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Effect of Storage Conditions on the Oil Quality of Chinese Tallow Tree Seeds

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Abstract The Chinese tallow tree (TT) has been widely considered to be an invasive species in the US without potential benefits. However, the literature on TT seeds is scarce and the effect of storage conditions on seed oil quality in particular has not been published to our knowledge. Prior research revealed that TT has a very high yield of seeds containing large percentages of long and short chain fatty acids (mainly palmitic fatty acid, along with some oleic, linoleic and linolenic fatty acids), which can be base transesterified into biodiesel. This study aims to address the issue of the quality of the kernel oil of TT seeds stored at different temperatures (4 °C and room temperature) and under controlled atmosphere storage conditions (3% CO_2, 6% CO_2, vacuum, normal headspace). The total storage time was 3 months with the oil quality being analyzed weekly. Extracted oil was analyzed by titratable acidity, peroxide value, oxidative stability index and fatty acid composition. These experiments provided evidence that, after 12 weeks of storage, a controlled atmosphere did not produce any remarkable advantage over low cost air storage. The results validate the belief that no elaborate storage conditions are required to store this economically promising high oil content biofuel feedstock.

Keywords Chinese tallow tree · *Sapium sebiferum* · Preservation · Storage · Oil quality · Biodiesel

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Introduction

The Chinese tallow tree (TT)—*Sapium sebiferum* is an ancient and valuable oil seed-producing tree with a long history of large scale commercial production in China and other parts of Asia. It is a deciduous tree, and it has been used in the US as an ornamental tree due to its exceptional foliage in the fall and flowering in the spring (Fig. 1a, b). Although it is known as an invasive tree in the US, TT has been regarded as a promising biomass candidate in the Gulf Coast regions, due to its ability to re-sprout, its rapid growth rate, and its drought and salt tolerance [1]. It is one of nature's most prolific producers of renewable hydrocarbons, yielding the equivalent of 500 gallons (12 barrels) of fats and oils per acre per year (4,700 L per hectare per year), far exceeding other traditional oil seed crops [2]. The seeds contain approximately 40–50% lipid [3], almost equally distributed in the external vegetable tallow coating and in the kernel as a drying (Stillingia) oil (Fig. 1c), suitable for conversion into biodiesel [4]. Chinese tallow can be grown over large areas by conventional agricultural methods and can provide woody biomass for direct burning or conversion to charcoal, ethanol, and methanol [4]. In addition, other value-added components have been identified in the leaves and bark of this tree [1, 4]. The oil has also been reported to have been used in Chinese medicine but overdoses might cause violent sickness and perhaps death [3].

Due to the seasonal nature of the TT and due to its potentially enormous worldwide economic importance, it is critical to recognize the various factors contributing to the deterioration of TT seeds. In general, once the seeds have reached their full maturity they are at the peak of their germinability and vigor. From that point, their lifespan diminishes due to aging, although the rate of aging depends

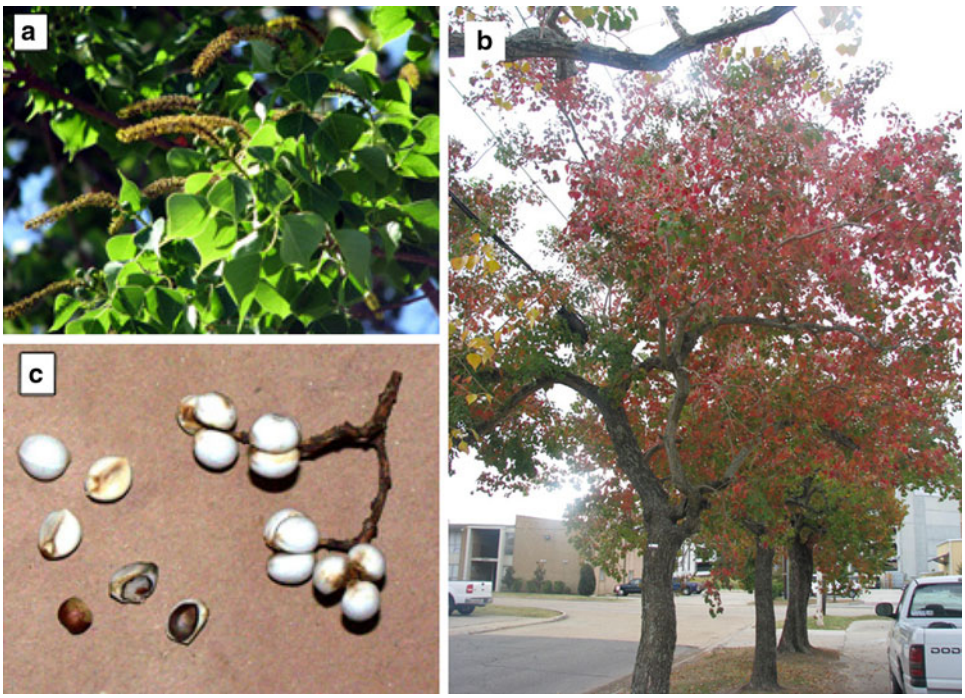

Fig. 1 Chinese tallow tree: **a** Green tree with flowers; **b** in the fall with multiple colors; **c** seeds with coating and kernel

