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Variability in oil content and composition and storage stability of seeds from *Jatropha curcas* L. grown in Mozambique

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ABSTRACT

This study evaluates the variation in oil content and composition of *Jatropha curcas* L. seeds from 12 origins, grown in Mozambique, under the same edapho-climatic and agronomic conditions. The seeds had oil contents from 37% to 45% (d.w.). The oils were rich in oleic (mean 40%) and linoleic (mean 40%) acids and poor in linolenic acid (mean 0.22%), with high content of beta-sitosterol (71% of total sterols), low acidity and low levels of oxidation products, making them adequate for biodiesel production by alkaline-catalyzed transesterification.

The high levels of gamma-tocopherol (69–182 mg/kg) may explain the high oxidative stability exhibited by the oil during 6 months of storage at 28 or 35 °C. Conversely, seeds storage under high temperatures and humidity promoted the growth of fungi responsible for oil degradation. Therefore, under tropical climate conditions, when oil conversion to biodiesel is not possible immediately after fruit harvest, it is better to extract the oil and store it under closed containers without contact with oxygen and light, to preserve its quality, than to store the seeds.

1. Introduction

The majority of the world's energy needs are supplied by petrochemical sources, coal and natural gases, in addition to hydroelectricity and nuclear energy. In a context of increased environmental concerns and enhancement of renewable energy sources, biodiesel production from vegetable oils is one of the possible options to reduce greenhouse gas (GHG) emissions, as well as the dependence on fossil fuels (Achten et al., 2008; Verrastro and Ladislaw, 2007).

The use of plants as energy sources to replace conventional fuels is however controversial, because of the risk that they might replace food crops in food-insecure areas (Harinder et al., 2009). To avoid competition with food crops, energy crops must be non-edible crops adapted to marginal lands. Many oils extracted from seeds or kernels of non-edible crops are potential feedstock for biodiesel production. Important non-edible oil plants include Jatropha (*Jatropha curcas* L.), karanja (*Pongamia pinnata* L.), mahua (*Madhuca indica* J.F. Gmel), castor (*Ricinus communis* L.) and cardoon (*Cynara cardunculus* L.) (Banković-Ilić et al., 2012; Sengo et al., 2010).

In the last decade, J. curcas L. is attracting plenty of interest as a feedstock crop for biodiesel production (Brittaine and Lutaladio, 2010). *J. curcas* L. is a small tree or shrub (up to 5 m), belonging to the family *Euphorbiaceae*, that grows mainly in tropical and sub-tropical climates, in low to high rainfall areas and surviving on poor soils. This plant is native from Central America, and was spread to Asia and Africa by the Portuguese traders in the 16th century as a hedge plant and for medicinal uses, via the Cape Verde islands and Guinea-Bissau (Brittaine and Lutaladio, 2010). It is now widespread throughout the tropical and sub-tropical areas.

Jatropha produces bunches of ellipsoidal green fruits, which normally contain three black seeds, about 2 cm × 1 cm in size. These seeds have thick black hulls protecting the grain kernels, containing 27–40% of non-edible oil. The first commercial applications of *J. curcas* oil were reported in Lisbon for soap production and for lamps with oil imported from Cape Verde (Kumar and Sharma, 2008). Nowadays, *Jatropha* is used either as a commercial oil crop, with a long productive period of 30–50 years, or as a hedge to protect fields and households from animals and wind erosion. *Jatropha* oil can be easily converted into biodiesel that meets American and European standards (Achten et al., 2007; Azam et al., 2005).

Jatropha has been reported as a wild, drought-resistant and well adapted to semi-arid-climates plant, able to conserve and re-establish soil fertility in degraded regions, and therefore

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a promising crop to prevent desertification and to improve socio-economic development in poor tropical rural areas (Foidl et al., 1996; Francis et al., 2005; Zahawi, 2005). As a wild plant, *Jatropha* seed yields from 0.4 to 12 t ha^{-1} have been reported (Achten et al., 2008). Although surviving in harsh edapho-climatic conditions, high productivity can not be obtained under such marginal agricultural environments; well-drained soils are preferred and mechanical weed control is essential, depending on climate conditions (Achten et al., 2010; Brouzos, 2013). Pest control is also a determinant practice to achieve acceptable yields.

In addition, as with many members of the *Euphorbiaceae* family, *J. curcas* contains phytotoxins (Becker, 1993; Sauerwein et al., 1993). All the products, including the oil and the seed cake, are toxic to humans and animals. Although *Jatropha* seed meal contains 50–62% protein, it is not adequate for animal feed due to the presence of a glycoprotein, curcin, which is a toxin that inhibits protein synthesis (Pal et al., 2012). *Jatropha* contains two types of curcin (curcin and curcin-L) which were detected in all parts of the plant including leaf, cotyledonary leaf, stem and root (Pal et al., 2012). However, several studies have shown that the major factor responsible for *J. curcas* toxicity is the high concentration in the seeds of phorbolesters (tetracyclic diterpenoids) with known tumour promoting activity (Goel et al., 2007; Makkar and Becker, 1997). Other toxic compounds and anti-nutritional factors in the kernel and the seed cake include flavonoids, vitexine and isovitexine and 12-deoxyl-16-hydroxyphorbol (Aregheore et al., 2003).

In order to detoxify the seed cake to make it usable as a livestock feed, several studies have been performed at laboratory scale using heat, chemical or biological treatments using white-rot fungi or *Bacillus* (Aregheore et al., 2003; Barros, 2009; Phasukarratchai et al., 2012; Phengnuam and Suntornsuk, 2013; Rakshit et al., 2008). However, it is not a simple process and is unlikely to be economically viable on a small scale.

Jatropha is indeed a challenging crop. The success of a profitable crop relies on the use of high yielding genotypes rather than low-yielding local genotypes (Das et al., 2010). The first *Jatropha* projects were established in unsuitable locations with the seed material collected from wild trees and without following the basic principles of professional agriculture and agronomic knowledge, therefore leading to unsuccess of 95% of the original projects (Joos, 2013).

To fulfil the EU mandatory addition of biofuels to road fuel, in 2008 Galp Energia started two agro-industrial projects in Mozambique aimed the establishment of a complete green energy chain based on *Jatropha*: plantation, seed oil extraction and oil transesterification to biodiesel. Partnerships were made between Petromoc, Ecomoz and Galp Energia (MOÇAMGALP) in the Lugela district (Zambezia Province) and between Companhia do Búzi and Galp Energia in Búzi District (Sofala Province). Also, a direct engagement was made with local communities, including one outgrowers supporting scheme involving community farmers and food crops grown to increase food security. R&D protocols with Mozambican academic institutions were also promoted.

The biofuel project Galp-Búzi started in 2008 with the first experimental plots of *J. curcas* L. grown in Mozambique. In order to maximize productivity, Galp performed a meticulous area selection, with climate and soil classification and a logistic study using geographic information system (GIS). Areas with soils with high farming potential were excluded for the *Jatropha* plantations as well as places where they could disrupt and impair natural carbon stocks, such as forests and biodiversity-rich areas. Selection of genotypes had been carried out based on field trials (Brouzos, 2013).

This agricultural project is environmentally and socially sustainable (Brouzos, 2013). It contributes towards reducing lifecycle GHC emissions: A study with real data from Mozambique indicates that *Jatropha* biofuels produce 65% less GHG emissions than mineral diesel. The *Jatropha* biodiesel production chain will result in important by-products namely, seed cake, fruit husks and glycerol. Their valorisation is very desirable to increase the economic profit of the complete product chain under the biorefinery concept (Manurungd et al., 2009) and to avoid considerable volumes of wastes with environmental negative impacts.

At the moment, more than 1.5 million plants grow in about 1000 ha, in the centre of Mozambique (Búzi and Lugela districts) where temperatures vary from 14 to 40 °C, with an average of 30 °C, and a relative humidity ranging from 43% to 100%, with an average value of 81%. The productivity in relation to oil content and quality of the plantation material remains as one determining aspect for the economic success of the crop.

The aim of the present study was to determine the variation in oil content in seeds from 12 different origins, grown in Mozambique plantations (Búzi), under the same edapho-climatic and agronomic conditions. In addition, fatty acid composition and tocopherols composition and content (natural antioxidants) of the oils were assessed, in order to evaluate the behaviour of these oils towards oxidation. Sterol fraction composition and content were also determined, since the sterol profile is characteristic of the botanical species from which the oil was obtained and may be used to discriminate accessions. At the moment, the lack of biodiesel production units near the *Jatropha* production areas in Mozambique brings problems related to the seed conservation until oil extraction and/or oil storage until conversion into biodiesel. Therefore, the evaluation of the stability of the oil in the seeds, during storage under controlled moisture and temperature conditions simulating tropical climate conditions, and of the extracted oil at the same temperatures, was carried out.

