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Storage stability of *Jatropha curcas* L. oil naturally rich in gamma-tocopherol

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ABSTRACT

Jatropha curcas L. is an interesting tropical oil crop for biodiesel production. However, seed conservation until oil extraction may be a problem under high temperature and humidity. In this study, *Jatropha curcas* L. seeds grown in Mozambique and presenting 160 mg/kg of gamma-tocopherol in their oil were stored for 42 days, in dark, at 35 °C and 75% or 92% relative humidity (RH). Along storage, the oil was extracted and analysed in terms of fatty acid composition, tocopherol content, acidity, initial and final oxidation products (monitored by K232 and K270 values, respectively).

Jatropha seeds presented an initial water content of 8.4% and an oil content of 45.7% (dry basis). The oil was rich in oleic (41.2%) and linoleic (38.8%) acids.

Along 42 days of storage, the acidity increased from 0.8% to 7.4% and 25.3% and K270 increased from 0.07 to 0.25 and 0.46 in oils from seeds stored at 75% and 92% RH, respectively. Simultaneously, a decrease in gamma-tocopherol content was observed, which was more pronounced at 92% RH than at 75% RH (96% decrease versus 57% decrease). Gamma-tocopherol showed to protect the oil against oxidation principally during the second stage of oxidation. During the storage at 35 °C, the fatty acid composition of the oils from seeds kept either at 75% or 92% of humidity, did not significantly vary throughout the test.

1. Introduction

Biofuels are an alternative to fossil fuels since they offer numerous advantages for the environment namely reduced emissions of greenhouse gases and particles, as well as for economy and development via energy security and stimulation of rural development (Smeets et al., 2007; AETS Consortium, 2013).

As other developing countries, Mozambique has been exploring the potential for renewable energy to fulfil its energy demands (FAO, 2008). A considerable percentage of Mozambican GDP is spent on fuel and energy, which explains the government's concern in investigating alternative energy sources, including biofuels (World Bank, 2008; Schut et al., 2010). During the last decade, the Mozambican government stimulated farmers to grow *Jatropha curcas* L. on fallow and marginal soils with the aim that Mozambique could become an oil exporting country (Schut et al., 2010). Ever since, investment in the agrofuel sector increased and expanded, with several multinational companies demonstrating interest in agro-industrial business centred on Jatropha.

J. curcas L. is a drought-resistant shrub or tree belonging to the genus *Euphorbiaceae*, which is cultivated in Central and South America, South-East Asia, India and Africa (Giibitz et al., 1999). It grows in semi-arid marginal sites and can be used for erosion control (Heller, 1996). The seed kernel of *J. curcas* L. contains 43–59% oil which in the majority of genotypes cannot be used for edible purposes without detoxification, making it attractive for biodiesel production (Mtinch and Kiefer, 1986; Liberalino et al., 1988; Sharma et al., 1997; Wink et al., 1997; Kumar and Sharma, 2008; Rodrigues et al., 2013). In fact, some edible varieties of *J. curcas* have been cultivated for human food from ancient times in the mountains of the Totonacapan (Mexico), but due to the "biofuel program", these non-toxic genotypes are in serious risk of being lost (King et al., 2009; Vera-Castillo et al., 2014).

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The storage of large volumes of Jatropha seeds or oil under tropical climate conditions without loss of quality is not an easy task. In order to maintain stability of Jatropha oil, a good storage method needs to be developed. The degradative reactions taking place in vegetable oils are mainly hydrolysis and oxidation. Oil oxidation occurs in the presence of catalysts such as light, heat, enzymes, metals and metalloproteins. Autoxidation is the most common process promoting oxidative deterioration and is defined as the reaction of atmospheric oxygen with lipids, which is faster at higher temperatures. It occurs via a free radical chain reaction. Lipid hydroperoxides have been identified as primary products of autoxidation (Shahidi and Zhong, 2005). In the presence of metals or at high temperatures, these compounds are split in alkoxy radicals to form aldehydes, ketones, acids, esters, alcohols, and short-chain hydrocarbons, which originate unpleasant odours, characteristic of rancid fats (Choe and Min, 2006).