on moisture content of the seed and storage conditions [5]. Deterioration in seed quality can occur during handling and storage after harvest until it reaches its end-user. Oil quality is directly related to the physiological conditions of the seeds from which oil is extracted. The aim of storage is to preserve properties of products and maintain the quality of extractable compounds. If suitable storage conditions are not employed for specific product varieties, qualitative and quantitative losses increase. Appropriate storage conditions and management preserves seed viability and vigor for relative extended periods by reducing the rate of seed deterioration. Optimal seed storage is achieved by preserving seeds in favorable climatic conditions and/or by modifying the environment around the seeds so that it is suitable for different storage periods of time [5]. In turn, post-storage losses may be also influenced by conditions during storage. For example, the storability of grains is affected mostly by their temperature, moisture content and environmental conditions [5]. Respiratory activity may accelerate some deterioration of the seeds and cause breakdown of the cell structure. Ultimately, oil extracted from deteriorated and damaged seeds can develop greater amounts of volatile acids and can be high in acidity and low in stability [6].

Loss may be considered in terms of either quantity or quality. Qualitative loss is more difficult to assess and is perhaps best identified through comparison with well defined standards. Nutritional loss and loss of seed are both aspects of quality losses. Fatty foods can be stored for only limited time periods because of their susceptibility to oxidation in air [7]. Natural oils are normally a mixture of triglycerides of saturated and unsaturated fatty acids. Oxygen can oxidize fatty acids in a multi-step reaction that occurs in principle according to a radical chain mechanism. The oxidation products formed by this reaction (mainly aldehydes) are volatile [7], and oxidative stability of natural oils is therefore a standard quality control method in the food industry [8].

Numerous handling, transportation and storage systems have evolved over the years for postharvest preservation of fresh fruits, vegetables and crop seeds. Depending upon the commodity and the specific preservation objective, there is a wide selection of techniques and systems to choose from. They vary in complexity from common storage involving little or no control of the postharvest environment to highly sophisticated systems such as hypobaric storage [9], controlling within very narrow limits: the temperature and humidity [10], concentration of oxygen [10], carbon dioxide [11] and other gases [12]. There are numerous studies that report about storage conditions affecting the quality of oil obtained from various feedstocks like olives, rice and sunflower [13–15].

The aim of this study is to evaluate the long-term effect of various storage conditions on oil quality and fatty acid composition in TT seeds during the storage period that follows the harvest. The results of this research will be used for optimum storage of TT seeds to preserve their quality for biofuel production.

Experimental Procedures

Seeds Collection

Seeds were manually harvested from tallow trees in and around the Baton Rouge area between October and November 2007. Another harvest of seeds left on other trees took place in March the following year, for comparison purposes. After harvest, the seeds were air dried for 2–3 days and separated from twigs and leaves by a combination of threshing, blowing and hand picking. The clean seeds with a final moisture content of approximately 4% were stored in a freezer at −4 °C until further use.

Parameters

TT seeds (50 g) were individually packaged using a Multivac system (Model C200, MultiVac Inc., Kansas City, MO) under different environmental conditions (3% CO_2, 6% CO_2, normal atmosphere and vacuum of 0.05 atm.). The packaged samples were stored at two temperatures, one under refrigeration (4 °C) and the other at controlled room temperature (24 °C) for 3 months. The storage temperatures were monitored and recorded daily. Due to the limited amount of seeds collected, only 3 months of storage at the most stringent parameters for food applications were considered in the study.

Oil Extraction

Every week, two packages from each storage temperature and under each storage condition were removed (total of eight packages), and the seeds were cracked manually in order to separate the endocarp from the seed shell. All seed and kernel samples were ground using a coffee grinder prior to oil extraction. A batch microwave system (model Ethos E, Milestone Inc., Monroe, CT) was used to extract the oil from the seed kernels using ethanol (ACS/UPS grade, 200 proof) as a solvent in a ratio of 1–3 (seed:ethanol, w/w) [16]. The extraction was performed in two steps: a gradual temperature increased to extraction temperature for 5 min, followed by holding at the extraction temperature of 130 °C for 15 min. The temperature of the sample inside the vessels was monitored with a fiber optic probe connected to the control system. After a ventilation period of 20 min in which the samples were cooled down, the vessels were unsealed and the oil and ethanol (miscella) were filtered from the cake through a Whatman® filter paper ($\Phi = 47$ mm) using a vacuum pump (Model SR 10/50, Thomas Compressors and Vacuum Pumps, Skokie, IL). The ethanol was evaporated from the miscella using a vacuum centrifuge evaporator (CentriVap Console Labconco, Kansas City, MO) running for approximately 14–16 h. The residual oil for each sample was used to determine the quality. Oil from whole seeds (coat and kernel) harvested in fall and spring was also extracted to estimate changes in yield and composition if the seeds are left on the trees past the seeds maturity date. Due to small sample sizes of the stored seeds, the oil yield values were compared only between the early and late harvested whole seeds. For comparison purposes, oil was extracted using hexane as a solvent from kernel, early and late harvested seeds using the same extraction method, FA also being analyzed using the same chromatographic procedure as for with ethanol extraction.