2. Material and methods

2.1. Field trial

One field experiment was planted in Búzi (19°56′S; 34°24′E), Sofala province, in central Mozambique, in December 2010. According to Köppen climate classification (Köppen and Geiger, 1939), the coastal region of Sofala province where Buzi is located has a "Tropical rainy climate – Aw" and the interior region has a "Mild humid climate – Cw".

The weather data for 2011 and 2012 recorded in a weather station located at 19 km from the experimental field, as well as the mean values of precipitation in this province from 1971 to 2000, are presented in Table 1. Considering average normal rainfall for the region (city of Beira 1971–2000), rainfall was quite low in the 2011/2012 growing season (Table 1). This factor is not negligible since *Jatropha* crop was only rain fed. The growing season at that latitude, considering normal temperature and rainfall regimes, is between middle September and late July, when air temperature drops significantly. Consequently, the harvest period is also very long, reaching 9 months (middle December to beginning August).

The plantation was made with 1 month old nursery pinched plants at 4 m × 3 m spacing, in a randomized complete block design, with 4 replicates of 9 plants per accession and per plot (total of 36 plants per accession in a total of 432 plants).

The soil is very deep (>1 m), sandy loamy, with a mean organic matter of 4.2%, pH neutral to slightly acid, 6.3 (water) and 4.7 (KCl), and very high contents of 210 mg kg^{-1} P_2O_5 (Egner–Riehm) and 420 mg kg^{-1} K_2O (Egner–Riehm) (Egner et al., 1960).

No fertilizer was used at planting or during the first growing season. Weed control was hand made with rudimental tools in the rows and with disk harrowing between rows. Pest control, mainly against flea beetle (*Aphtona* sp.), was assured with three sprays, two with acetamiprid and one with cypermethrin. Weed

Table 1
Weather data (temperature, relative humidity and rain) of Búzi, Sofala province, in 2011 and 2012, and mean values of precipitation in this province from 1971 to 2000, along the growing season of *Jatropha curcas* (Sept to July).

	Month	Temperature (°C)			Relative humidity (%)	Rainfall (mm)	
		Mean	Min abs	Max abs	Mean	Growing season	1971–2000
2011	September	22.7	12.9	36.1	78.0	2	25
	October	25.1	13.6	40.3	77.0	20	47
	November	26.4	19.0	35.8	77.8	78	105
	December	26.8	18.9	36.5	83.9	128	236
2012	January	27.5	20.5	35.5	92.1	166	238
	February	28.0	20.7	38.9	100	32	296
	March	27.8	19.8	39.9	98.5	112	273
	April	23.5	15.3	35.7	100	65	132
	May	23.0	13.8	32.4	100	12	73
	June	21.2	9.7	34.8	97.4	5	48
	July	20.0	9.3	33.5	97.3	8	56

and pest control was made as needed, during the growing season, from December to May.

2.2. Seed material

The *Jatropha* plants were grown from seeds of 12 accessions, coded from 1 to 12. Accessions 1 to 9 have different origins and are included in an international breeding and cultivar development program carried out by Quinvita N.V. (Ghent, Belgium); accession 10 is from Brazil and it is present in most of the plantation area; accessions 11 and 12 were obtained in Ghana and Mozambique, respectively.

The studied *Jatropha* seeds from the 12 accessions were collected manually from healthy and ripened fruits that were harvested along 2012. Periodic harvests were made in the following dates: 31 January, 3 March, 3 April, 12 June, 27 June and 3 August 2012. The samples of each block and accession are a composite mixture of all the seeds collected in the 6 harvest moments, since January to August 2012. Thus, one sample per block and accession was obtained, i.e. four samples per accession in a total of 48 samples. After harvesting, the seeds were hand dehulled, air dried, and stored in perforated plastic bags when seed water content was lower than 10%, according to Quinvita's procedure guide.

2.3. Evaluation of the oil content in seeds

The air dried whole seeds were ground with a hammer, the coarse fraction was removed by sieving and the fraction with particles lower than 2 mm was kept for analysis. The oil contained in 4.5 g of this seed meal was extracted in a Soxtec apparatus for 4 h, using petroleum ether *p.a.* (boiling point of 40–60 °C) as extraction solvent. The extracted lipids were recovered by solvent evaporation using a Soxhlet apparatus to remove the majority of the solvent followed by rotatory evaporation at 40 °C under reduced pressure. The extracted seed oil was weighed and stored in amber glass flasks, at −18 °C, for subsequent analysis. The amount of oil in seeds was converted to dry basis (kg of oil/100 kg of seed dry matter) using the dry matter content of the seeds determined previously by drying of the ground seeds at 101 °C until constant weight.

For each accession, the oil content of four samples (one from each block) was determined in triplicate tests.

2.4. Oil and seed storage stability tests

Storage studies for seeds and for the extracted oil were carried out under conditions of temperature and humidity similar to those observed at the plantation field in Búzi (Mozambique).

Intact seeds from accession 10 with an initial moisture content of 7.7% (d.w.) were stored at two temperatures (28 and 35 °C) and, for each temperature, at two relative humidity (RH) values (75% and 92%). These moisture values were achieved by contacting the seeds, suspended in a plastic net, with the vapor phase of saturated salt solutions of sodium chloride (NaCl; RH = 75.09%, T = 30 °C) or potassium nitrate (KNO_3; RH = 92.31%, T = 30 °C) in closed glass vessels (Greenspan, 1977).

The oil used in these experiments was obtained from seeds of accession 10 by mechanical extraction in a screw-press Täby Press type 20 (Skeppsta Maskin AB, Sweden). The oil was stored in 24 amber glass 10 mL flasks (full of oil to avoid contact with oxygen) and submitted to the same temperature conditions (28 and 35 °C) during 6 months. Aliquots, corresponding to individual flasks, were collected every 15 days.

Concerning the experiments on seed storage, one aliquot of seeds (c.a. 65 g) stored at each temperature and humidity was collected every 15 days, along 45 days, and the oil was solvent extracted (cf. Section 2.3).

The acidity and the presence of oxidation products (cf. Sections 2.6.1 and 2.6.5) were analyzed in the oils extracted from the stored seeds and in the oils stored in glass flasks.

2.5. Water equilibrium isotherms for seeds

In order to preview the moisture content of seeds stored under different temperature and humidity conditions, equilibrium water sorption isotherms were established for *Jatropha* seeds stored at 28 and 35 °C. A similar procedure to that followed for seed storage stability studies was carried out. Seeds from accession 10, with an initial moisture content of 8.5% (d. w.), were suspended in a plastic net and stored in closed flasks containing saturated solutions of the following salts, at 28 or 35 °C: potassium acetate (CH_3CO_2K; RH = 21.61%, T = 30 °C) magnesium chloride ($MgCl_2$; RH = 32.44%, T = 30 °C), potassium carbonate (K_2CO_3; RH = 43.17%, T = 30 °C), sodium bromide (NaBr; RH = 56.03%, T = 30 °C), sodium chloride (NaCl; RH = 75.09%, T = 30 °C), ammonium sulphate ($(NH4)_2SO_4$; RH = 80.63%, T = 30 °C) and potassium nitrate (KNO_3; RH = 92.31%, T = 30 °C) (Greenspan, 1977). Along 14 days, seed samples were taken and weighted until attaining a constant mass. At the end of the experiments, the equilibrium humidity of the seeds was measured at 28 or 35 °C in a Rotronic Hygroskop DT humidity sensor (DMS-100H). The corresponding water content of the seeds was determined by drying in an oven at 100 ± 3 °C until constant mass. The determinations were carried out in triplicate.

2.6. Chemical analysis of seed oil

2.6.1. Acidity

The acidity (% of free fatty acids, FFA) of seed oil was determined according to ISO standard 660:2009. The FFA content was assayed

by titration with a 0.1 N sodium hydroxide (NaOH) aqueous solution using phenolphthalein as indicator. The mass percentage was calculated on the basis of the molecular weight of oleic acid (282.5). For each oil sample, the analysis was carried out at least in triplicate.

