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Seed Storage Indicates the High Stability of *Babaçu* Oil (*Attalea vitrivir* Zona)

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Abstract

Because the *babaçu* palm (*Attalea vitrivir*) has been considered for incorporation into programs of biofuel production, in this work we evaluated the influence of the geographic origins and storage conditions of babaçu palm seeds on the quantity and quality of their extracted oils. Seeds harvested from three different areas were stored for 12 months under natural environmental conditions (mean temperature 25 ºC), under refrigeration (4 ºC), or in a freezer (-20 °C). We determined their water and oil contents, acidity and peroxide levels, refractive indices, and their fatty acid profiles. The original water content of the seeds was 4.9%, becoming reduced by half during storage. Oil represented 53% of the seed weight, with lauric acid predominating throughout storage. Theseed oil demonstrated high oxidative stability, with low levels of acidity and peroxide formation, independent of seed origin or storage conditions. The high quality of *Attalea vitrivir* oil and its stability confirm its potential for agroindustrial use.

Keywords: oxidative stability, palm oil, fatty acid profile, oil quality

1. Introduction

Vegetable oils are widely used in industry to produce food items, cosmetics, paints and fuels (Ferreira et al., 2012; Siddique et al., 2010). Oleaginous plants are the second most commonly cultivated species in the world (Almeida et al., 2010), with palm trees (Arecaceae) being one of the most economically important groups in tropical regions (Nascimento, 2010). Among the Brazilian oil-producing palms, *babaçu* (*Attalea* sp., including the former genera *Orbignya*, *Scheelea,* and *Maximiliana*) is one of the most important natural resources for traditional communities subsistence (Teixeira, 2008; Pintaud, 2008; Lorenzi et al., 2010).

Attalea vitrivir Zona (synonym *Orbignya oleifera*) occurs in southeastern Brazil (Lorenzi et al., 2010) in areas receiving less rainfall than required by most other species of its group. These palms produce large quantities of fruits from which a starchy flour can be extracted from the mesocarp, the woody endocarp is used for making charcoal, and the oil extracted from the seeds (Lorenzi et al., 2010; Albieiro et al., 2007) can be used for manufacturing foodstuffs, cosmetics, and lubricants (González-Pérez et al., 2012). Because of its wide distribution in Brazil, rusticity, and wide variety of traditional uses, the *babaçu* palm has been considered for incorporation into programs of biofuel production (EMBRAPA, 1984; Trzeciak et al., 2008; MME, 2014; Pinto et al., 2005). Little is known, however, about the quality of its oil, especially its stability under storage.

Raw material storage is often problematic in agroindustrial production chains. Monitoring the physical and chemical properties of oils is essential to determining adequate conditions for seed storage to minimize deterioration as well as broaden use alternatives (Almeida et al., 2010; Azevedo et al., 2003; Davide et al., 2003). Inadequate storage is associated with autocatalytic and enzymatic hydrolysis of the oil, as well as the

proliferation of lipolytic microorganisms that facilitate triglyceride hydrolysis (Sambanthamurthi et al., 2000; Piña-Rodriguez & Jesus, 1992). In spite of the huge potential for the commercial use of natural products from Brazil, there is a notable lack of information available concerning the storage of seeds of native species. Studies focusing on seed storage have largely concentrated on cultivated grains (Orozco-Segovia et al., 2003), and interest has been shown for investigating palm seeds (Carvalho et al., 2015; Neves et al., 2013).

As such, the present study quantified and characterized the oil derived from *A. vitrivir* seeds and evaluated the influence of storage time and storage conditions on seeds from different regions in terms of oil yields and quality.

2. Material and Methods

2.1 Origin of the Plant Material

A. vitrivir seeds were harvested from natural populations occurring in the River Pandeiros Environmental Protection Area (APA-Pandeiros), in northern Minas Gerais State, Brazil (15°26′10″ S × 44°40′44″ W). The APA-Pandeiros is located in a transition zone between the Caatinga and Cerrado biomes. The predominant vegetation formations there are Cerrado *sensu stricto*, gallery forests, and seasonally deciduous forests (Silva et al., 2009). The regional climate is semiarid, with well-defined wet and dry seasons. The mean annual temperature varies between 21 and 24 °C, and the mean annual rainfall varies between 900 and 1200 mm, with rainfall concentrated between November and January (INM, 2012).