Determination of Oil Quality

Titratable acidity (TA), peroxide value (PV), oxidative stability index (OSI) and fatty acid (FA) compositions were determined according to the IUPAC standard methods for analysis of oil and fats [17]. Titratable acidity was measured by titrating 1 g of oil with 0.1 N KOH solution, and 3 g of oil were titrated with $Na_2S_2O_3$ for peroxide value analysis. The OSI of the oil samples was evaluated using a Rancimat oxidative stability instrument (model 743, Metrohm USA, Inc., Riverview, FL) operating at 110 °C on 3 g samples exposed to airflow at 0.01 m^3/h. The volatile products formed as a result of the oxidation reaction were collected in distilled water and the change in electrical conductivity of the water was measured as an indication of the oil's oxidative stability.

The FA compositions were determined by quantifying the methyl esters through gas chromatography (Varian 450-GC, Scientific Instruments, Lake Forest, CA) coupled with a Varian 240-MS Ion Trap Mass Spectrometer (Scientific Instruments). A Varian FactorFour Capillary column WAXms (30 m × 0.25 mm i.d., 0.25 μm) was used at 245 °C with helium at 1 ml/min as carrier gas, a split injector at 270 °C with a split ratio of 1:20, and a detector temperature of 270 °C. Components were identified using a standard FAME mix (Supelco, Bellefonte, PA).

Statistical Analysis

All measurements were carried out in duplicates and expressed as mean values. Statistical analysis was performed in order to test significant differences among the different storage conditions in time. A two-way ANOVA using Proc Mixed 2 × 5 factorial (SAS system, SAS Institute Inc., Cary, NC) was used to test significant differences among temperatures (4 and 24 °C) and environmental atmospheric conditions. Two-way ANOVA using Proc Multiple comparison tests were performed by using Tukey adjustment to determine the significant difference ($\alpha = 0.05$) between treatments.

Results and Discussion

Titratable Acidity and Peroxide Value

The chemical properties of oil can be used to ascertain its quality. Titratable acidity is an important indicator of vegetable oil quality and is expressed as the amount of KOH (in mg) necessary to neutralize free fatty acids contained in 1 g of oil [17]. The TA value of the oil must not be too high (≥5 mg KOH/g) as this denotes an excessively high content of free fatty acids, which causes the oil to turn acidic [18]. TA was measured across the 12 weeks of storage and is presented in Table 1. Compared to the value at harvest, neither air nor controlled atmosphere significantly modified TA values within the storage conditions. Moreover, temperature of storage did not seem to have an influence on the TA.

The peroxide value (PV) indicates the extent to which oil has been oxidized. It quantifies the amount of peroxides, the intermediate product of the autoxidation reaction, contained in the oil. The PV of the oil extracted from the seeds ranged from 0.72 to 0.82 mequiv/kg (Table 1) during the entire storage period. Irrespective of the storage conditions, PV of the stillingia oil extracted from seeds during any week of storage (within the tested period) did not vary significantly. Though the PVs of the oil were not significantly different, changes in the biological make-up of the oil cannot be discounted which could have an influence on the OSI values. Peroxide value has shown to be inversely related to the α-tocopherol (an antioxidant) content in shelled and roasted almonds stored over 9 months [19], and the rate of increase in the peroxide value in stored pistachio has been shown to be greater when stored in air than when stored in a CO_2 rich environment [20]. A study conducted by Kaul et al. [21] on the effect of aging on jojoba (*Simmondsia chinensis*) oil quality revealed that the TA, PV and iodine value of solvent extracted oil increased with storage time (over 18 months storage). Similar changes in PV over time (but for 12 months) has been reported by Mexis et al. [22] in their study on the storage condition of oil from shelled walnuts. The PV of the oil from the stored walnuts was found to increase at a faster rate at higher storage temperatures (20 °C) when compared at a storage temperature of 4 °C. Temperature influences the rate of rancidity of fats and oils and also has an influence on the gaseous diffusion rate (through the packaging material). Even though our study agrees with other research describing the positive increase in PV with storage temperature [20], an in-depth investigation involving an increase in the storage period, multiple storage temperatures, inclusion of seed moisture content and humidity would provide a better picture of the long term quality of tallow tree seeds.