The presence of natural antioxidants in vegetable oils, such as tocopherols, may delay the beginning or may slow the rate of lipid oxidation reaction, either by quenching free radical reactions or by scavenging oxygen. Tocopherols are primary or chain breaking antioxidants, which inhibit or slow down lipid oxidation by interfering either with chain propagation or with initiation by donating hydrogen atoms to lipid peroxyl radicals. Tocopherols have two principal oxidation mechanisms: (i) they may be oxidised in a one electron-transfer reaction to a tocopheryl-radical or (ii) they may react with singlet oxygen to form a hydroperoxide (Neely et al., 1988; Krieger-Liszkay and Trebst, 2006). These reactions can be reversed, since both the tocopheryl-radical and the hydroperoxide can be re-reduced to tocopherol by ascorbate. However, under mild acidic conditions, the hydroperoxide is split to tocopherylquinone which is an irreversible reaction (Krieger-Liszkay and Trebst, 2006). Tocopherols are powerful antioxidants since they produce stable antioxidant radicals and have the capacity to compete with the lipid substrate for oxygen (Van Aardt et al., 2004).

Several experimental works have demonstrated that oil resistance towards oxidation is a function of its tocopherol content (Emanuel and Lyaskovskaya, 1967; Reinton and Rogstad, 1981; Jung and Min, 1990; Fuster et al., 1998; Kamal-Eldin, 2006). In a previous study performed by our group (Rodrigues et al., 2013), *J. curcas* L. oil samples from 12 accessions and grown under the same edaphoclimatic conditions in Mozambique were analysed with respect to their oil content, fatty-acid composition and sterol and tocopherol composition. In all these samples, only gamma-tocopherol, which is a powerful natural antioxidant (Kamal-Eldin and Appelqvist, 1996), was detected in contents ranging from 68.3 mg/kg to 181.8 mg/kg.

Storage stability tests were also performed with seeds from the accession present in most of the plantation area, with an average content of 89 mg of gamma-tocopherol per kg of oil. These studies were carried out under tropical climate conditions (28 °C and 35 °C, at relative humidities of 75% and 92%) for 45 days. The oxidation rate of the oil in the seeds increased with high relative humidity and temperature (Rodrigues et al., 2013). A higher resistance to oxidation would be expected in oils with higher gamma-tocopherol content.

The aim of this study was to investigate if Jatropha seed oil, from the accession containing the highest amounts of gamma-tocopherol, presents a higher oxidative resistance during storage, under tropical conditions, than the oil with lower content of gamma-tocopherol (89.1 mg/kg) used in the previous studies (Rodrigues et al., 2013). Thus, along 42 day storage of seeds, the oil was extracted and analysed in terms of fatty acid composition, tocopherol composition, acidity and oxidation products.

2. Materials and methods

2.1. Seed material

J. curcas L. plants were planted in December 2010, in Búzi (19°56′S; 34°24′E), Sofala province, in central Mozambique, characterised by a "Tropical rainy climate – Aw" (Köppen and Geiger, 1939). The seeds used in this study were collected from plants included in an international breeding and cultivar development programme carried out by N.V. Quinvita (Ghent, Belgium). The seeds were collected manually from healthy and ripened fruits that were harvested from January to July 2013 (Rodrigues et al., 2013). After harvesting, the seeds were manually dehulled, air dried until a seed water content below 10%, and stored in perforated plastic bags, according to Quinvita's procedure guide.

2.2. Seed storage stability tests

Storage studies were carried out under the highest average temperature observed at the plantation field in Búzi (Rodrigues et al., 2013). Thus, intact seeds were stored at 35 °C, at 75% or 92% relative humidity (RH) values, in the dark. These moisture values were achieved by contacting the seeds, suspended in a plastic net, with the vapour phase of saturated salt solutions of sodium chloride (NaCl; RH = 75.1%, T = 35 °C) or potassium nitrate (KNO_3; RH = 92.3%, T = 35 °C) in closed glass vessels (Greenspan, 1977). One aliquot of seeds (ca. 65 g) stored at each temperature and humidity was collected every 7 days, along 42 days and the oil was extracted and analysed (cf. Sections 2.3 and 2.4).

2.3. Seed oil extraction

The seed samples collected along the storage experiments were crushed with a hammer. The oil contained in the fraction with particles lower than 2 mm was extracted in a Soxtec apparatus for 4 h, using petroleum ether p.a. (boiling point 40–60 °C) as extraction solvent, as previously described (Rodrigues et al., 2013). The extracted seed oil was stored in amber glass flasks, at −18 °C, for subsequent analysis.

2.4. Chemical analysis of seed oil

2.4.1. 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 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.

2.4.2. Oxidation compounds

Oil thermoxidation was indirectly evaluated by UV absorbance at 232 nm (K232) and at 270 nm (K270) of 1% (w/v) oil solution in isooctane.