Palm seeds were harvested from three areas (A, B and C): Area A showed denser formations of *babaçu* palms close to the Pandeiros River; Area B was intermediate, with a denser vegetation cover but also with open areas used for pasture and small-scale agro-extractivism; Area C was composed of open pasture with wide spacing between the trees and shrubs, with a predominance of herbaceous plants. Mature fruits (brown fruits that separate easily from the bunches) were collected directly from five palm trees in each area. The seeds were extracted manually from the harvested fruits using a hatchet.

2.2 Seed Storage Times and Conditions

The seeds were stored in polyethylene sacks under room temperature conditions in the laboratory (mean temperature 25 °C), or under refrigeration (4 °C), or in a freezer (-20 °C). Oil analyses and evaluations were performed before initiating storage (zero time) and after three, six, nine, and twelve months.

2.3 Water Contents

Seed water contents were determined by the difference between their fresh and dry masses; drying was accomplished in a forced air oven at 105 °C (MAPA, 2009).

2.4 Oil Extraction

To evaluate the physical and chemical properties, the oil was cold extracted by crushing and pressing them using a table-mounted vice. The crude oil was centrifuged for 15 min. at 3500 RPM to remove impurities and then stored in a freezer until analyzed.

2.5 Oil Contents

Only fresh non-stored seeds (zero time) and seeds stored for 12 months were used to compare oil contents before and after storage. The oil contents of the seeds were determined through solvent extraction using a *Goldfish*-type apparatus (Detmann et al., 2012).

2.6 Determination of Acidic and Peroxide Levels, and the Refractive Index of the Oil

Analyses were performed on the oil extracted from seeds before storage and after storage for three, six, nine, and twelve months under room temperature, refrigeration, and freezer conditions. The acidic and peroxide levels and refractive indices were determined following the methodologies of the AOCS (1990).

2.7 Fatty Acid Profiles

Analyses were performed on *babaçu* oil extracted from seeds that had not been stored, and from seeds stored for periods of six and twelve months under room temperature, refrigeration, and freezer conditions.

Approximately 12 mg of *babaçu* oil was dissolved in 100 μL of a 95% ethanol/5% potassium hydroxide (1 mol L^{-1}) solution, in a 2 mL cryogenic tube. After agitation in a vortex mixer for 10 s, the oil was hydrolyzed in a domestic microwave oven (Panasonic Piccolo) for 5 min at 80 W. After cooling, 400 μL of 20% hydrochloric acid, ~20 mg of NaCl, and 600 μL of ethyl acetate were added. After agitation in the vortex mixer for 10 s and being left to stand for 5 min, a 300 μL aliquot was removed from the organic layer, transferred to a

micro-centrifuge tube, and dried by evaporation—yielding the free fatty acids (adapted from Christie1998). Gas chromatographic analyses were performed as follows: the free fatty acids were methylated using 100 μL BF_3/methanol (14%) by heating for 10 minutes in a water bath at 60 °C, diluted with 900 μL of methanol, and subsequently analyzed in a HP7820A Gas Chromatograph (Agilent) equipped with a flame ionization detector. We used a HP-INNOWAX (Agilent) 15 m × 0.25 mm × 0.25 μm column with a 70 °C, 0 min; 10 °C min^{-1} to 240 °C temperature gradient; injector (1/50 split) at 250 °C with the detector at 260 °C. Hydrogen was used as the carrier gas (3 mL min^{-1}) and the injection volume was 1 μL. The identifications of the chromatograph peaks were made by comparisons with FAME C14-C22 methylated fatty acid standards (Supelco cat nº 18917).