Fatty Acid Composition

The major fatty acids were selected to examine any changes in oil composition as a function of time and storage parameters. Although stillingia oil is composed of more than seven kinds of fatty acids, five major fatty acids

Table 1 Average titratable acidity (TA) (mg KOH/g sample) and peroxide values (PV) (mequiv/kg) of the stored TT seeds

			Storage time (weeks)												
			0	1	2	3	4	5	6	7	8	9	10	11	12
Air	4 °C	TA	1.62	1.64	1.65	1.65	1.67	1.65	1.67	1.65	1.65	1.69	1.67	1.70	1.71
		PV	0.72	0.72	0.73	0.73	0.74	0.73	0.75	0.78	0.74	0.76	0.80	0.79	0.79
	24 °C	TA	1.62	1.63	1.66	1.66	1.68	1.68	1.67	1.66	1.69	1.72	1.70	1.70	1.71
		PV	0.72	0.72	0.72	0.74	0.74	0.75	0.76	0.75	0.78	0.76	0.80	0.83	0.82
Vacuum	4 °C	TA	1.62	1.62	1.64	1.64	1.64	1.63	1.65	1.68	1.66	1.68	1.72	1.69	1.70
		PV	0.72	0.72	0.72	0.72	0.72	0.75	0.74	0.77	0.80	0.83	0.79	0.80	0.81
	24 °C	TA	1.62	1.63	1.65	1.65	1.67	1.68	1.67	1.70	1.68	1.69	1.72	1.70	1.71
		PV	0.72	0.72	0.73	0.73	0.75	0.74	0.77	0.80	0.79	0.77	0.82	0.80	0.81
3% CO_2	4 °C	TA	1.62	1.63	1.63	1.65	1.67	1.67	1.65	1.65	1.64	1.65	1.69	1.67	1.67
		PV	0.72	0.72	0.72	0.74	0.74	0.73	0.75	0.73	0.76	0.76	0.78	0.76	0.77
	24 °C	TA	1.62	1.64	1.64	1.65	1.64	1.66	1.66	1.65	1.66	1.68	1.70	1.67	1.69
		PV	0.72	0.72	0.73	0.74	0.74	0.72	0.76	0.74	0.75	0.79	0.79	0.77	0.79
6% CO_2	4 °C	TA	1.62	1.62	1.63	1.63	1.62	1.64	1.64	1.67	1.68	1.72	1.69	1.70	1.71
		PV	0.72	0.72	0.72	0.74	0.75	0.75	0.73	0.76	0.78	0.77	0.80	0.79	0.80
	24 °C	TA	1.62	1.64	1.64	1.66	1.66	1.68	1.66	1.69	1.67	1.71	1.70	1.69	1.70
		PV	0.72	0.72	0.74	0.74	0.74	0.76	0.76	0.78	0.75	0.76	0.79	0.81	0.81

(palmitic, stearic, oleic, linoleic and linolenic acids) comprise about 95% of the total fatty acid content. The FA composition of stillingia oil extracted from the kernel of freshly harvested seeds was 0.5% myristic, 4.29% palmitic, 1.18% stearic, 11.07% oleic, 18.30% linoleic, 63.21% linolenic, 0.5% arachidic and 1.13% "japanic acid" (eicosanedioic and docosanedioic acids). The TT kernel oil contains a high degree of unsaturated fatty acids (oleic, linoleic and linolenic) comprising more than 93% of the total fatty acids. This makes this oil highly susceptible to oxidation resulting in the development of rancidity and off-flavors, but it is suitable for transesterification into biodiesel. The presence of japanic acid is believed to be an important factor in the dryness of stillingia oil when used as a coating material or for biodiesel production. Similar values for FA composition for this crop have been reported earlier [23].

Storage of TT seeds for 3 months had no significant effect on the FA composition of kernel oil between the studied controlled atmospheres. Since no significant differences were observed between the storage times under the same storage conditions, the average values ± standard deviations for the 12 weeks of study are presented in Table 2.

Although there were no significant changes in the oil composition between the different storage conditions, the amount of myristic and "japanic acid" could only be detected in trace amounts after the first week of study. One good explanation for its disappearance could be that japanic acid in the presence of atmospheric oxygen could have degraded to form other compounds. As japanic acid was not detected even in vacuum packaging, we can infer that its degradation occurs mostly in the several days between harvesting time and packaging.

TT oil has a higher percentage of linolenic (>63%) and linoleic (>19%) acids than oleic acid (between 10 and 11%). The greater percentage of unsaturated fatty acids present in this oil could lead to faster rate of rancidity in the oil during extended storage and exposure to higher storage temperature. A study conducted on the sensory stability of two peanut varieties containing different ratios of oleic–linoleic acid content reveals the importance of studying the fatty acid composition in oils [24]. The peanuts containing an oleic–linoleic acid (O/L) ratio of 17.2 had a shelf life of 25 times longer (202 days as opposed to 8 days) than the peanuts having an O/L ratio of 1.4 when stored at 23 °C. The shelf life of the peanuts was determined based on the storage period required for the peroxide values in the peanuts to cross 10 mequiv O_2 kg^{-1}. The study also concluded that the shelf life was ten times longer (99 days) for the high O/L ratio peanuts than the low O/L ratio peanuts (10 days) when stored at 40 °C.