2.4.3. 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 PerkinElmer Autosystem 9000 gas chromatograph (GC), equipped with a FID and a fused silica capillary column SPTM-2380 (60 m × 0.25 mm × 0.2 μm film thickness), as previously described (Rodrigues et al., 2013). The results were expressed as area percentage of each peak relative to the total area.

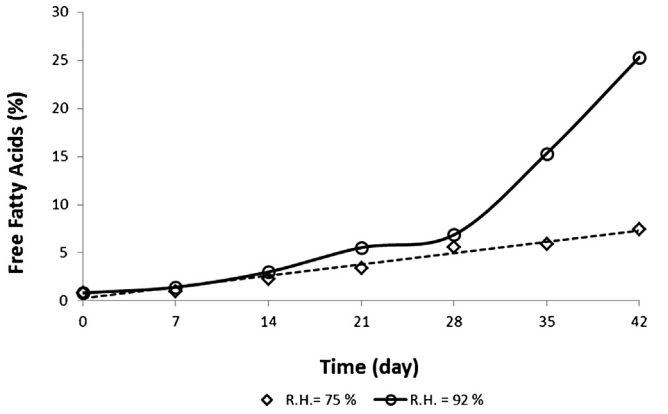

Fig. 1. Variation of free fatty acid content (%) of *Jatropha curcas* L. oil from seeds stored at 35 °C under different relative humidity (RH = 92% and RH = 75%) during 42 days.

2.4.4. Gamma-tocopherol oil content

Tocopherol composition was performed according to ISO 9936:2006, using a Perkin Elmer HPLC 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 m), as previously described (Rodrigues et al., 2013).

2.5. Statistical analysis

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

3. Results and discussion

3.1. Oil acidity

Jatropha seeds used in this study presented an initial water content of 8.4% and an oil content of 45.7% (dry basis). The oil was rich in oleic (41.2%) and linoleic (38.8%) acids (Tables 1 and 2), contained 160 mg/kg of gamma-tocopherol, and had an acidity of 0.8%, a K232 of 1.1 and a K270 of 0.07.

Along the experiments, the quality of Jatropha oils was evaluated in terms of their acidity and content of oxidation products. When the oil is to be used for biodiesel production, it is important that it does not have a high content of oxidation products and free fatty acids, which inhibit the chemical alkaline catalysts.

Fig. 1 shows the evolution of acidity in oils extracted from seeds stored at 35 °C and 75% or 92% RH, along 42 days. A linear increase of acidity of the oils (by 0.17% FFA/day) extracted from seeds stored at 75% RH was observed along the storage. For the oils from seeds stored at 92% RH, the acidity also increased linearly during the first 28 days of storage, at a rate of 0.23% FFA/day and from 28 to 42 days of storage, about a six-fold increase in the hydrolysis rate (1.32% FFA/day) was observed. This may be due to the presence of higher amounts of water available for the hydrolysis reaction at 92% RH. In presence of high humidity, after 28 days of storage, growth of storage fungi were observed that were microscopically identified as belonging to *Penicillium* sp. and *Aspergillus* sp. (Rodrigues et al., 2013). These fungi produce enzymes, such as lipases (EC 3.1.1.3., triacylglycerol acylhydrolases) that catalyse oil hydrolysis.

At the end of the experiment, the oil from seeds kept under 92% RH reached about 25% FFA while the oil from seeds stored at 75% humidity presented a much lower acidity of about 8%. However, in our previous work performed with *J. curcas* seeds containing