2.6.2. Fatty acid composition

The analyses of fatty acid profile of *Jatropha* seed oil were performed according to the official method of the European Community Regulation (1991), as fatty acid methyl esters (FAME) using a Perkin Elmer Autosystem 9000 gas chromatograph (GC), equipped with a FID and a fused silica capillary column SP™—2380 (60 m × 0.25 mm × 0.2 μm film thickness). The detector and the injector temperatures were 260 and 250 °C, respectively. Hydrogen was used as the carrier gas. The column temperature program was: 45 min at 165 °C, followed by a rate of 7.5 °C/min up to 230 °C and an isotherm for 15 min, then a second rate of 10 °C/min up to 250 °C and a final isotherm for 20 min.

The identification of the different fatty acids was achieved by comparing retention times with FAME standards (GLC-10 FAME mix, 1891-1AMP, from Sigma-Aldrich) analyzed under the same conditions. The results were expressed as area percentage of each peak relative to the total area. For each accession, the fatty acid composition of two samples (one from block 1 and one from block 3) was determined in duplicate.

2.6.3. Sterol composition

The analyses of sterols were performed following the EU standard method for olive oil, with some modifications (1991R2568-PT-01.04.2011). The oil was added with alfa-cholestanol (internal standard, from Sigma-Aldrich) and saponified with 2 N sodium hydroxide in ethanol solution. The unsaponified fraction containing the sterols was extracted by ethyl ether. The sterolic fraction was separated from the unsaponified fraction by thin layer chromatography in silica-gel G60 plates, using benzene:acetone (95:5, v/v) as developing eluent. The sterol band was scrapped and converted to trimethylsilylethers using a solution of pyridine, hexamethyldisilazane and trimethylchlorosilane (9:3:1; v/v/v) and analyzed in a Perkin Elmer 8600 gas chromatograph, equipped with a FID and a capillary column SGL-5 (30 m × 0.25 mm × 0.25 μm film thickness). Helium was used as the carrier gas. The oven temperature was kept constant at 255 °C during the analysis (40 min); the injector and the detector were at 320 °C.

The identification of the different sterols was achieved by comparing retention times with those of the standards (cholesterol, beta-sitosterol, campesterol and stigmasterol, from Sigma-Aldrich) previously analyzed under the same conditions and referred in EU standard method 1991R2568-PT-01.04.2011. Each sterol was expressed in percentage of the area in the chromatogram and the total amount of the sterolic fraction was quantified in mg/100 g of oil using the internal standard method. For each accession, the sterol composition of two samples (one from block 1 and one from block 3) was determined in duplicate.

2.6.4. Tocopherol composition

Tocopherol composition was performed according to ISO 9936:2006, using a Perkin Elmer high performance liquid chromatographic system equipped with a Perkin Elmer UV/VIS LC295 detector, a Perkin Elmer Series 200 Pump and a Peltier Column Oven Perkin Elmer Series 200. The column used was a Li ChroCART® 250-4, LiChrospher®, Si 60 (5 μm). The oil (0.8 g) was solubilized in hexane (10 mL) and 20 μL of this solution were injected in the HPLC using propan-2-ol/hexane (0.5/99.5, v/v) at a flow rate of 1 mL/min for 30 min. The identification and quantification were achieved by comparison with the retention times and areas of the tocopherol external standards (alfa-, beta-, gamma- and delta-tocopherols from Sigma-Aldrich). For each accession, the tocopherol composition of two samples (one from block 1 and one from block 3) was determined in duplicate.

Table 2
Average oil content (%, dry mass) and standard deviation of seeds from 12 *Jatropha curcas* L. accessions. Superscript indexes indicate differences based on Fisher LSD test ($p < 0.05$). The same indexes indicate no differences between samples.

Accession	Oil content (%)
1	45.04 ± 0.95^{d}
2	40.44 ± 2.78^{abc}
3	39.05 ± 2.91^{ab}
4	41.18 ± 2.15^{bcd}
5	42.38 ± 2.86^{bcd}
6	39.10 ± 2.44^{ab}
7	40.99 ± 4.55^{abc}
8	43.11 ± 2.50^{cd}
9	37.17 ± 2.28^{a}
10	39.03 ± 3.50^{ab}
11	40.21 ± 1.17^{abc}
12	42.02 ± 2.41^{bcd}
Mean	40.81 ± 2.14

2.6.5. Oxidation compounds

Thermal oxidation of the fats was indirectly evaluated by UV absorbance at 232 nm ($k_{232\,nm}$: related to the presence of initial products of oxidation, i.e. conjugated hydroperoxides) and at 270 nm ($k_{270\,nm}$: related with the presence of final oxidation products, i.e. FFA, aldehydes and ketones) of 1% (w/w) oil in isooctane. For each accession, the analysis of four samples (one from each block) was performed in triplicate.

2.7. Statistical analysis

For each set of data, one-way analysis of variance (ANOVA) was performed using the programme Statistica, version 6, Statsoft, Tulsa, USA. Post-hoc comparisons were carried out at a p value of 0.05 and using Fisher LSD test.

3. Results and discussion

3.1. Oil content in seeds

The oil content of *Jatropha* seed samples of the 12 *Jatropha* accessions grown under the same conditions, in Mozambique, is presented in Table 2. Significant differences were observed in seed oil content from the various accessions. Maximum oil content of *Jatropha* seeds was 45% d.w., observed in seeds from accession 1; the minimum oil content was observed in accession 9 (37% d.w.) and the mean value was 41% d.w. However, the oil content of seeds from accession 1 is not significantly different from that of seeds from accessions 4, 5, 8 and 12. The oil content of seeds from accession 9 was significantly different from those from accessions 1, 4, 5, 7, 8 and 12.

These results show that the oil content of *Jatropha* seeds depends on the accession.

3.2. Oil quality

The quality of *Jatropha* oils was evaluated in terms of their acidity and content of oxidation products (Table 3).

The acidity of oils, caused by the presence of free fatty acids (FFA), is the result of the hydrolysis of the triacylglycerols. This degradative reaction is promoted by temperature and it mainly occurs in damaged seeds by the action of lipases in the presence of water.

Jatropha oil extracted from the seeds of 12 different accessions has an average FFA content of 0.92%.The maximum content of FFA in

Table 3
Average values and standard deviation of UV absorbance related to the presence of primary oxidation products (k_{232}) and secondary oxidation products (k_{270}) and average acidity values (% FFA) of the different accessions. Superscript indexes indicate differences based on Fisher LSD test ($p < 0.05$). The same indexes indicate no differences between samples.

Accession	Acidity (% FFA)	k_{232}	k_{270}
1	0.84 ± 0.29^{ab}	2.00 ± 0.59^{a}	0.24 ± 0.06^{ab}
2	0.94 ± 0.10^{ab}	2.04 ± 0.47^{a}	0.20 ± 0.02^{a}
3	1.06 ± 0.11^{b}	2.11 ± 0.73^{a}	0.19 ± 0.05^{a}
4	0.95 ± 016^{ab}	1.81 ± 0.31^{a}	0.25 ± 0.03^{ab}
5	0.82 ± 0.25^{ab}	1.73 ± 0.31^{a}	0.22 ± 0.05^{ab}
6	1.10 ± 0.22^{b}	2.08 ± 0.71^{a}	0.22 ± 0.05^{ab}
7	0.94 ± 0.55^{ab}	1.83 ± 0.16^{a}	0.20 ± 0.05^{a}
8	0.60 ± 0.21^{a}	2.22 ± 0.40^{a}	0.18 ± 0.03^{a}
9	0.93 ± 0.41^{ab}	2.16 ± 0.85^{a}	0.21 ± 0.03^{ab}
10	0.76 ± 0.1^{ab}	2.09 ± 0.35^{a}	0.27 ± 0.05^{b}
11	1.07 ± 0.29^{b}	1.83 ± 0.53^{a}	0.23 ± 0.03^{ab}
12	1.00 ± 0.20^{ab}	1.97 ± 0.33^{a}	0.24 ± 0.05^{ab}
Mean	0.92 ± 0.14	$1.99 + 0.16$	0.22 ± 0.03

the oil was 1.10% (accession 6) and the minimum was 0.60% (accession 8) (Table 3). The oil acidity from accession 8 was significantly lower than that of oil from accessions 3, 6 and 11 while the FFA content in oil from accession 6 was only significantly different from that from accession 8 (Table 3).

The FFA and moisture contents have important effects on the transesterification of oils with an alcohol (methanol or ethanol) to produce fatty acid methyl (ethyl) esters using an alkali catalyst. FFA will react with the catalyst to form soaps, hindering the separation of the product from the glycerol, due to an increase in viscosity or the formation of gels, and also reducing the yield and transesterification rate (Canakci and Van Gerpen, 2001; Erickson, 1995; Fernandes and Ferreira-Dias, 2001; Freedman et al., 1984; Goodrum, 2002). When alkali catalysts are used, the oils must have free fatty acid content lower than 1–2% (Freedman et al., 1984).