Table 2 Fatty acid composition of stillingia oil (%)

Fatty acid	Air		Vacuum		3% CO_2		6% CO_2	
	4 °C	24 °C	4 °C	24 °C	4 °C	24 °C	4 °C	24 °C
Myristic, C 14:0	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]
Palmitic, C 16:0	4.18 ± 0.38[b]	4.35 ± 0.38[b]	4.30 ± 0.58[b]	4.40 ± 0.37[b]	4.19 ± 0.58[b]	4.11 ± 0.65[b]	4.02 ± 0.18[b]	4.20 ± 0.40[b]
Stearic, C 18:0	1.14 ± 0.07[b]	1.19 ± 0.13[b]	1.22 ± 0.12[b]	1.23 ± 0.07[b]	1.14 ± 0.11[b]	1.11 ± 0.17[b]	1.11 ± 0.07[b]	1.13 ± 0.11[b]
Oleic, C 18:1	10.88 ± 0.85[b]	10.79 ± 1.07[b]	11.13 ± 0.67[b]	10.88 ± 0.56[b]	10.74 ± 0.58[b]	10.84 ± 1.03[b]	10.61 ± 0.61[b]	11.04 ± 0.61[b]
Linoleic, C 18:2	19.39 ± 0.65[b]	19.68 ± 0.70[b]	19.59 ± 1.07[b]	19.67 ± 0.73[b]	20.25 ± 0.90[b]	19.95 ± 0.82[b]	19.70 ± 0.74[b]	19.84 ± 0.62[b]
Linolenic, C 18:3	64.26 ± 1.15[b]	63.72 ± 1.85[b]	63.67 ± 1.68[b]	63.54 ± 0.99[b]	63.64 ± 1.66[b]	63.92 ± 1.56[b]	64.54 ± 0.082[b]	63.63 ± 0.91[b]
Arachidic, C 20:0	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]

[a] Trace ≤0.3%

[b] Values in the same row for each treatment, i.e., storage condition and temperature, are not significantly different ($P \geq 0.05$)

Table 3 Fatty acid composition of stillingia oil (%) extracted with hexane

Fatty acid	
Myristic, C 14:0	–
Palmitic, C 16:0	2.42 ± 0.17
Stearic, C 18:0	2.20 ± 0.16
Oleic, C 18:1	14.36 ± 0.06
Linoleic, C 18:2	46.80 ± 0.76
Linolenic, C 18:3	34.23 ± 1.14
Arachidic, C 20:0	–

Oil extracted using hexane as a solvent was also analyzed for comparison purposes. Results indicated different amounts of fatty acids extracted from the kernel (Table 3) especially for linoleic and linolenic acids. The amount of linoleic acid was found to be double, while the amount of linolenic acid was approximately 50% less than in the oil extracted with ethanol. The amount of oleic acid was 4% higher when using hexane as an extraction solvent. Since hexane is a non-polar solvent in comparison with the polar solvent ethanol, it is natural that the components or the amount of components extracted from oil to be different. Using the percentage values of oleic, linoleic and linolenic acids determined from GC analysis (Tables 2, 3), the iodine values (IV) of the extracted stillingia oil were computed by the procedure outlined by Kyriakidis and Katsiloulis [25]. The iodine values of the ethanol and hexane extracted stillingia oils were 207.84 and 174.24, respectively. The ethanol-extracted oil has a higher IV than the hexane-extracted oil. According to EN14214 biodiesel specifications the maximum acceptable limit for IV in the oil is 140 [26] and hence, stillingia oil extracted by both solvents does not meet this specification. However, this does not mean that stillingia oil is not useful as biodiesel as it satisfies most of the other requirements.

Oxidative Stability Index

During long term storage, oxidation due to contact with air (autoxidation) represents a legitimate concern with respect to maintaining oil quality. Figure 2 shows the changes in oxidation stability of the oils obtained from seed kernels stored at different temperatures and under different storage conditions. Although no differences were detected in TA, PV and FA compositions of the oils, there was a significant change in the OSI. The stability of the oils was shown to be influenced by time, temperature and the atmosphere used for storage. Significant differences could not be found between the first week and the last week of storage; however, the OSI for all the storage conditions increased to a maximum after the seventh week of storage. The conditions of storage also influenced the OSI. The oils from the seeds stored in 6% CO_2 had the highest OSI of 16 h, followed by the ones stored at 3% CO_2 and vacuum (Fig. 2). As it was expected based on the existing literature data, the oils from the seeds stored in the normal headspace had the lowest OSI, most commonly resulting from a higher autoxidation reaction that took place in contact with air. The lower values obtained (approximately 4 h) are similar to the other values reported in the literature for other vegetable oils (i.e. soybean oil, canola oil) with a minimum of 3 h [27].