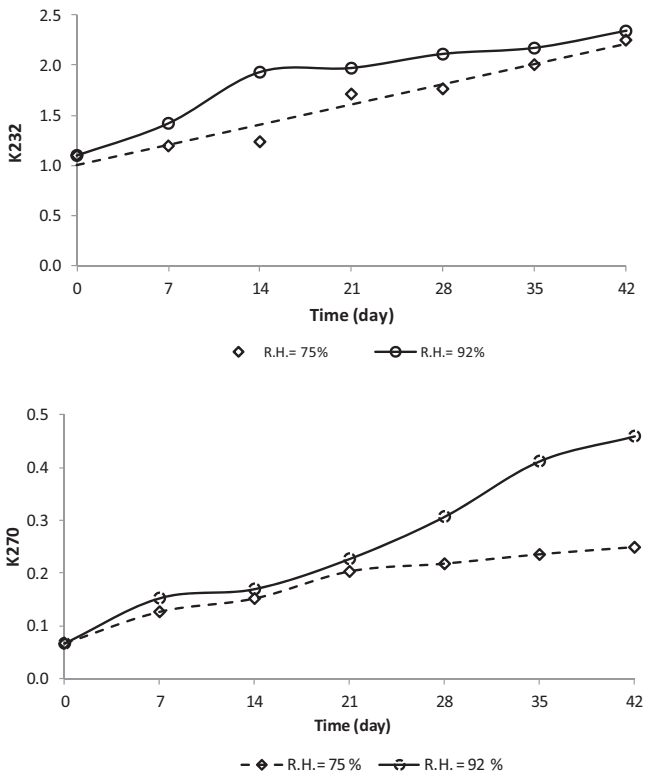

Fig. 2. Absorbances at 232 nm (K232) and at 270 nm (K270) in the oil extracted from *Jatropha curcas* L. seeds stored for 42 days at 35 °C under different relative humidity values (RH = 92%, RH = 75%).

lower amounts of gamma-tocopherol, lower final acidity values were found: 17% FFA and 1.2% after 45 days of seed storage at 35 °C, at 92% or 75% RH, respectively (Rodrigues et al., 2013). The higher acidity in the oil with higher gamma-tocopherol content may be explained by higher levels of lipases in seeds, either from internal or external origin.

An increase in FFA content of Jatropha seed oil during storage is also reported by other authors. Tadakittisarn et al. (2011) observed an increase in FFA in oil from three Jatropha accessions from different regions of Thailand, along seed storage. A linear increase in acidity from 7.8 to 32.1% was also observed in the oil extracted from Jatropha seeds stored for 3 months, at 35 °C and under a RH varying from 71 to 75% (Akowuah et al., 2012). An increase of FFA content of Jatropha oil, along 72 days storage of seeds, was also reported (Gupta and Rao, 2008).

In a study by Worang et al. (2008), FFA content increased with the storage duration of Jatropha seeds packed in plastic material. The increase in oil acidity was also reported by Sushma (2014) when Jatropha seeds were collected and stored at room temperature (open air condition) for 15 months.

3.2. Oxidation products and gamma-tocopherol content in oil

The evolution of oil oxidation in seeds along the 42 days experiments at 35 °C, under different relative humidity values, is presented in Fig. 2.

A linear increase in K232, related with the presence of initial products of oxidation, i.e. conjugated hydroperoxides, was observed along the experiment, for the oil in seeds stored at 75% RH (increase of 0.029 absorbance units/day). The K232 value increased faster in the oil extracted from seeds kept at 92% of humidity than in the oil from seeds stored at lower RH (75%), during the first two weeks of storage (increase of 0.059 absorbance units/day). After 14

Table 1
Variation in fatty acid composition (%) of the oil extracted from the seeds of Jatropha stored at 35 °C and 75% RH over 42 days. C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C17:0 (margaric acid), C17: 1 (heptadecanoic 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) and C24:0 (lignoceric acid). Standard deviations lower than 0.01 are not presented.

Fatty acids	Time (day)						
	0	7	14	21	28	35	42
C14:0	0.20	0.20	0.20	0.20	0.20	0.20	0.20
C15:0	0.10	0.10	0.10	0.20	0.10	0.10	0.10
C16:0	11.60	11.75 ± 0.07	11.35 ± 0.07	11.75 ± 0.07	11.70	11.65	11.80
C16:1	0.60	0.60	0.60	0.60	0.60	0.60	0.60
C17:0	0.10	0.10	0.10	0.10	0.10	0.10	0.10
C17:1	0.10	0.10	0.10	0.10	0.10	0.10	0.10
C18:0	6.60	6.40	6.65 ± 0.07	6.65 ± 0.07	6.70	6.80	6.70
C18:1	41.2	40.5	41.25 ± 0.07	41.65 ± 0.21	41.2	41.8	41.3
C18:2	38.80	39.55 ± 0.07	38.90	38.10 ± 0.28	38.60	37.95 ± 0.07	38.40
C18:3	0.20	0.20	0.20	0.20	0.20	0.20	0.20
C20:0	0.20	0.20	0.20	0.20	0.20	0.20	0.20
C20:1	0.10	0.10	0.10	0.10	0.10	0.10	0.10
C22:0	0.10	0.1	0.10	0.10	0.10	0.10	0.10
C24:0	0.10	0.10	0.10	0.10	0.10	0.10	0.10

Table 2
Variation in fatty acid composition (%) of the oil extracted from the seeds of Jatropha stored at 35 °C and 92% RH over 42 days. C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C17:0 (margaric acid), C17:1 (heptadecenoic 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) and C24:0 (lignoceric acid). Standard deviations lower than 0.01 are not presented.