Thus, the oils extracted from all the *Jatropha* seeds presented FFA contents low enough to be directly used as feedstock for biodiesel production by alkali transesterification.

Oxidation of unsaturated fatty acids is one of the most important reactions responsible for lipid degradation and production of rancid flavors. Under mild conditions, molecular oxygen reacts with the double bonds in glyceride molecules, following a free radical mechanism (Muik et al., 2005). The more unsaturated the oil is, the more susceptible to oxidative breakdown it is.

Fat and oil oxidation takes place in two main steps: Induction period with the formation of conjugated hydroperoxides (containing conjugated *trans* double bonds) and the final oxidation period where hydroperoxides split into short chain aromatic organic compounds (mainly aldehydes, ketones, alcohols, and short chain fatty acids), which cause the rancidity (Sherwin, 1978).

Oxidation varies according to the oxidative stability of the oils and oxidation conditions. The oxidative stability of an oil depends on: (i) fatty acid composition and (ii) the presence of antioxidant molecules, such as tocopherols and sterols.

According to Table 3, the minimum value of UV absorbance at 232 nm (k_{232}), related with the presence of primary oxidation products (conjugated hydroperoxides) in *Jatropha* oil was 1.73 (accession 5), the maximum value was 2.22 (accession 8) and the mean value was 1.99. No significant differences were found in the absorbance at 232 nm for the oils from the 12 accessions, showing that all of them presented the same level of conjugated hydroperoxides. The minimum value of UV absorbance at 270 nm (k_{270}), which is related with the presence of secondary oxidation products in *Jatropha* oil was 0.18 (accession 8), the maximum value was 0.27 (accession 10) and the mean value was 0.22. Concerning the content of secondary oxidation products, only accession 10 showed significantly higher levels than those found in oil samples from accessions 2, 3, 7 and 8. However, the values found in all *Jatropha* oil samples show low levels of oxidation products.

3.3. Fatty acid composition

According to Table 4, *Jatropha* oil has in average a content of 81% of unsaturated fatty acids and 19% of saturated fatty acids. Oleic (C18:1) and linoleic (C18:2) acids dominate the fatty acid composition of *Jatropha* oil, presenting the highest percentage (c.a. 40% for each). Thus, *Jatropha* seed oil can be classified as an oleic–linoleic oil (Akbar et al., 2009). Those fatty acids are followed by palmitic (C16:0) and stearic (C18:0) fatty acids with 13% and 6%, in average, respectively.

For all fatty acids of *Jatropha* seed oil, significant differences in contents were found among accessions (Table 4). The content of oleic acid found in oils from accessions 1, 2 and 9 (41.4% to 42.6%) showed to be similar and significantly higher than the amounts found in oils from the other origins. The level observed in oil from accession 8 (36.8%) was significantly lower than the amounts found in the oils from the other accessions.

Concerning linoleic acid, the highest content was found in oil from accession 8 (43.4%), which was significantly higher than those in the other oil samples. The lowest content of linoleic acid was found in oil samples from accession 9 (37.5%) which was significantly lower than the levels found in oils from all the other accessions, except accessions 1 and 2.

With respect to palmitic acid, the highest amount was found in oils from accession 12 (13.7%) while the lowest content was found in oils from accession 8 (12.3%). Oils from accessions 1, 2, 8 and 9 showed palmitic acid contents significantly lower than the contents present in oils from accession 12.

In terms of stearic acid content, the highest values were observed in oils from accession 8 (6.0%) which were significantly higher that the levels found in all other samples except those from accessions 1, 2 and 9. The oil from accessions 10 and 11 presented the lowest content of stearic acid (5.3%) which was significantly different from the contents found in oils from accessions 1, 2, 3, 5, 7, 8 and 9.

According to the European standard for biodiesel (DIN EN 14103), the concentration of linolenic acid (C18:3) in fatty acid methyl esters should not exceed the limit of 12%. *Jatropha* oil samples only contained linolenic acid in amounts from 0.215% to 0.232% (Table 4). The linolenic acid content in *Jatropha* oil is much lower than the values observed in rapeseed (5.0–13.0%) and soybean (4.5–11.0%) oils (CODEX STAN 210, 2001).

As a result of its fatty acid composition, *Jatropha* oil of the 12 accessions under study showed to be as feedstock for biodiesel production.

3.4. Sterol composition

Fifteen sterols were identified in all the samples of *J. curcas* oil and significant differences were observed among accessions in the contents of each sterol (Table 5). Beta-sitosterol was the major sterol in the oil (mean of 71.0%, varying from 68.3%, in accession 5, to 72.2% in accession 1) followed by stigmasterol (mean of 9.8%, varying from 9.0%, in accession 2, to 11.5%, in accession 5) and campesterol (mean of 8.3%, varying from 7.9%, in accession 12, to 9.0%, in accession 5). The oil from accession 5 was significantly different from all the others in terms of beta-sitosterol, stigmasterol and campesterol contents. The content of beta-sitosterol in the oil from accession 1 was significantly higher than that of oils from accessions 5, 6, 10, 11 and 12. The amount of stigmasterol found in the oil from accession 2 was significantly lower than the contents of this sterol in oils from accessions 5, 6, 8 and 10. The oil from accession 12 presented the lowest content of campesterol, which

Table 4
Fatty acid composition (% of total fatty acids) of *Jatropha curcas* L. oil from different accessions. C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C17:0 (margaric acid), C17:1 (heptadecaenoic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C20:0 (arachidic acid), C18:3 (linolenic acid), C20:1 (gadoleic acid), C22:0 (behenic acid) and C24:0 (lignoceric acid). Mean values and standard deviation. Superscript indexes indicate differences based on Fisher LSD test ($p < 0.05$). The same index indicates no differences between samples.

