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Methods to prevent acidification of Macaúba (*Acrocomia aculeata*) fruit pulp oil: A promising oil for producing biodiesel

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ABSTRACT

Macaúba (*Acrocomia aculeata*) produces large amounts of oil (more than 2.5 tons/ha/year) with a high oleic acid content. The oil is unsuitable for use in common industrial biodiesel processes (e.g., alkaline transesterification) because it rapidly acidifies after the fruit matures. The present study investigated simple procedures to prevent this acidification. Preliminary evaluation of the enzyme(s) involved in pulp oil degradation did not indicate the presence of plant lipase, but did identify eight species of lipolytic microorganisms colonizing the macaúba fruit pulp. Some methods maintained the acidity of the fruit pulp oil statistically equal to the fresh fruit for 15 days: immersing the fruit in either pH 10 solution, brine (1% and 3%) or potassium sorbate solution (0.3%); autoclaving; steam; and pasteurization at 85 °C. Autoclaving and then drying the fruits gave the best result, preserving the level of pulp oil acidity and fatty acid profile equal to those of fresh fruit for up to 180 days.

1. Introduction

Increasing environmental concerns and petroleum depletion have led to intensified use of biofuels (UNCTAD, 2014). Biodiesel is a biofuel produced from vegetable and/or animal oils, composed of monoalkylesters of fatty acids with a short-chain alcohol, usually methanol (Agarwal, 2007). World biodiesel production now exceeds 20 billion L/year (UNCTAD, 2014), and demand for biodiesel is increasing sharply.

The macaúba palm (*Acrocomia aculeata*) is a promising oil crop for biodiesel production, primarily due to its high oil productivity. Currently, more than 2.5 tons of oil/ha/year is obtained from semi-wild palms (Poetsch et al., 2012) and it is believed that production may reach 6.5 tons of oil/ha/year after macaúba domestication (Mariano et al., 2011), close to the amount obtained from the African oil palm (*Elaeis guineensis*). The ecophysiological requirements of macaúba make it even more interesting: this native South American palm occurs in temperate and tropical regions with limited rainfall and less-fertile soils, and therefore does not compete with rainforest or fertile food-producing land (Poetsch et al., 2012). Furthermore the fruit pulp oil has a favorable fatty-acid composition for use as a biodiesel feedstock: due to the high oleic acid content (53–66%; Aguieiras et al., 2014; Hiane et al., 2005), the final biodiesel product has a low cloud point and high oxidation stability.

Despite these advantages, macaúba is not yet employed in industrial biodiesel production. The alkaline transesterification route (current industrial biodiesel production process) requires oil with 0.5% (w/w) maximum acidity in order to achieve high yield (Freire et al., 2011). However, macaúba pulp oil rapidly acidifies after the fruit matures, and normally far exceeds this limit. Due to difficulties in harvesting macaúba fruits, they are not picked but fall to the ground when mature. This requires an efficient method to quickly treat them and retard pulp decomposition and oil acidification (Poetsch et al., 2012). Oil acidification represents the increase of free fatty acids in the oil, which are derived from oil hydrolysis. Lipases (E.C. 3.1.1.3; glycerol ester hydrolases) are a group of enzymes that are frequently present in plants and

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microorganisms, and catalyze the oil hydrolysis reaction, generating free fatty acids (Freire et al., 2011). The lipases involved in macaúba pulp oil acidification have not yet been analyzed.

Some studies have examined methods to deacidify acid macaúba oil (Mariano et al., 2011) and to produce biodiesel from this oil by routes other than the industrial alkaline transesterification (Aguieiras et al., 2014; Bressani et al., 2015; Silva et al., 2014; Nogueira et al., 2010; Freitas et al., 2008). Among fruit preservation procedures, only dehydration has been described for macaúba fruit pulp, although with no evaluation of the quality of the resulting oil (Munhoz et al., 2014). To the best of our knowledge, no published study has examined methods to prevent acidification of macaúba fruit pulp oil.

The aim of this study was to understand how to prevent macaúba fruit pulp oil acidification, in order to make this oil crop suitable for use in the production of biodiesel by current industrial methods.

2. Material and methods

2.1. Macaúba fruits and reagents

Mature macaúba fruits were collected directly from palm trees, to avoid contact with soil, near the Riacho d'Antas community in Montes Claros, Brazil. The fruits were stored at 4 °C until use, for a maximum of 60 days. Soybean oil was purchased in the local market. All reagents were of analytical grade.

2.2. Origin of lipases involved in pulp oil acidification

2.2.1. Plant enzyme: preparation and utilization for oil hydrolysis

Pulp of macaúba fruits was triturated in 4 °C acetone in a Turmix blender and washed twice with acetone to completely remove the oil, as described by Cavalcanti et al. (2007). The fat-free powder (acetone powder) was stored at 4 °C until use.