Fatty acids	Time (days)						
	0	7	14	21	28	35	42
C14:0	0.20	0.20	0.20	0.20	0.20	0.20	0.20
C15:0	0.10	0.10	0.10	0.10	0.10	0.10	0.10
C16:0	11.60	11.55 ± 0.07	10.95 ± 0.07	11.60	11.80	11.50	11.80
C16:1	0.60	0.60	0.60	0.60	0.60	0.60	0.60
C17:0	0.10	0.10	0.10	0.10	0.10	0.10	0.10
C17:1	0.10	0.10	0.10	0.10	0.10	0.10	0.10
C18:0	6.60	6.65 ± 0.07	6.60	6.70	6.60	6.60	6.70
C18:1	41.20	41.25 ± 0.07	41.30	40.85 ± 0.07	40.60	41.40	41.25 ± 0.07
C18:2	38.80	38.75 ± 0.07	39.35 ± 0.07	39.05 ± 0.07	39.20	38.70	38.45 ± 0.07
C18:3	0.20	0.20	0.20	0.20	0.20	0.20	0.20
C20:0	0.20	0.20	0.20	0.20	0.20	0.20	0.20
C20:1	0.10	0.10	0.10	0.10	0.10	0.10	0.10
C22:0	0.10	0.10	0.10	0.10	0.10	0.10	0.10
C24:0	0.10	0.10	0.10	0.10	0.10	0.10	0.10

days storage and until the end of experiments, a slower increase in K232 was observed for the oil from seeds at 92% RH. This is probably due to the decomposition of the hydroperoxides in smaller molecules (final oxidation products). At the end of the experiments, K232 values were similar for both oils.

The K270 values, related with the presence of final oxidation products (i.e. FFA, aldehydes and ketones), increased from 0.07 to 0.22 in oils from seeds stored at both RH, during the first 21 days of storage. For the oil in seeds stored at 75% R.H., K270 remained approximately constant thereafter. However, in seeds stored at 92% RH, a linear increase in K270 was observed subsequently, reaching an absorbance value of around 0.46 after 42 days of storage.

Similar K232 and K270 values were obtained for the oil from seeds stored at 75% humidity when compared with those previously obtained for Jatropha oil with lower amounts of gamma-tocopherol (89 mg/kg) (Rodrigues et al., 2013).

With respect to the oils from seeds stored at 92% RH, K232 and K270 values of the oil with higher content of gamma-tocopherol were 1.5 or 2.8 times lower than the values observed for the oil extracted from seeds of the previous study, respectively (Rodrigues et al., 2013). It seems that gamma-tocopherol protects the oil against oxidation principally during the second stage of oxidation where hydroperoxides are broken down into secondary products. Also, this protective effect seems to be particularly important if the seeds are stored under high humidity environments.

The evolution of the average gamma-tocopherol content in the oil extracted from the seeds along the storage experiments is shown in Fig. 3. A significant decrease in gamma-tocopherol content ($p < 0.05$) was observed during the course of the experiment for both relative humidities. This decrease may explain the increase in oxidation products (related with K232 and K270 values) in the oils, observed along Jatropha seed storage. However, the decrease in gamma-tocopherol content was more pronounced at 92% RH than at 75% RH (96% decrease versus 57% decrease on gamma-tocopherol content, after 42 days of storage). Probably, this may be ascribed to the presence of higher amounts of water which will promote the

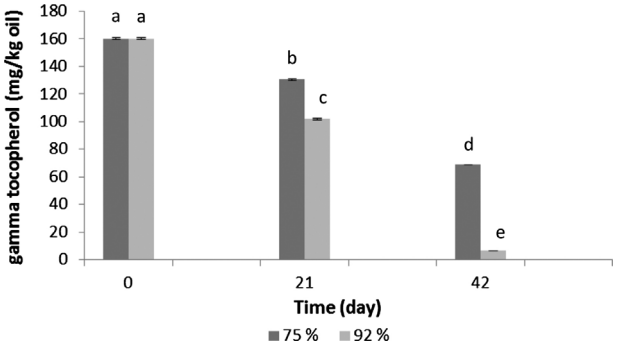

Fig. 3. Average gamma-tocopherol content of the oil extracted from Jatropha seeds stored at 35 °C and 75% or 92% RH along 42 days. Different letters indicate significant differences based on Fisher LSD test ($p < 0.05$).

growth of fungi that will produce oxidative enzymes responsible for lipid oxidation.