Storage temperatures also had an impact on OSI. Oils from seeds stored at 4 °C showed that they have a higher stability over ones stored at ambient temperature, with the exception of a 3% CO_2 environment where a room temperature conditions gave a slightly higher value (Fig. 2). Studies on olive oil storage [13] also showed a high decrease in OSI for olive oil stored at ambient temperature compared to one stored at 5 °C under different conditions and an increase in oxidized triglycerides and diglycerides with storage time. It is notable that other researchers have recently found that the OSI values of biodiesel derived from stillingia oil were approximately 0.6 h, which is lower than the accepted value for biodiesel of 6 h [28], but nonetheless this biodiesel will still be useful as it satisfied most other requirements.

One of the most important parameters that influences lipid oxidation and its stability is the degree of unsaturation of its fatty acids. The reason for autoxidation is due to the presence of double bonds in unsaturated fatty acids. The rate of autoxidation varies according to the number and position of the double bonds. The oxidation of the unsaturated fatty acids components in the stillingia oil might occur easily and lead to degradation of the oil during storage. Although the unsaturated level in our study did not have a significant change between the storage conditions and storage time, there could be other changes in oil composition that could influence the OSI. The increase in OSI may also be an effect of the presence of natural compounds that exhibit antioxidant activity. Lipid oxidation is known as one of the major forms of spoilage in foods, and leads to the formation of off-flavors and also potentially toxic compounds. Autoxidation is an extremely complex process involving numerous reactions that result to a variety of chemical and physical changes in lipids, sometimes difficult to discover with typical standard analysis. The possible species formed during the low oxidation process in the first 6 weeks of storage could lead to the possible formation of extraneous species that might act as a barrier to oxidation in the seventh week of study. Another possible alteration mechanism is lipid hydrolysis, with consequent free fatty acid (FFA) generation, by chemical or enzymatic action. Although the original causes and consequences of oxidative

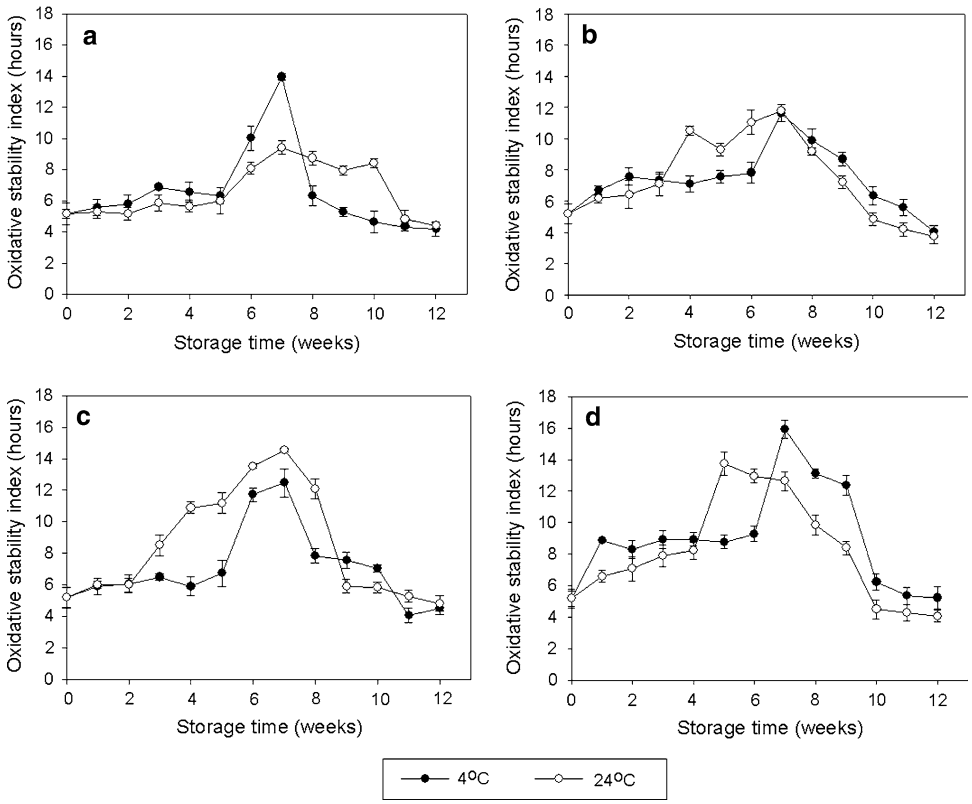

Fig. 2 Changes in the stability to oxidation (h) stored under **a** air atmosphere; **b** vacuum; **c** 3% CO_2; **d** 6% CO_2

and hydrolytic degradation processes are quite different, they might interact with each other and contribute to the observed changes in the oil stability. In fact, the pro oxidant action of FFA, which seems to be exerted by the carboxylic molecular group, accelerating the rate of decomposition of hydroperoxides has been reported [29]. It could be also hypothesized that amino groups, from amino acids in the proteins transferred in the oil during extraction, could react in the presence of oxygen with radicals present in the FA, the reaction that would lead to the formation of stable products [30]. The amino acids, most probably histidine and tyrosine present in the tallow tree seeds [3], are able to convert in imino acids and liberate phenolic or pyrroline groups that could further bond to hydrogen, and display higher antioxidant activity.