2.8 Experimental Design and Statistical Analyses

The evaluations of seed water contents, the acidic and peroxide levels of the oil and its refraction index were performed using a random factorial scheme of 3 (seed origin) × 3 (storage conditions) × 5 (time periods), with three repetitions for each treatment. To evaluate the oil content, only the zero and 12 month time periods were used; to evaluate the fatty acid profiles, the zero, six, and 12 month time periods were considered. The data were submitted to analyses of variance, and the means compared using the Tukey test at a 5% level of probability.

3. Results

3.1 Water Content

There were no significant differences at time zero between the water contents of the seeds harvested in the three different areas (mean 4.9%; P = 0.1858). During storage, the seed water content decreased to 2.67%, independent of the storage conditions. Maintaining seeds under refrigeration (M = 3.08%) resulted in a greater reduction in water content than maintaining them in a freezer (M = 3.61%). Storage at room temperature resulted in an intermediate, but not statistically significant, loss of water content (M = 3.20%).

3.2 Oil Content

No statistically significant differences were observed between the oil contents of seeds harvested from the three different areas at time zero, with a mean of 53.24% by weight (P = 0.5061). There were slight decreases in lipid contents of these seeds when analyzed after 12 months under all storage conditions, with lesser oil losses from area C and greater losses from area A; area B demonstrated intermediate oil content values. Nonetheless, storage conditions did not influence seed oil contents (mean 53.31%; P = 0.991).

3.3 Acidity Levels

The *babaçu* seed oil had a mean acidic index of 0.20% at time zero (Figure 1). Seed storage under freezer and refrigeration conditions maintained the initial acidic level, although a very discreet increase in acidity after nine months of storage under refrigeration conditions was noted. Seeds maintained under room temperature conditions demonstrated greater oil acidity than the other storage methods at all times, with greater acidity values than seen at time zero after nine months of storage; the final acidity level was very slight, however (M = 0.40%).

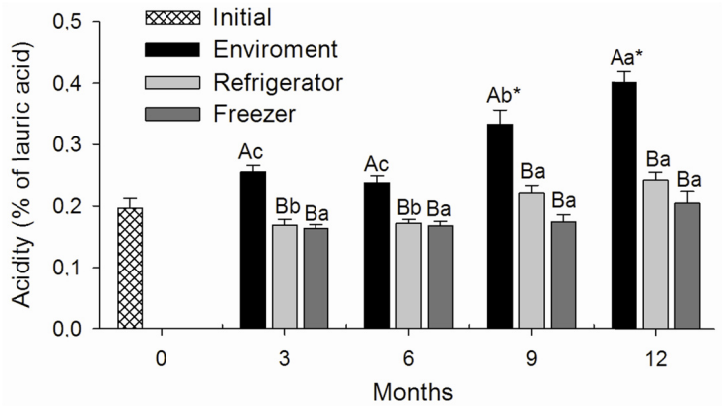

Figure 1. Acidity indices of the oil extracted from *Attalea vitrivir* seeds stored under different conditions for 12 months. Different letters indicate significant differences by the Tukey test at a 5% level of probability. Uppercase letters compare storage methods at different times, while lowercase letters compare times within each storage technique. Asterisks indicate significant differences between the treatments and the initial condition. Vertical bars indicate the mean standard errors

3.4 Peroxide Levels

Peroxide levels showed interactions between the factors: seed origin x time of storage, and storage method × time (Figure 2). At time zero, the peroxide index of the oil did not differ between seeds harvested in areas B and C, with means of 6.94 and 5.17 meq 1000 g^{-1}, respectively; these means were significantly higher, however, than those observed in area A (M = 1.35 meq 1000 g^{-1}) (Figure 2A). No effect of seed origin on the peroxide index during storage was observed, however, and the low peroxide indices observed in all samples after nine and 12 months of storage were not significantly different.