The antioxidant activity of tocopherols is not only due to their ability to donate their phenolic hydrogens to lipid free-radicals, but can also be explained by their participation in certain side-reactions (Kulås et al., 2002).

The following order for relative antioxidant activity of the tocopherols in vivo is generally accepted: alpha> beta > gamma > sigma. However, when relative antioxidant activities were compared in vitro, in fats, oils, and lipoproteins, a reversed order was obtained (sigma > gamma ~ beta > alpha) (Kamal-Eldin and Appelqvist, 1996). The reasons behind this reversed order are not yet clearly understood but the "absolute" and "relative" in vitro activities of the tocopherols depend on their absolute chemical reactivities towards hydroperoxy and other free radicals, and also on many other possible side reactions. These side reactions may be highly propagative and their extent greatly depends on tocopherol concentrations, temperature and light, type of substrate and solvent, and on other chemical species acting as prooxidants and synergists in the system (Kamal-Eldin and Appelqvist, 1996).

In our study, the effect of gamma-tocopherol occurs inside the seed, along storage, and not in the extracted oil. Thus, a similar behaviour to that observed in vivo systems is to be expected. The effect of tocopherols on the oxidative stability of vegetable oils during storage has been extensively evaluated. However, according to our knowledge, few works have reported the effect of tocopherols on the oxidative stability of Jatropha oil during seed storage (Rodrigues et al., 2013).

Diwani et al. (2009) observed that the addition of natural antioxidants obtained from Egyptian Jatropha crude root extracts, on Jatropha oil and its biodiesel promoted higher oxidation stability than that observed with the addition of alpha-tocopherol.

Antioxidants may have different effects on the formation and decomposition of hydroperoxides. In a study on fish oil stability along storage, the tocopherol type and concentration showed to affect not only the overall formation of volatile secondary oxidation products, but also the composition of this group of oxidation products (Kulås et al., 2002). The effects of individual tocopherols and tocopherol mixtures on the oxidative stability of corn oil were evaluated by Huang et al. (1995). In this study, gamma-tocopherol showed to promote the formation of hydroperoxides but, when in very high concentrations (5 g/kg oil), it strongly inhibited hydroperoxide decomposition. Conversely, in the presence of tocopherols, the rate of hydroperoxides breakdown and induction of further oxidation was markedly inhibited in rapeseed oil stored at 40 °C in the dark. A concentration as low as 11 mg of gamma-tocopherol per kg of rapeseed oil was enough to markedly inhibit hydroperoxide and secondary product formation (Lampi et al., 1997). The antioxidant properties of alpha- and gamma-tocopherols in the oxidation of rapeseed oil at 40 °C, in the dark, for 16 days was also evaluated (Lampi et al., 1999). In vitro, at concentrations higher than 100 mg/kg, gamma-tocopherol is better antioxidant than alpha-tocopherol.

3.3. Fatty acid composition of oils

The fatty acid composition of Jatropha oil extracted from the seeds stored at 35 °C under 75% or 92% humidity, was evaluated along the 42 days storage experiments (Tables 1 and 2). As previously reported (Rodrigues et al., 2013), this is a highly unsaturated oil, rich in oleic (c.a. 41%) and linoleic (c.a. 38.8%) acids and it also contains 11.6% of palmitic acid and 6.6% of stearic acid. During the storage at 35 °C, the fatty acid composition of the oil from seeds kept either at 75% or 92% of humidity, did not significantly vary throughout the test (Tables 1 and 2). Fatty acid composition and the proportions of different fatty acids of oilseeds during storage are dependent on the degradation rate of different fatty acids (Khan and Shahidi, 2000). In a study by Balešević-Tubić et al. (2007), sunflower seeds were stored under uncontrolled conditions for 12 months. During that period of time, the autooxidation of lipids occurred and led to modifications in fatty acid composition of the seeds. Although oleic acid content did not significantly vary, linoleic acid content was drastically reduced from 23%, in fresh sunflower seeds, to 5.49% at the end of the experiment.

4. Conclusions

The storage of *J. curcas* L. seeds under tropical climate conditions of high temperature and humidity is difficult without affecting oil quality. However, the high content of gamma-tocopherol (160 mg/kg of oil) in Jatropha oil showed to promote a high resistance to oxidation during seed storage.

Along storage, a decrease in gamma-tocopherol content in the oil was observed, which may explain the increase in oxidation products. High humidity conditions promoted the growth of storage fungi such as *Penicillium* sp. and *Aspergillus* sp., which were also responsible for oil degradation, via hydrolysis and enzymatic oxidation.