	Average fatty acid composition (%)												
Accession	C14:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C22:0	C24:0
1	0.06 ± 0.00^{a}	12.88 ± 0.35^{bc}	0.80 ± 0.06^{bc}	0.09 ± 0.01^{a}	0.05 ± 0.01^{bc}	5.74 ± 0.18^{de}	41.41 ± 0.64^{cde}	38.46 ± 1.07^{ab}	0.18 ± 0.01^{bc}	0.22 ± 0.01^{ab}	0.07 ± 0.01^{bc}	0.02 ± 0.01^{ab}	0.03 ± 0.01^{a}
2	0.06 ± 0.01^{a}	12.70 ± 0.27^{ab}	0.81 ± 0.04^{bcd}	0.09 ± 0.00^{abc}	0.05 ± 0.00^{bc}	5.79 ± 0.17^{de}	41.48 ± 0.93^{de}	38.48 ± 0.81^{ab}	0.18 ± 0.01^{c}	0.22 ± 0.01^{abc}	0.07 ± 0.00^{c}	0.03 ± 0.01^{b}	0.04 ± 0.01^{a}
3	0.06 ± 0.01^{a}	13.33 ± 0.29^{cd}	0.89 ± 0.03^{cde}	0.09 ± 0.00^{abc}	0.05 ± 0.01^{ab}	5.54 ± 0.29^{bcd}	39.71 ± 1.86^{b}	39.81 ± 1.91^{bc}	0.17 ± 0.01^{ab}	0.23 ± 0.00^{c}	0.07 ± 0.01^{abc}	0.03 ± 0.01^{ab}	0.04 ± 0.01^{a}
4	0.06 ± 0.01^{a}	13.24 ± 0.28^{bcd}	0.86 ± 0.05^{cde}	0.09 ± 0.01^{bc}	0.06 ± 0.01^{c}	5.39 ± 0.19^{abc}	39.14 ± 0.91^{b}	40.66 ± 0.79^{c}	0.17 ± 0.01^{a}	0.22 ± 0.01^{a}	0.06 ± 0.00^{a}	0.03 ± 0.01^{ab}	0.03 ± 0.01^{a}
5	0.09 ± 0.02^{b}	13.58 ± 0.46^{d}	0.92 ± 0.07^{e}	0.09 ± 0.00^{abc}	0.05 ± 0.01^{ab}	5.63 ± 0.28^{cd}	39.04 ± 1.03^{b}	40.10 ± 1.20^{c}	0.17 ± 0.01^{a}	0.23 ± 0.01^{abc}	0.06 ± 0.00^{a}	0.03 ± 0.01^{ab}	0.03 ± 0.01^{a}
6	0.07 ± 0.01^{a}	13.54 ± 0.43^{d}	0.91 ± 0.05^{e}	0.09 ± 0.00^{abc}	0.06 ± 0.01^{c}	5.36 ± 0.14^{ab}	38.96 ± 0.48^{b}	40.50 ± 0.67^{c}	0.17 ± 0.01^{a}	0.23 ± 0.01^{abc}	0.06 ± 0.01^{ab}	0.03 ± 0.01^{ab}	0.04 ± 0.01^{a}
7	0.08 ± 0.01^{ab}	13.19 ± 0.59^{bcd}	0.82 ± 0.08^{bcd}	0.09 ± 0.01^{a}	0.05 ± 0.01^{ab}	5.59 ± 0.19^{bcd}	40.08 ± 0.99^{bcd}	39.60 ± 0.73^{bc}	0.17 ± 0.01^{abc}	0.23 ± 0.01^{abc}	0.07 ± 0.01^{c}	0.02 ± 0.01^{ab}	0.04 ± 0.01^{a}
8	0.23 ± 0.03^{c}	12.25 ± 0.27^{a}	0.66 ± 0.05^{a}	0.09 ± 0.01^{ab}	0.04 ± 0.00^{a}	5.99 ± 0.13^{e}	36.77 ± 0.61^{a}	43.44 ± 0.62^{d}	$0.18 \pm 0,01^{bc}$	0.22 ± 0.01^{ab}	0.08 ± 0.01^{d}	0.03 ± 0.01^{ab}	0.04 ± 0.01^{a}
9	0.06 ± 0.00^{a}	12.67 ± 0.26^{ab}	0.75 ± 0.01^{b}	0.09 ± 0.01^{ab}	0.05 ± 0.01^{abc}	5.74 ± 0.09^{de}	42.58 ± 0.99^{e}	37.53 ± 1.00^{a}	0.18 ± 0.00^{bc}	0.23 ± 0.01^{bc}	0.07 ± 0.01^{bc}	0.03 ± 0.01^{ab}	0.04 ± 0.01^{a}
10	0.07 ± 0.01^{a}	13.31 ± 0.46^{cd}	0.89 ± 0.07^{de}	0.09 ± 0.00^{abc}	0.05 ± 0.01^{bc}	5.26 ± 0.02^{a}	39.38 ± 0.92^{b}	40.44 ± 0.91^{c}	0.17 ± 0.01^{a}	0.22 ± 0.01^{ab}	0.06 ± 0.01^{ab}	0.02 ± 0.01^{ab}	0.04 ± 0.01^{a}
11	0.07 ± 0.01^{a}	13.42 ± 0.35^{cd}	0.89 ± 0.06^{cde}	0.09 ± 0.01^{ab}	0.05 ± 0.00^{bc}	5.27 ± 0.03^{a}	39.95 ± 0.65^{bc}	39.76 ± 0.88^{bc}	0.16 ± 0.01^{a}	0.23 ± 0.01^{bc}	0.06 ± 0.01^{ab}	0.03 ± 0.01^{ab}	0.03 ± 0.01^{a}
12	0.07 ± 0.01^{a}	13.65 ± 0.74^{d}	0.92 ± 0.09^{e}	0.10 ± 0.01^{c}	0.06 ± 0.01^{c}	5.38 ± 0.18^{ab}	39.47 ± 1.55^{b}	39.88 ± 1.41^{bc}	0.16 ± 0.02^{a}	0.22 ± 0.02^{ab}	0.06 ± 0.01^{ab}	0.02 ± 0.00^{a}	0.03 ± 0.01^{a}
Mean	0.08 ± 0.05	13.15 ± 0.43	0.84 ± 0.08	0.09 ± 0.00	0.05 ± 0.00	5.55 ± 0.23	39.83 ± 1.49	39.89 ± 1.47	0.17 ± 0.01	0.22 ± 0.00	0.07 ± 0.01	0.03 ± 0.00	0.04 ± 0.00

Table 5
Sterol composition (% of total sterols) of *Jatropha curcas* L. oil from different accessions. Mean and standard deviation. Superscript indexes indicate differences based on Fisher LSD test ($p < 0.05$). The same index indicates no differences between samples.

Genotype	Cholesterol	Brassicasterol	24-methilen-cholesterol	Campesterol	Campestanol	Stigmasterol	Delta-7-campesterol	Delta-5-23-stigmastadienol	Clerosterol	Beta-sitosterol	Sitostanol	Delta-5-avenasterol	Delta-5-24-stigmastadienol	Delta-7-stigmasterol	Delta-7-avenasterol
1	0.22 ± 0.02^{a}	0.02 ± 0.00^{a}	0.10 ± 0.04^{a}	8.11 ± 0.17^{ab}	0.03 ± 0.01^{a}	9.57 ± 0.29^{ab}	0.30 ± 0.04^{abc}	0.04 ± 0.00^{a}	1.20 ± 0.09^{a}	72.22 ± 0.25^{e}	5.25 ± 0.08^{bc}	0.16 ± 0.02^{a}	0.80 ± 0.04^{a}	1.06 ± 0.02^{abc}	0.94 ± 0.03^{bc}
2	0.22 ± 0.04^{a}	0.02 ± 0.00^{a}	0.16 ± 0.08^{a}	8.21 ± 0.00^{abc}	0.03 ± 0.01^{a}	9.04 ± 0.85^{a}	0.35 ± 0.05^{c}	0.04 ± 0.01^{a}	1.30 ± 0.17^{a}	72.16 ± 0.33^{e}	5.40 ± 0.02^{bc}	0.16 ± 0.01^{a}	0.81 ± 0.01^{a}	1.14 ± 0.09^{bc}	1.01 ± 0.10^{bcd}
3	0.20 ± 0.03^{a}	0.03 ± 0.01^{a}	0.13 ± 0.04^{a}	8.23 ± 0.01^{abc}	0.10 ± 0.14^{a}	9.55 ± 0.25^{ab}	0.33 ± 0.01^{bc}	0.07 ± 0.04^{a}	1.28 ± 0.04^{a}	71.39 ± 0.60^{bcde}	5.77 ± 0.42^{bcd}	0.16 ± 0.01^{a}	0.83 ± 0.04^{ab}	1.04 ± 0.05^{abc}	0.93 ± 0.04^{bc}
4	0.25 ± 0.01^{a}	0.03 ± 0.01^{a}	0.13 ± 0.01^{a}	8.43 ± 0.03^{bcd}	0.04 ± 0.01^{a}	9.34 ± 0.33^{ab}	0.34 ± 0.01^{bc}	$0,04 \pm 0.01^{a}$	1.36 ± 0.03^{a}	71.44 ± 0.45^{bcde}	5.64 ± 0.12^{bc}	0.15 ± 0.01^{a}	0.83 ± 0.07^{ab}	1.03 ± 0.03^{abc}	0.99 ± 0.04^{bcd}
5	0.24 ± 0.03^{a}	0.04 ± 0.01^{a}	0.15 ± 0.00^{a}	9.04 ± 0.12^{e}	0.03 ± 0.00^{a}	11.52 ± 0.07^{d}	0.29 ± 0.02^{ab}	0.04 ± 0.01^{a}	1.22 ± 0.04^{a}	68.27 ± 0.18^{a}	6.39 ± 0.13^{def}	0.14 ± 0.01^{a}	0.86 ± 0.01^{abc}	0.92 ± 0.09^{a}	0.91 ± 0.08^{ab}
6	0.26 ± 0.02^{a}	0.03 ± 0.00^{a}	0.15 ± 0.03^{a}	8.42 ± 0.10^{bcd}	0.02 ± 0.02^{a}	10.06 ± 0.23^{bc}	0.33 ± 0.01^{bc}	0.04 ± 0.01^{a}	1.36 ± 0.04^{a}	70.60 ± 0.20^{bcd}	5.85 ± 0.08^{cde}	0.14 ± 0.01^{a}	0.85 ± 0.03^{abc}	$0,97 \pm 0.08^{ab}$	0.96 ± 0.04^{bc}
7	0.20 ± 0.01^{a}	0.03 ± 0.01^{a}	0.11 ± 0.01^{a}	8.24 ± 0.03^{abc}	0.03 ± 0.00^{a}	9.43 ± 0.13^{ab}	0.33 ± 0.01^{bc}	0.04 ± 0.01^{a}	1.32 ± 0.04^{a}	71.67 ± 0.19^{de}	5.43 ± 0.14^{bc}	0.17 ± 0.03^{a}	0.87 ± 0.01^{abc}	1.13 ± 0.09^{bc}	1.03 ± 0.01^{cd}
8	0.23 ± 0.01^{a}	0.03 ± 0.01^{a}	0.11 ± 0.01^{a}	8.68 ± 0.33^{d}	0.01 ± 0.00^{a}	10.51 ± 0.37^{c}	0.26 ± 0.00^{a}	0.05 ± 0.03^{a}	1.26 ± 0.06^{a}	71.43 ± 0.39^{bcde}	4.51 ± 0.24^{a}	0.17 ± 0.01^{a}	0.89 ± 0.04^{bc}	0.99 ± 0.04^{ab}	0.81 ± 0.04^{a}
9	0.23 ± 0.06^{a}	0.02 ± 0.01^{a}	0.13 ± 0.02^{a}	8.54 ± 0.16^{cd}	0.03 ± 0.01^{a}	9.56 ± 0.12^{ab}	0.33 ± 0.01^{bc}	0.04 ± 0.01^{a}	1.28 ± 0.06^{a}	71.57 ± 0.23^{cde}	5.09 ± 0.11^{ab}	0.14 ± 0.03^{a}	0.80 ± 0.01^{a}	1.18 ± 0.16^{c}	1.08 ± 0.06^{d}
10	0.26 ± 0.09^{a}	0.03 ± 0.01^{a}	0.25 ± 0.03^{ab}	8.03 ± 0.22^{a}	0.03 ± 0.01^{a}	10.15 ± 0.95^{bc}	0.33 ± 0.02^{bc}	0.04 ± 0.01^{a}	1.30 ± 0.10^{a}	70.40 ± 0.87^{b}	6.35 ± 0.35^{def}	0.16 ± 0.01^{a}	0.86 ± 0.05^{abc}	0.93 ± 0.08^{a}	0.92 ± 0.07^{bc}
11	0.24 ± 0.07^{a}	0.03 ± 0.01^{a}	0.15 ± 0.01^{a}	8.02 ± 0.24^{a}	0.13 ± 0.02	9.37 ± 0.35^{ab}	0.30 ± 0.06^{abc}	0.07 ± 0.05^{a}	1.24 ± 0.19^{a}	70.95 ± 0.73^{bcd}	6.51 ± 0.83^{ef}	0.18 ± 0.08^{a}	0.84 ± 0.02^{abc}	1.02 ± 0.01^{abc}	0.98 ± 0.03^{bcd}
12	0.27 ± 0.02^{a}	0.04 ± 0.01^{a}	0.45 ± 0.44^{b}	7.93 ± 0.10^{a}	0.10 ± 0.11^{a}	9.52 ± 0.14^{ab}	0.34 ± 0.02^{bc}	0.08 ± 0.05^{a}	1.29 ± 0.08^{a}	70.44 ± 1.03^{bc}	6.74 ± 0.37^{f}	0.18 ± 0.01^{a}	0.92 ± 0.05^{c}	1.06 ± 0.06^{abc}	0.98 ± 0.02^{bcd}
															Mean
	0.23 ± 0.02	0.03 ± 0.01	0.17 ± 0.1	8.32 ± 0.32	0.05 ± 0.04	9.80 ± 0.68	0.32 ± 0.02	0.05 ± 0.01	1.28 ± 0.05	71.04 ± 1.06	5.74 ± 0.66	0.16 ± 0.01	0.84 ± 0.04	1.04 ± 0.08	0.96 ± 0.07

