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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.