If *J. curcas* oil extraction from the seeds is not possible immediately after harvest, *J. curcas* genotypes rich in gamma-tocopherol must be chosen, in order to avoid a high extent of oil oxidation.

The control of storage conditions of the seeds (temperature and humidity) is extremely important in order to preserve the quality and the yield of Jatropha oil, with economic benefits.

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References

AETS Consortium, February 2013. Assessing the impact of biofuels production on developing countries from the point of view of Policy Coherence for Development – Final report.

Akowuah, J.O., Addo, A., Kemausuor, F., 2012. Influence of storage duration of *Jatropha curcas* seed on oil yield and free fatty acid content. J. Agric. Biol. Sci. 7 (1), 41–45.

Balešević-Tubić, S., Tatić, M., Miladinović, J., Pucarević, M., 2007. Changes of fatty acids and vigor of sunflower seed during natural aging. Helia 30 (47), 61–68.

Choe, E., Min, D.B., 2006. Mechanisms and factors for edible oil oxidation. Compr. Rev. Food Sci. Food Saf. 5, 169–186 (Institute of Food Technologists).

Diwani, G.E., Rafie, S.E., Hawash, S., 2009. Protection of biodiesel and oil from degradation by natural antioxidants of Egyptian Jatropha. Int. J. Environ. Sci. Technol. 6 (3), 369–378.

Emanuel, N.M., Lyaskovskaya, Y.N., 1967. The Inhibition of Fat Oxidation Processes. Pergamon Press Ltd., Headington Hill Hall, Oxford 4 & 5 Fitzroy Square, London.

FAO, 2008. Soaring food prices: facts, perspectives, impacts and actions required. In: Proceedings of the High-Level Conference on World Food Security: The Challenges of Climate Change and Bioenergy, Rome, Italy.

Fuster, M.D., Lampi, A.M., Hopia, A., Kamal-Eldin, A., 1998. Effects of alfa- and gamma-tocopherol on the autoxidation of purified sunflower triacylglycerols. Lipids 33, 715–722.

Giibitz, G.M., Mittelbach, M., Trabi, M., 1999. Exploitation of the tropical oil seed plant, *Jatropha curcas* L. Bioresour. Technol. 67, 73–82.

Greenspan, L., 1977. Humidity fixed points of binary saturated aqueous solutions. J. Res. Natl. Bur. Stand. Sect. A 81, 89–96.

Gupta, R.M., Rao, D.G., 2008. Effect of storage time on yield and free fatty acid (FFA) content of raw Jatropha oil. In: 32nd National Systems Conference (NSC 2008), Roorkee, India, December 17–19, pp. 440–443.

Heller, J., 1996. Physic nut. *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. 1. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome.