The longer shelf-life with no appreciable change in oil properties of the in-shelled materials could be attributed to the role of the shell which can behave as a barrier to moisture and atmospheric exchange, retaining the quality aspects of the kernel. Prior research [31] reported that cardinal flower seeds stored under an oxygenated environment lost viability rapidly and that storage in carbon dioxide, nitrogen, or partial vacuum extended storage life. In contrast, another study on sorghum and crimson seeds [32] showed no significant advantages in using a partial vacuum or carbon dioxide. nitrogen, argon or helium atmospheres instead of air.

When comparing to other semi-dry oils used for biodiesel production, Jatropha oil was found to have a high content of FFA (15%) [33] and high AV (3.5 mg KOH/g) [34] but technologies (such as acid or enzyme catalysis) do exist that can handle the transesterification of FFA [18]. Another drying oil, tung oil has about 80% eleostearic acid (a conjugated C18:3) and hence, low OSI (0.5 h) [35].

Early and Late Harvested Seeds

Early and late harvested TT seeds were collected and the whole seeds oil compositions and chemical properties are presented in Table 4. Whereas the oil yield did not change between the two harvesting times, the oil composition showed itself to be significantly affected by the time of harvest. Percentages of myristic, palmitic, stearic and oleic acids significantly decreased in the oil from late harvested seeds. The change in seed coating could be observed visually in the late harvested samples, becoming darker than the earlier harvested seeds, as a possible consequence of the presence of a particular species of fungi (mold) on the seeds' surface. This could be the direct cause of this decreased value, especially for myristic and palmitic acids that are present in the outer coat of the seed. Japanic acid, in a similar way to the phenomenon observed in kernel oil, did not appear in the late harvested samples due to a higher degree of oxidation, while stearic acid could only be found

Table 4 Properties and composition of the oil from early and late harvested seeds (values reported as %, unless otherwise noted) extracted with ethanol

	Early	Late
Myristic, C 14:0	13.63 ± 1.33[a]	3.83 ± 0.96[a]
Palmitic, C 16:0	14.61 ± 1.75[a]	3.86 ± 0.37[a]
Stearic, C 18:0	2.42 ± 0.11[a]	Tr.[a, b]
Oleic, C 18:1	8.96 ± 0.14[a]	3.94 ± 1.14[a]
Linoleic, C 18:2	26.01 ± 0.73[a]	25.01 ± 0.17[a]
Linolenic, C 18:3	30.89 ± 0.98[a]	36.17 ± 2.13[a]
Arachidic, C 20:0	2.44 ± 0.28[a]	21.36 ± 0.95[a]
Eicosanedioic, $C_{20}H_{38}O_4$	0.51 ± 0.06[a]	–[a]
Docosanedioic, $C_{22}H_{42}O_4$	0.59 ± 0.04[a]	–[a]
Total unsaturated	65.85 ± 1.85[a]	70.97 ± 1.85[a]
Total saturated	33.10 ± 3.48[a]	29.06 ± 1.42[a]
TA (mg KOH/g)	4.2 ± 0.61[a]	5.1 ± 0.39[a]
PV (mequiv/kg)	2.66 ± 0.36[a]	3.35 ± 0.48[a]
OSI (h)	24.18 ± 1.5[a]	11.64 ± 1.35[a]
Oil yield (%)	34.55 ± 0.32[c]	34.33 ± 0.65[c]

[a] Value in the same row for each component, are significantly different ($P < 0.05$)

[b] Trace <0.3%

[c] Value in the same row for each component, are not significantly different ($P \geq 0.05$)

in traces. An increase in unsaturated fatty acid composition and a decrease in saturated fatty acid composition during seed maturation has been reported in previous studies on the maturity of cannabis and jatropha seeds [36, 37].