The oil hydrolysis reaction was conducted using a reaction medium composed of soybean oil (20% v/v), acetone powder (5% w/v) and buffer (100 mM; pH 4, 7 or 10), at 35 °C, for 24 h, in a magnetically stirred reactor. After the reaction ended, the oil was extracted with hexane and concentrated in a rotary evaporator, for subsequent determination of its acidity (Section 2.4).

2.2.2. Lipolytic microorganisms: isolation and identification

Macaúba fruit pulp-colonizing microorganisms were isolated by the serial dilution procedure, using an indicative culture medium for lipase-producing microorganisms (2% w/v peptone, 0.1% w/v yeast extract, 0.5% w/v sodium chloride and 1% v/v tributyrin) (Freire, 1996). Lipase production was indicated by a transparent halo around the colony, and only positive strains were isolated.

The isolated microorganisms were identified by Genotyping BPI enterprise (Botucatu, SP, Brazil), employing 16S or 18S ribosomal RNA homology.

2.3. Fruit treatments

2.3.1. Chemical treatments

Six different solutions were evaluated for conservation of macaúba fruits: (1) buffer solution pH 3 (universal buffer 0.04 M); (2) buffer solution pH 10 (universal buffer 0.04 M); (3) NaCl solution 1% (w/v) (brine 1%); (4) NaCl solution 3% (w/v) (brine 3%); (5) potassium sorbate solution 0.03% (w/v); (6) potassium sorbate solution 0.5% (w/v). Groups of 10 fruits were incubated in each solution for 15 days at room temperature. After the incubation period, the fruit pulp oil was extracted with hexane and concentrated in a rotary evaporator. The control fruits were incubated at room temperature for 15 days without any treatment. "Fresh fruit" are the fruits analyzed at the beginning of the experiment.

2.3.2. Physical treatments

Four different physical treatments were evaluated for conservation of macaúba fruits: (1) autoclaving: 121 °C for 15 or 30 min; (2) steam: fruits were incubated in an autoclave with fluent steam at 100 °C for 15 or 30 min; (3) pasteurization: fruits were immersed in 65 °C water for 30 min or 85 °C water for 1 min; (4) drying: fruits were incubated in an oven at 60 °C for 15 days, in order to reach 0.4% water content (an incubation period of 15 days was necessary to reach <1% water content). Groups of 10–20 fruits were submitted to each physical treatment (or a treatment followed by drying at 60 °C for 15 days) and stored for 15–180 days. After the storage period, the fruit pulp oil was extracted with hexane and concentrated in a rotary evaporator. The control fruits were incubated at room temperature for 15 days without any treatment. "Fresh fruit" are the fruits analyzed at the beginning of the experiment.

2.4. Oil acidity determination

The acidity of the pulp oil from each fruit was determined by titration of free fatty acids with 0.25 M NaOH solution, and the result was expressed in grams of free fatty acid per 100 g of sample (% w/w) (Sousa et al., 2010). Oleic acid molar mass was used in the calculation because it is the predominant fatty acid in the oil (Aguieiras et al., 2014).

2.5. Oil fatty-acid profile determination

Oils from fruits submitted to the same treatment were mixed (after the samples for acidity determination were collected) and the fatty-acid profile of this mixture was determined. The results of Figs. 4 and 5 are from the same fruits as in Fig. 3 (samples of 15 days originated Fig. 4 and samples of zero and 180 days originated Fig. 5).

An 8.0 μL aliquot of oil was added to 3.0 mL methanol:hexane (4:1 v/v) and 300 μL acetyl chloride, followed by heating at 100 °C/1 h. To stop the reaction, 3.0 mL of $KHCO_3$ (10% w/v) was added, followed by centrifugation (2000 rpm/10 min/23–28 °C) and the upper phase was collected for analysis (modified from Lepage and Roy, 1986).

The fatty-acid methyl esters were analyzed with a gas chromatograph (Shimadzu 2010) equipped with a flame ionization detector and an Omegawax capillary column (30 m × 0.25 mm × 0.25 μm). Analyses were carried out at an initial temperature of 200 °C for 5 min followed by an increase of 20 °C/min until 260 °C and then a fixed temperature of 260 °C for 6 min. Detector and injector temperatures were 250 °C and 260 °C, respectively. Helium was the carrier gas with 2.0 mL/min flow and 1:20 split injection. The peaks were identified utilizing the Supelco 37 standard, and the fatty-acid profile was determined by the peak area percentage.

3. Results and discussion

3.1. Lipases involved in pulp oil acidification

In order to evaluate the causes of macaúba fruit pulp oil acidification, we investigated the presence of lipase(s) produced by the macaúba itself (plant enzyme) and produced by macaúba-colonizing microorganism(s) (microbial enzyme). This preliminary evaluation was important to direct the choice of the oil acidification-prevention methods to be studied later.

To investigate the presence of plant lipase(s) in the pulp, pulps of fresh fruits were separated and delipidated with acetone to obtain the enzyme preparation called acetone powder, which has