Huang, S., Frankel, E.N., German, J.B., 1995. Effects of individual tocopherols and tocopherol mixtures on the oxidative stability of corn oil triglycerides. J. Agric. Food Chem. 43 (9), 2345–2350.
Jung, M.Y., Min, D.B., 1990. Effects of α-, γ-, and δ-tocopherols on oxidative stability of soybean oil. J. Food Sci. 55, 1464–1465.
Kamal-Eldin, A., 2006. Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. Eur. J. Lipid Sci. Technol. 58, 1051–1061.
Kamal-Eldin, A., Appelqvist, L.Å., 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31, 671–701.
Khan, M.A., Shahidi, F., 2000. Oxidative stability of stripped and nonstripped borage and evening primrose oils and their emulsions in water. J. Am. Oil Chem. Soc. 77 (9), 963–969.
King, A.J., He, W., Cuevas, J.A., Freudenberger, M., Ramiaramanana, D., Graham, I.A., 2009. Potential of *Jatropha curcas* as a source of renewable oil and animal feed. J. Exp. Bot. 60 (10), 2897–2905.
Köppen, W., Geiger, R., 1939. Handbuch der Klimatologie. Borntraeger, Berlin.
Krieger-Liszkay, A., Trebst, A., 2006. Tocopherol is the scavenger of singlet oxygen produced by the triplet states of chlorophyll in the PSII reaction centre. J. Exp. Bot. 57 (8), 1677–1684.
Kulås, E., Olsen, E., Ackman, R.G., 2002. Effect of α-, γ-, and δ-tocopherol on the distribution of volatile secondary oxidation products in fish oil. Eur. J. Lipid Sci. Technol. 104, 520–529.
Kumar, A., Sharma, S., 2008. An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.). A review. Ind. Crop. Prod. 28, 1–10.
Lampi, A.M., Hopia, A., Piironen, V., 1997. Antioxidant activity of minor amounts of γ-tocopherol in natural triacylglycerols. J. Am. Oil Chem. Soc. 74, 549–555.
Lampi, A.M., Kataja, L., Kamal-Eldin, A., Vieno, P., 1999. Antioxidant activities of α- and γ-tocopherols in the oxidation of rapeseed oil triacylglycerols. JAOCS 76 (6), 749–755.
Liberalino, A.A., Bambirra, E.A., Moraes-Santos, T., Vieira, E.C., 1988. *Jatropha curcas* L. seeds: chemical analysis and toxicity. Arq. Biol. Technol. 31, 539–550.
Mtinch, E., Kiefer, J., 1986. Die Purgiernu B (*Jatropha curcas* L. – Botanik, Okologie, Anbau, Ernteprodukt, Verwendungsalternativen, wirtschaftliche Uberlegungen, Diplomarbeit. Universitiit Hohenheim.
Neely, W.C., Martin, M., Barker, S.A., 1988. Products and relative reaction rates of the oxidation of tocopherols with singlet molecular oxygen. Photochem. Photobiol. 48, 423–428.
Reinton, R., Rogstad, A., 1981. Antioxidant activity of tocopherols and ascorbic acid. J. Food Sci. 46, 970–971.
Rodrigues, J., Miranda, I., Gominho, J., Vasconcelos, M., Barradas, G., Pereira, H., Bianchi-de-Aguiar, F., Ferreira-Dias, S., 2013. Variability in oil content and composition and storage stability of seeds from *Jatropha curcas* L. grown in Mozambique. Ind. Crops Prod. 50, 828–837.
Schut, M., Slingerland, M., Locke, A., 2010. Biofuel developments in Mozambique. Update and analysis of policy, potential and reality. Energy Policy 38, 5151–5165.
Shahidi, F., Zhong, Y., 2005. Lipid oxidation: measurement methods. In: Fereidoon Shahidi (Ed.), Bailey's Industrial Oil and Fat Products, vol. 6, Sixth ed. John Wiley & Sons Inc.
Sharma, G.D., Gupta, S.N., Khabiruddin, M., 1997. Cultivation of *Jatropha curcas* as a future source of hydrocarbon and other industrial products. In: Gubitz, G.M., Mittelbach, M., Trabi, M. (Eds.), Biofuels and Industrial Products from *Jatropha curcas*. DBV Graz, pp. 19–21.
Smeets, E.M.W., Faaij, A.P.C., Lewandowski, I.M., Turkenburg, W.C., 2007. A bottom-up assessment and review of global bioenergy potentials to 2050. Prog. Energy Combust. Sci. 33, 56–106.
Sushma, 2014. Analysis of oil content of Jatropha curcas seeds under storage condition. J. Environ. Biol. 35, 571–575.
Tadakittisarn, S., Janchai, P., Vaithanomsat, P., Apiwatanapiwat, W., 2011. Effect of ripening stage and temperature on free fatty acid content of *Jatropha curcas* oil during storage. Nat. Sci. 45, 275–283.
Van Aardt, M., Duncan, S.E., Long, T.E., O'Keefe, S.F., Marcy, J.E., Sims, S.R., 2004. Effect of antioxidants on oxidative stability of edible fats and oils: thermogravimetric analysis. J. Agric. Food Chem. 52, 587–591.
Vera-Castillo, Y.B., Cuevas, J.A., Valenzuela-Zapata, A.G., Urbano, B., González-Andrés, F., 2014. Biodiversity and indigenous management of the endangered non-toxic germplasm of *Jatropha curcas* L. in the Totonacapan (Mexico), and the implications for its conservation. Genet. Resour. Crop Evol. 61, 1263–1278.
Wink, M., Koschmieder, C., Sauerwein, M., Sporer, F., 1997. Phorbol esters of *J. curcas*—biological activities and potential applications. In: Gubitz, G.M., Mittelbach, M., Trabi, M. (Eds.), Biofuels and Industrial Products from *Jatropha curcas*. DBV Graz, pp. 160–166.
Worang, R.L., Dharmaputra, O.S., Syarief, R., Miftahudin, 2008. The quality of physic nut (*Jatropha curcas* L.) seeds packed in plastic material during storage. Biotropia 15, 25–36.
World Bank, 2008. Mozambique at a glance.