Table 6
Total sterol and tocopherol contents (mg/kg) of *Jatropha curcas* L. oil from different accessions. Mean and standard deviation. Superscript indexes indicate differences based on Fisher LSD test. The same index indicates no differences between samples.

Genotype	Total sterol content (mg/kg)	Gamma-tocopherol content (mg/kg)
1	2650 ± 65[a]	92.56 ± 8.22[ab]
2	2610 ± 24[a]	86.42 ± 2.00[ab]
3	2436 ± 388[a]	68.34 ± 2.87[a]
4	2532 ± 188[a]	73.05 ± 15.61[ab]
5	2419 ± 100[a]	89.31 ± 34.91[ab]
6	2528 ± 72[a]	68.81 ± 1.90[ab]
7	2575 ± 78[a]	107.26 ± 7.56[b]
8	2267 ± 231[a]	181.82 ± 35.65[c]
9	2525 ± 132[a]	94.41 ± 16.93[ab]
10	2533 ± 129[a]	89.08 ± 3.02[ab]
11	2432 ± 337[a]	76.40 ± 13.02[ab]
12	2386 ± 409[a]	74.92 ± 5.03[ab]
Mean	2491 ± 107	92.04 ± 30.65

was significantly lower than the contents in oils from accessions 4, 5, 6, 8 and 9 (Table 5).

The presence of delta-5-avenasterol (mean of 0.15%, varying from 0.14%, in oils from accessions 5, 6 and 9, to 0.18% in oils from accessions 11 and 12) in the unsaponifiable fraction of *Jatropha* oil is extremely desirable. This sterol has an ethylidene group in the side chain and sterols with such structure are believed to impart resistance to darkening and polymerization of the oils (Johansson and Appelqvist, 1978). However, the content of this sterol, as well as of cholesterol, brassicasterol, campestanol and delta-5-23-stigmastadienol, was not significantly different among the samples from the 12 accessions (Table 5).

Total sterol content in *Jatropha* oil varied with the accession but no significant differences were observed at a *p*-value of 0.05 and using the LSD post-hoc multiple comparison test (Table 6). The maximum total sterol content determined was 2650 mg/kg in accession 1, the minimum content was 2267 mg/kg in accession 8 and the average content was 2491 mg/kg.

3.5. Tocopherol composition

Tocopherols are considered to be a major group of natural lipid-soluble chain-breaking antioxidants. Tocopherols and tocotrienols can inhibit lipid oxidation due to their ability to donate their phenolic hydrogen to lipid free radicals and slowdown the autocatalytic lipid peroxidation process (Seppanen et al., 2010).

Oils can be classified on the basis of their prevalent tocopherol isomers: oils containing predominantly alfa-tocopherol (e.g. sunflower seed oil, olive oil, safflower oil), gamma-tocopherol (e.g. linseed oil, cocoa butter), alfa and gamma-tocopherol (e.g. rapeseed and cottonseed oil), or gamma and delta-tocopherols (e.g. soybean oil) (Coors, 1991).

In the samples of *J. curcas* oil of the 12 accessions analysed in this study, only gamma-tocopherol was detected. The content of this tocopherol varied from 68.3 mg/kg (accession 3) to 181.8 mg/kg (accession 8). The average content was 92.04 mg/kg (Table 6). The gamma-tocopherol content of the oil from accession 8 is significantly higher than the values found in the oils from all the other accessions. Therefore, different resistance of *Jatropha* oils towards oxidation is expected, as a function of their gamma-tocopherol content.

3.6. Comparison with other studies on Jatropha curcas oil

In a study on the variability in 82 accessions of *J. curcas* from five ecogeographic zones of peninsular India, the oil content ranged from 11.5% to 45.5% and the level of unsaturated fatty acids in the oils ranged from 75.5% to 85% (Bhagat et al., 2011).

In another study on the variability in seven accessions from seven sites of Jammu and Kashmir State in Northwest India, the oil content from the seeds varied from 24.5% to 37.9% (Wani et al., 2012). The quantities of palmitic (5–25%), stearic (5–43%), oleic (17–48%) and linoleic acids (26–51%) varied among samples and accessions (Wani et al., 2012).

Oil content of 47.3%, with 72.7% of unsaturated fatty acids (41.3% oleic acid and 31.4% of linoleic acid) was reported for *J. curcas* seeds grown in Nigeria (Akintayo, 2004). In a different study also with *Jatropha* seeds grown in Nigeria, the oil was poorer in unsaturated fatty acids (c.a. 62%), being linoleic the major fatty acid (47.3%) while only 12.8% of oleic acid was detected (Adebowale and Adedire, 2006).

Higher oil contents, from 61% to 64%, were found in *Jatropha* seeds from Malaysia, Indonesia and Thailand (Emil et al., 2010). These oils were richer in oleic (42.4–48.8%) than in linoleic acid (28.8–34.6%). The saturated fatty acids, palmitic and stearic acids, varied from 13.2% to 14.5% and from 7.0% to 7.7%, respectively (Emil et al., 2010). Also, *Jatropha* oil from seeds grown in Nepal contained approximately 80% of unsaturated fatty acids with 46% of oleic and 32% of linoleic acids (Chhetri et al., 2008).

