter %	10.05 ± 0.01	0.79 - 3.80
Data are expressed as mean ± standard deviation		

The proximal analysis of the Paraguayan jatropha cake resulted from the press extraction is shown in Table 5.

Table 5. Proximal analysis of the Paraguayan jatropha cake obtained from the press extraction.

Humidity	Fat (d.b.%)	Proteins (d.b.%)	Ashes (d.b.%)	Carbohydrates (d.b.%)
10.2 ± 0.5	10.94 ± 0.01	26.2 ± 0.2	6.38 ± 0.01	56.5 ± 0.5
Data are expressed as mean ± standard deviation				

It can be appreciated that an important concentration of carbohydrates, together with the ashes, indicates high values of fiber. This, combined with a high protein value, is indicating that the residue should be considered for animal feeding. Nevertheless, antinutritional factors like curcin should be previously removed [19]. A second alternative for oil extraction residue is as land fertilizing, as pointed out in the bibliographic data [10].

The use of the residue as fuel for boilers can be another possibility. Its energy value was calculated from the cake's composition as indicated in 2.4. The result is shown in table 6 and compared with some renewable and nonrenewable energy sources. Although more accurate estimations should be done through calorimetric studies, the jatropha cake seems to be better than wood and peat for calorific uses.

Table 6. Calorific values from some common sources (MJ/Kg) [28].

Coal[a]	Wood[b]	Petrol	Ethanol	Peat[c]	Jatopha curcas L.[d] (calculated)
32-42	16	44.8-46.9	30	16	19.8
[a] 5–10% water [b]15% water [c] 20% water [d]10.2% water					

Conclusions

Jatropha curcas L. can be a revolutionary crop in the Argentinian biodiesel industry, by replacing or complementing the use of soy to produce the renewable fuel. It has many advantages in comparison to soy and rapeseed, (e.g. it does not compete for land use, it is not an edible crop and it has more oil). Nevertheless, nowadays lack of information about some crucial aspects, like vulnerability to national pests, diseases and climatic adaptability, are the main obstacle to its large scale development. The main difference between jatropha's and other oil sources for fuel production is the higher acid number, which affects the processing for biodiesel production.

About the jatropha cake obtained by pressing, three different uses can be pointed out: 1- as animal feed (studies should be performed); 2- as fertilizer for exhausted lands, and 3- as boiler fuel.

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