The increase in linolenic and arachidic acids is a good evaluation of the oxidation between the harvesting times. The increase in these compounds is the direct consequence of the decrease in oxidation stability, also observable from the OSI (Table 4), where the stability against oxidation decreased from 24 to 11.6 h for the two harvested periods. Another plausible explanation for observing an increase in arachidic acid content in the extracted oil from the seeds is the biochemical changes occurring within the seeds as a result of maturity. A study on the fatty acid composition of chia seeds at different stages of maturity indicate an increase in palmitic, stearic, oleic and arachidic acid contents with increasing growth stage accompanied by a decrease in the linolenic acid content [38]. Sunflower oil undergoes a similar transformation during maturation, in which the elongase enzyme increases the length of the triglyceride chain into unsaturated fatty acids [39]. The difference observed in FA composition from the TT seeds at the different stages of maturity could also be correlated to the biochemical changes as a result of the presence growth and of fungi. Certain fungi are well capable of up-converting significant amounts of short chain FA into long chain unsaturated FA [40]. It is probable that either of these mechanisms occurred during seed maturation on the tree, with the further transformation into saturated chains as a result of long-term exposure to various environmental factors while still on the tree prior to late-season harvesting. Chemical properties of the seeds also changed between the two harvesting periods. TA increased from 4.2 to 5.1 (mg KOH/g) of oil, due to the prolonged oxidation processes and also due to the presence of the fungi on the tallow coating, which has a direct consequence on the increased FFA content in the late harvested seeds. An increase in value was also observed for the PV, where change from 2.66 to 3.35 (mequiv/kg) was measured between the two harvesting times, also indicating a higher level of rancidity of the oil.

When using hexane as an extraction solvent instead of ethanol, the relative amount of oil extracted decreased by approximately 5.8% (i.e. from 34.6 to 32.6% oil extracted on an average), and small but significant changes in oil composition took place for both early and late harvested seeds. Significant changes occurred for myristic acid (68.16% relative decrease), linoleic acid (27.8% relative increase) and linolenic acid (a relative increase of 23.57%) for the early harvested seeds (Table 5). The results indicate that the oil extracted using ethanol (polar solvent) contained more saturated fatty acids (myristic acid) than unsaturated fatty acids (linoleic and linolenic acids).

Change in composition was also noticed in the late harvested seed oil, where no myristic and palmitic acids

Table 5 Properties and composition of the oil from early and late harvested seeds (values reported as %, unless otherwise noted) extracted with hexane

	Early	Late
Myristic, C 14:0	4.34 ± 0.43[a]	–[a]
Palmitic, C 16:0	13.21 ± 0.76[a]	–[a]
Stearic, C 18:0	0.66 ± 0.06[a]	3.63 ± 0.23[a]
Oleic, C 18:1	6.62 ± 1.20[b]	7.74 ± 0.70[b]
Linoleic, C 18:2	33.24 ± 0.49[a]	29.35 ± 0.20[a]
Linolenic, C 18:3	38.17 ± 1.13[b]	37.70 ± 0.73[b]
Arachidic, C 20:0	3.75 ± 0.29[a]	22.24 ± 1.45[a]
Eicosanedioic, $C_{20}H_{38}O_4$	–	–
Docosanedioic, $C_{22}H_{42}O_4$	–	–
Total unsaturated	78.03 ± 2.82[a]	74.79 ± 1.63[a]
Total saturated	21.96 ± 1.54[a]	25.87 ± 1.68[a]
TA (mg KOH/g)	5.8 ± 0.52[b]	5.4 ± 0.76[b]
PV (mequiv/kg)	2.49 ± 0.86[a]	3.77 ± 0.59[a]
OSI (h)	22.36 ± 2.23[a]	13.48 ± 2.56[a]
Oil yield (%)	32.57 ± 0.22[b]	32.49 ± 0.32[b]

[a] Value in the same row for each component, are significantly different ($P < 0.05$)

[b] Value in the same row for each component, are not significantly different ($P \geq 0.05$)

were detected in the hexane-extracted oil while traces of stearic acid was found in the ethanol-extracted oil (as opposed to 3.63% in hexane-extracted). The relative percentage increase in oleic and linoleic acid contents were respectively, 96.45 and 17.35% when compared with the ethanol-extracted oil. These small changes in FA composition also affected the unsaturation levels in the oil, which increased by 18.5% in early and by 5.38% in late harvested seed oil extracted by hexane. However, these differences are not critical to the usage of the oil for biodiesel production. These results, comparing the quality of the oil from seeds harvested at different times, provides important information about the behavior of the material depending on the intended end use (transesterification into biodiesel or other uses). First of all, the percentage of oil extracted did not significantly change between the seeds harvested at the two different times (Table 3). Moreover, the change in FA composition does not affect the oil quality requirements for being used as a feedstock for biodiesel.

Overall, from this study, it can be concluded that after 12 weeks of storage, a controlled atmosphere did not produce any remarkable advantage over low cost air storage for stillingia oil. This was evident from the absence of any significant changes found in the TA, PV and FA compositions of the extracted oil. The storage temperature did have an influence on the OSI with refrigeration temperature favoring higher OSI values in the oil than room temperature storage. For whole seeds lipids, there was little to no change in overall lipid content between early and late harvested seeds, even though changes in the FA makeup did occur. The results validate the belief that no elaborate storage conditions are required to store this economically promising high oil content biofuel feedstock. Further investigations of increases in the storage period for whole seeds, varying moisture content, relative humidity and storage temperature are warranted to obtain a better understanding of the effect of the storage environment on the TT oil quality.

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