The physicochemical properties of *Jatropha* seed oils from 21 accessions collected from nine origins (Malaysia, Borneo, India, Indonesia, Cape Verde, South Africa, the Philippines, Vietnam, and Thailand) and 24 candidate plus plants (CPPs) were evaluated by Islam et al. (2012). The yield of seed oil varied from 40.0% (Malaysia) to 48.4% (Vietnam) among seeds from different origins and 32.1% to 48.8% among CPPs. The FFA content of these oils varied from 0.3% in a South African sample and in an Indonesian sample, to 2.3% in a sample from Borneo. Most of the CPPs in this study had a FFA content lower than 1% (Islam et al., 2012).

Average FFA values of approximately 14% in *Jatropha* oils from seeds from Nepal, of 1.8% and 4.5% of *Jatropha* oils from seeds grown in Nigeria and of 1.0% of seeds grown in India, were reported (Akintayo, 2004; Adebowale and Adedire, 2006; Bhagat et al., 2011; Chhetri et al., 2008). *Jatropha* oils from Malaysia, Indonesia and Thailand presented FFA content varying from 1.7% to 9.2% (Emil et al., 2010).

Variability on the oxidative stability of *Jatropha* oil was also observed among 82 accessions of *J. curcas* from five ecogeographic zones of India (Bhagat et al., 2011).

With respect to sterol composition, the presence of campesterol, stigmasterol, beta-sitosterol, delta-5-avenasterol and delta-7-stigmasterol, with beta-sitosterol representing 71.9% of the sterolic fraction, was also reported in *Jatropha* oil from seeds grown in Nigeria (Akintayo, 2004).

No information was found on the tocopherol content in *Jatropha* oils. However, the tocochromanol fraction was evaluated in *J. curcas* seeds from 52 accessions grown in the south of Spain. The following tocopherols were detected in these seeds: gamma-tocopherol (up to 15.4% of the tocochromanol fraction, in August, and 18% in November), gamma-tocotrienol (up to 83.8% of the tocochromanol fraction, in August, and 80.4% in November), and delta-tocotrienol (up to 0.8% of the tocochromanol fraction in August and 1.6% in November). Also, the composition of this fraction in developing seeds was different from mature seeds since they contained primarily alpha-tocopherol and gamma-tocopherol, with gamma-tocotrienol and delta-tocotrienol being practically undetectable (Corzo-Valladares et al., 2012). In our study, only gamma-tocopherol was detected in the *Jatropha* oils in values ranging from 68.3 to 181.8 mg/kg.

In our study, the oil content of *Jatropha* seeds from 12 different accessions (37–41%) grown in Mozambique, under similar agricultural and edapho-climatic conditions, is in the upper range of oil

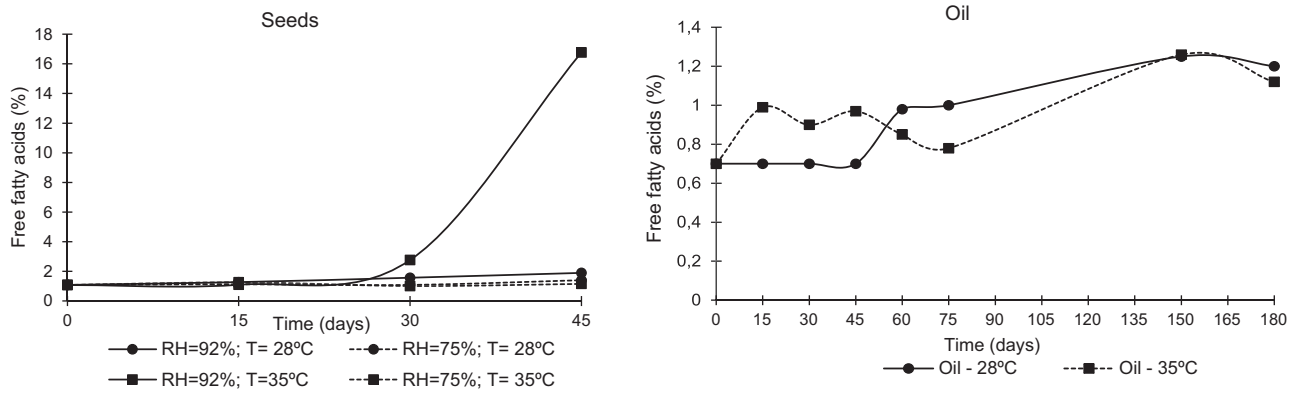

Fig. 1. Variation of free fatty acid content (%) in oil in seeds (RH = 92%, T = 35 °C; RH = 92%, T = 28 °C; RH = 75%, T = 35 °C; RH = 92%, T = 28 °C) and in oil stored in amber flasks (T = 28 °C and T = 35 °C) during the experiment.

content reported in the literature for these seeds. Also, FA and sterol fraction compositions are according to the literature. The low levels of both FFA and oxidation products show the importance of extracting the oil from intact seeds, stored under adequate conditions of temperature and relative humidity.

3.7. Oil and seed storage stability

During seed and oil storage, the main degradative reactions that occur are hydrolysis and oxidation. In order to minimize the effect of such reactions, it is necessary to store the oil and seeds at optimal temperature and relative humidity. In the specific case of oil for biodiesel production, it is important that the oil does not present high contents of oxidation products and free fatty acids, which inhibit the catalysts. The presence of phospholipids may also have an inhibitory effect.

The seeds from accession 10 used in these experiments presented an initial moisture content of 7.7% (d.w.). Fig. 1 shows the evolution of FFA content in the oil in seeds and in the oil stored in glass flasks, along storage experiments. FFA increased with temperature and relative humidity of storage. In seeds stored at 35 °C and 92% relative humidity, the FFA content of their oil was 1.2% during the first 15 days, but a steep increase in FFA content (of 0.93%/day) was observed subsequently, up to 17% after 30 days of storage. At that time, the seeds were covered with fungi, which were microscopically identified as belonging to *Penicillium sp*. and *Aspergillus sp*. (storage fungi).

In the seeds stored at 28 °C and 92% RH, a linear increase in FFA of 0.02%/day was observed along the 45 days of storage. When the seeds were stored at the same temperature but under 75% RH, the FFA content of their oil was kept approximately constant (ca. 1%) for the first 30 days of storage, showing an increase of 0.02%/day thereafter.

The FFA content of the oil from the seeds stored at 28 °C at 75% RH only increased from 1.1% to 1.4%, along the 45 days of experiments.

When the oil was stored in closed containers, the acidity did not increase much along the 180 days of storage either at 28 or 35 °C. Thus, the presence of large amounts of free water available for the hydrolysis seems to be more important than the temperature of storage. It is worth to notice that in presence of high humidity, the growth of fungi producers of enzymes, such as lipases (E.C. 3.1.1.3., triacylglycerol acylhydrolases) that catalyze oil hydrolysis, is promoted.

Concerning lipid oxidation, the oil stored at 28 or 35 °C in closed containers without access to oxygen showed to be more stable than the oil in seeds stored at those temperatures and under 75% or 92% RH (Fig. 2). Thus, the oil in seeds is highly prone to oxidation and the oxidation rate increases with moisture content and temperature.

The k_{232} values (related with the first step of oxidation) reached in the oil extracted from the seeds stored at 28 or 35 °C, at 75% or 92% RH, during 45 days of storage, were similar to the k_{232} values measured in the oils stored for 180 days, at the same temperatures in closed glass containers.

Also, after 180 days, the amounts of final oxidation products (related with the absorbance at 270 nm values, k_{270}) in the oils were similar to the values reached in the oils extracted from the seeds after 45 days of storage at 35 °C, 75% RH, or at 28 °C at 75% or 92% RH. When the seeds were stored at 35 °C and 92% RH, the Abs 270 nm of the extracted oil was about 6 times higher than the value observed in the oil stored for 180 days at this temperature.

In spite of the high content of unsaturated fatty acids, *Jatropha* oil showed a high stability to oxidation even under tropical temperatures. This behaviour may be ascribed to the high content of gamma-tocopherol, which is a powerful natural antioxidant (Kamal-Eldin and Appelquist, 1996). The seeds used in these experiments were from accession 10 and contained 89.01 mg of gamma-tocopherol per kg of oil. Probably, higher stability would be observed for the oil from accession 8, since it had the highest gamma-tocopherol content (181.8 mg/kg).