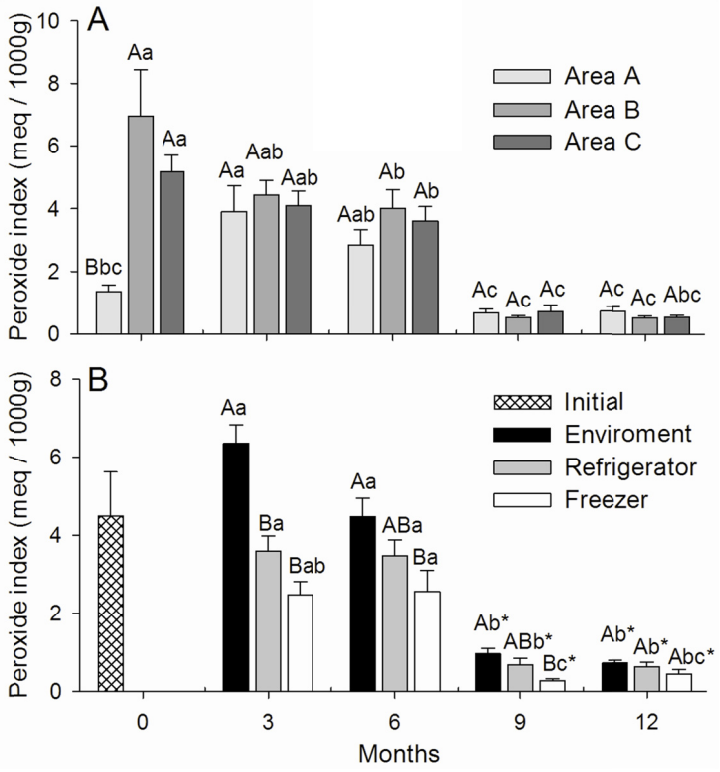

Figure 2. Peroxide indices of the oil extracted from *Attalea vitrivir* seeds stored for 12 months, as a function of the harvesting site (A) and the storage technique (B). Different letters indicate significant differences by the Tukey test at a 5% level of significance. Uppercase letters compare harvesting sites (A) or storage techniques (B) within each time frame, while lowercase letters compare the times within each harvesting site or storage technique. The asterisks (B) indicate significant differences between the treatments and the original condition. Vertical bars indicate the mean standard errors

In relation to seed storage conditions, the highest peroxide indices were observed in the oil of seeds stored at room temperature after three months; peroxide levels remained higher in seeds stored at room temperature than in those stored in a freezer after six and nine months (Figure 2B). The peroxide index was consistently lower at time zero than after nine months under all storage conditions; at 12 months there were no statistically significant differences between the three storage methods.

3.5 Refractive Index

The refractive indices of the seed oils derived from the three different areas did not differ among themselves at time zero (mean 1.4506; P = 0.1319). Their refractive indices likewise were not affected by storage conditions (P = 0.5842).

3.6 Fatty Acid Profiles

A. vitrivir oil demonstrated a predominance of lauric acid, with the presence of other saturated fatty acids such as myristic (C14:0), palmitic (C16:0), capric (C10:0), caprylic (C8:0), and stearic (C18:0) (Table 1). Unsaturated fatty acids were also identified, including oleic (C18:1) and linoleic (C18:2). There were no significant differences in the proportions of the predominant fatty acids in the seed oils derived from the three different

areas at time zero: lauric acid (M = 45.38%; P = 0.1847), oleic acid (M = 15.90%; P = 0.0595), and myristic acid (M = 12.12%; P = 0.1643).

Table 1. Fatty acid compositions of *Attalea vitrivir* oils obtained from seeds before and after 6 and 12 months of storage at room temperature (25 °C)

Name	RT* (min)	Before storage			6 months-room temperature			12 months-room temperature		
		Area			Area			Area		
		A	B	C	A	B	C	A	B	C
C8:0	1.58	7.03	6.47	5.77	6.83	6.91	6.06	7.24	6.83	5.85
C10:0	3.14	7.94	7.36	6.64	7.85	7.64	6.99	8.22	7.55	6.80
C12:0	5.10	46.57	45.24	44.32	46.73	45.85	45.84	47.26	45.69	45.41
C14:0	7.00	11.65	12.34	12.38	11.63	12.20	12.26	11.55	12.35	12.49
C16:0	8.83	7.23	8.01	8.16	7.17	7.72	7.65	7.01	7.82	7.99
C18:0	10.54	2.92	3.04	3.08	2.90	2.99	3.05	3.14	3.09	3.36
C18:1	10.69	14.80	15.50	17.41	14.65	14.76	16.06	13.93	14.82	16.10
C18:2	11.05	1.87	2.03	2.24	2.24	1.93	2.09	1.66	1.86	1.99

Note. * Retention Time.