The water sorption isotherms for *Jatropha* seeds, at 28 and 35 °C, are presented in Fig. 3. The seeds used contained an initial moisture content of 8.5% (d.w.). They were put in contact with the vapour phase of saturated salt solutions at different RH (cf. 2.5.). For relative humidity lower than 75%, a decrease in seed moisture content (and weight) was observed during water migration until to attain equilibrium. Conversely, an increase in seed moisture content was observed for RH of 75% or higher (data not shown). At 35 °C, the equilibrium was attained in less than 3 days, for all the humidity values, while at 28 °C, only after 5 days this situation was attained for the higher relative humidity values (RH of 75%, 80% and 92%). This is ascribed to a faster diffusion of water molecules at higher temperatures. This observation shows that *Jatropha* seeds, when under humid and hot environments, can rapidly gain water and therefore become prone to the attack by fungi with consequent oil degradation by hydrolysis and oxidation.

According to our knowledge, little work has been reported on the effect of storage time and conditions on *Jatropha* seeds and on oil yield and quality for biodiesel production.

In a study carried out by Tadakittisarn et al. (2011), seed samples of three *Jatropha* accessions from different regions of Thailand were collected at different ripening stages (yellow and black fruits). The seeds were stored for 2 months and the pressed oil from one of the accessions was stored for 6 months, at 4, 37 and 50 °C, without any relative humidity control. Throughout the storage, an increase

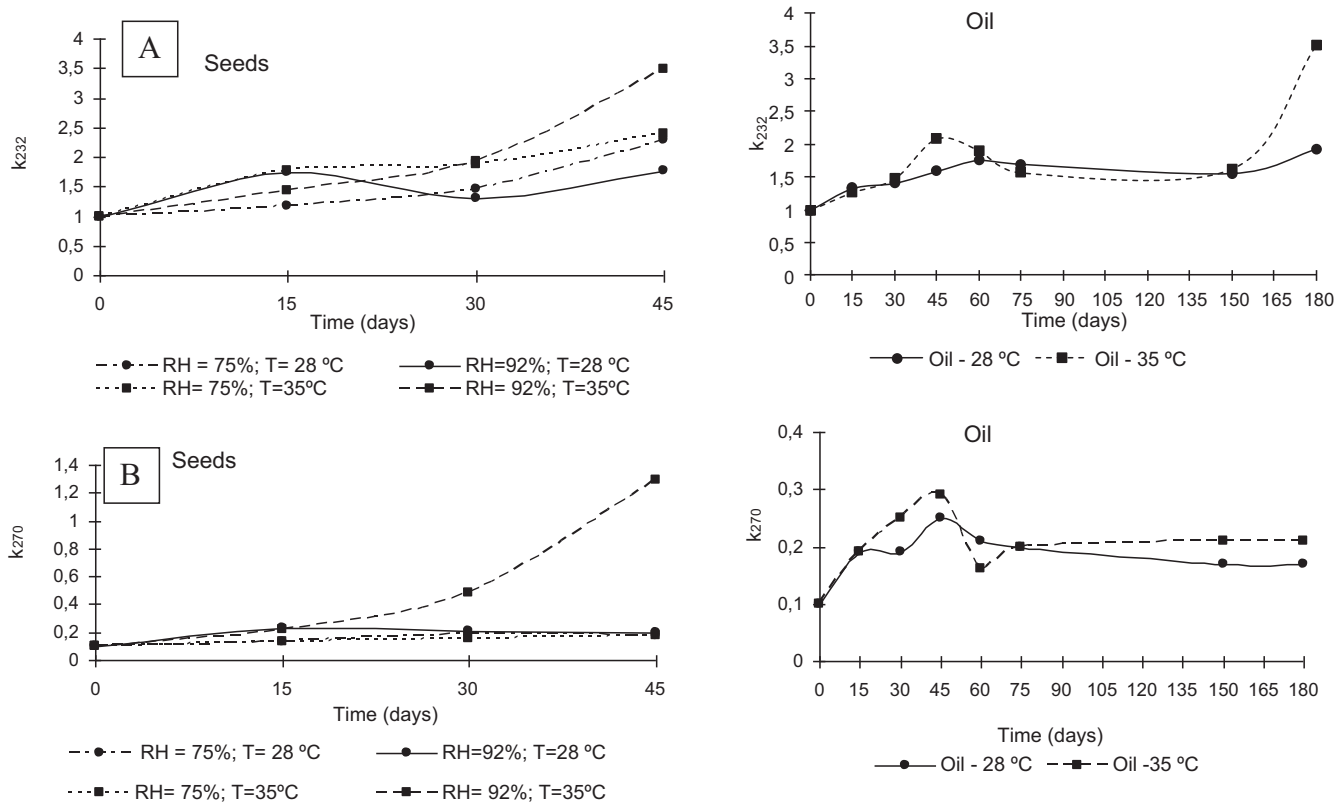

Fig. 2. Variation of absorbance at 232 and 270 nm, related to the presence of hydroperoxides content (A) and final oxidation products (B) in oil stored in amber flasks (T = 28 °C and T = 35 °C) and in oil in seeds (RH = 92%, T = 35 °C; RH = 92%, T = 28 °C; RH = 75%, T = 35 °C; RH = 92%, T = 28 °C) during the experiment.

in FFA content of seed oil from black-fruits was observed. On the contrary, the FFA content of yellow-fruit seed oil kept at 4, 37 and 50 °C did not change along 4 months storage. Also, the moisture content of the seeds did not very much increase during storage under different temperatures. These results also show the higher protection of the oil towards hydrolysis when kept in closed flasks than when oil bearing seeds are stored at the same temperatures, as observed in our experiments.

In *Jatropha* seeds stored for 3 months, at 35 °C and under a RH varying from 71% to 75%, a decrease of the oil content from 35.6% to 31.1% was observed and the acidity of this oil linearly increased from 7.8% to 32.1% (Akowuah et al., 2012). A decrease in oil yield and an increase of FFA content of *Jatropha* oil were also observed along 72 days storage of *Jatropha* seeds in India (Gupta and Rao, 2008). The oil content and quality decrease was related with the formation of oxidation products, responsible for the rancidity, during storage (Akowuah et al., 2012; Gupta and Rao, 2008).

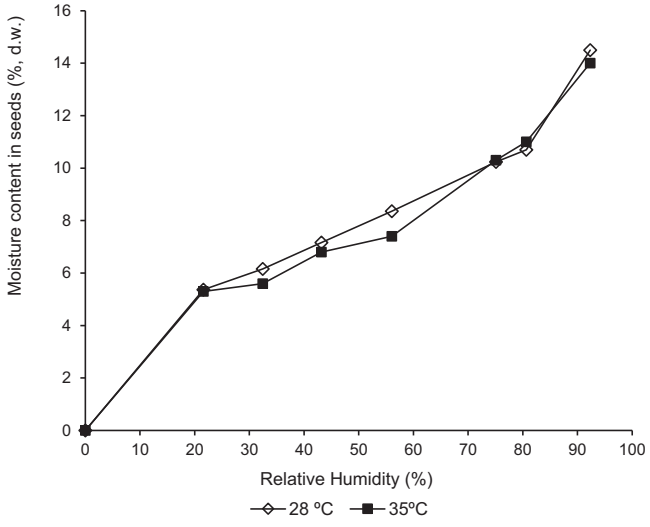

Fig. 3. Water sorption isotherms for *Jatropha curcas* L. seeds, at 28 and 35 °C.

4. Conclusions

The *J. curcas* L. seeds obtained from 12 different accessions, grown under similar edapho-climatic conditions in Mozambique, presented oil contents from 37% to 45% d.w. These oils showed to be unsaturated oils rich in oleic (c.a. 40%) and linoleic (c.a. 40%) acids and poor in linolenic acid (c.a. 0.2%) which makes them adequate for biodiesel production. Also, their low acidity and low levels of oxidation products make them adequate to be used in alkaline-catalyzed transesterification reactions for biodiesel production.

The high levels of gamma-tocopherol in *Jatropha* oils are responsible for a high oxidative stability exhibited by the oil during 6 months of storage at 28 or 35 °C. On the contrary, the storage of the seeds under tropical conditions (high temperatures and humidity) promoted the growth of fungi such as *Penicillium sp*. and *Aspergillus sp.* (storage fungi), responsible for oil degradation.

Under the tropical climate conditions in Mozambique, where the biodiesel will be produced, it is better to extract the oil from the seeds and to store it under closed containers without contact with oxygen and light to preserve its quality than to store the seeds.

The results obtained in this study show that seeds of *J. curcas* L., grown in Mozambique, have high oil content and good composition characteristics for industrial applications, such as biodiesel feedstock.

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