The storage conditions and times of storage did not significantly affect the proportions of fatty acids, with significant differences between the oils of seeds derived from the three different areas being noted only for C18:1 (P = 0.470). In this case, area C demonstrated the greatest proportion of that fatty acid (M = 16.4%) in comparisons to areas B (M = 14.8%) and A (M = 15.0%). The oil from seeds harvested in area A showed a higher percentage of saturated fatty acids (M = 83.3%) at time zero than the oil from area C (M = 80.4%); the oil from area B (M = 82.5%) did not differ significantly from the others in that respect. The storage periods did not significantly alter the percentages of saturated oils from any of the three areas (P = 0.1691), with only a slightly lower value in area C (M = 81.5) than observed in areas A (M = 83.0%) or B (M = 83.2).

4. Discussion

The oil yields from the seeds analyzed in the present work (mean 53.24%) were lower than the means reported in most studies of *babaçu* species (*Attalea* sp.), with lipidic contents generally above 60% (Machado et al., 2006; Soler et al., 2007; Gioielli, 1996; Cadernos NAE, 2005). The value found here was, however, greater than that reported by Guedes et al. (2015), who encountered a mean oil content of 45.7% in *Attalea vitrivir* seeds from the same general region as the present work, although they used a different extraction technique. Lipidic values near those reported here were encountered in *macaúba* palm (*Acrocomia aculeata*) seeds (54%) (Hiane et al., 2005), and higher than the values found for three populations of *Acrocomia emensis*, with a mean of 13.45% (Neiva et al., 2018). The value found here was also higher than the found in Bacaba, Buriti, Inajá, Pupunha and Tucumã, 22.1%, 10.6%, 14.6%, 5.9%, 11.8%, respectively (Santos et al., 2013a); in Inajá with 35.5% (Rodrigues et al., 2010).

The water contents of the seeds analyzed in the present study were very close to those previously reported for the same species that disperse seeds with low humidity levels and demonstrate orthodox behavior under storage (Silva et al., 2009). Oleaginous seeds should always be stored with low humidity levels that favor lipid conservation, as alterations of their oils are principally due to hydrolysis, whether enzymatic or autocatalytic (Peske et al., 2012; McDonald, 1999). The low initial humidity levels of freshly harvested seeds of *A. vitrivir*, and their tendency to dehydrate during storage, contribute to the maintenance of oil quality and will facilitate their management for agroindustrial purposes.

The low acidity of the oil in recently-harvested seeds and the absence of any discernible effects due to their geographic origins have been reported for other palms of the genus *Attalea* (Ferreira et al., 2012; Lima et al., 2007). The maximum acidic values reported here, even under conditions of room temperature storage, are still below the recommended limits for human consumption of unrefined oils, and even below that expected for refined oils (ANVISA, 2005). These acidic values are considered adequate for the oil to be used for biodiesel production, as the transesterification reactions that use hydroxides as catalyzing agents are sensitive to excessive free fatty acids (which favor saponification reactions and compete with transesterification) (Lima et al., 2007).

Oxidation is also an important cause of lipid deterioration through the formation of free radicals that subsequently form peroxides and hydroperoxides. The occurrence of oxidation can therefore be estimated by

determining the peroxide index (Silva et al., 1999; Madhavi et al., 1996; Farmer et al., 1942; Toledo et al., 1985). The significant differences observed between the peroxide values in the three different harvesting areas may be related to genetic or environmental factors—although these differences were later neutralized by the dynamics of chemical reactions associated with storage. Nascimento et al. (2009) reported the peroxide concentration in *babaçu* oil to be 1.136 meq 1000 g^{-1}, a value very similar to that observed in area A in the present work. Variations in peroxide indices are quite common among oleaginous seeds(Ferreira et al., 2012; Hiane et al., 2005), although the *A. vitrivir* oil analyzed here demonstrated peroxide values significantly lower than commonly encountered in unprocessed oils (15 meq 1000 g^{-1}) (ANVISA, 2005), indicating a low level of oxidative degradation (Malacrida & Jorge, 2003).

It is interesting to note that there were actually reductions in peroxide levels after nine months of storage related to the dynamics of oil degradation—as the free radicals initially formed will normally be converted into other radicals, forming peroxides and hydroperoxides that combine and give rise to stable products such as aldehydes, alcohols, and hydrocarbons (Silva et al., 1999; Madhavi et al., 1996; Farmer et al., 1942; Toledo et al., 1985). The peroxide index therefore represents the difference between the formation and decomposition of reactive species. It is important to note, however, that the maximum values reported here for *A. vitrivir* were still very much below the levels considered indicators of significant oil degradation (ANVISA, 2005; Malacrida & Jorge, 2003).

Hydrolysis and oxidation are the principal mechanisms of fat and oil rancidification, and they are associated with reactions of water or atmospheric oxygen with fatty acids, principally unsaturated fatty acids (P. A. Bobbio & F. O. Bobbio, 1992). The absence of double bonds in the carbon backbones of saturated fatty acids makes them more stable and more resistant to degradation processes (Luz et al., 2011). The refractive indices of fatty acids are a reflection of their degrees of saturation, and the values encountered in the present study are similar to those reported in the literature (Machado et al., 2006; Codex, 2001). *A. vitrivir* oil was found to be rich in saturated fatty acids, especially lauric acid—a result expected according to previous descriptions of *babaçu* oil in the literature (Ferreira et al., 2012; Lima et al., 2007; Santos et al., 2013b; Gonzalez et al., 2008). Similar results have also been reported for oils extracted from the seeds of other palm trees, such as *Astrocaryum aculeatum* G. Mey (Barbosa et al., 2009) and *Cocos nucifera* L., among others (Bereau et al., 2003). Storage time was not found to have a significant influence on the quantity or the composition of the oil extracted from *A. vitrivir* seeds, with its low acidity and peroxide levels even after storage for one year at room temperature. It is probable that the chemical stability of the oil is largely due to its high concentrations of saturated fatty acids. However, Queiroga et al. (2015) in their studies with *Orbignya* sp. report that the storage time can influence from the physical-chemical characteristics to the oil content.

The maintenance of *A. vitrivir* oil in intact seeds contributes to its conservation during storage, as only small changes were observed in its physical and chemical properties even after storage for one full year at room temperature. Another advantage of this situation resides in the ease of storage and transport of the seeds, as they will not require complex or costly storage conditions as compared to extracted oil. The storage of *A. vitrivir* seeds for agroindustrial uses requires only the simplest conditions and will thus complement the exceptional agricultural/commercial potential of the species (Silva et al., 2009; Guedes et al., 2015). In situations where more sophisticated applications of the oil might demand greater levels of conservation, refrigeration will provide numerous benefits, as is usual in seed storage (Delouche, 1968; Ordóñez, 2005).

5. Conclusions

Attalea vitrivir seeds contain high oil concentrations, equivalent to more than half of their total mass. The oil is largely composed of saturated fatty acids, such as lauric acid. The composition of the oil lends it a considerable level of oxidative stability, which is confirmed by its low observed acidic and peroxide indices. Simple storage conditions will guarantee the maintenance of seed oil quality, with low temperatures promoting even more efficient conservation of its characteristics. No significant differences in oil quality were observed between the harvesting sites, or between freshly harvested seeds and those stored for up to a year. The characteristics of the oil and its resistance to chemical alterations during storage indicate this species' exceptional potential for agroindustrial use.

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