Energy 36 (2011) 5403–5408

Contents lists available at ScienceDirect

Energy

journal homepage: www.elsevier.com/locate/energy

The influence of seed and oil storage on the acid levels of rubber seed oil, derived from *Hevea brasiliensis* grown in Xishuangbanna, China

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ARTICLE INFO

Article history:
Received 12 December 2010
Received in revised form
20 June 2011
Accepted 24 June 2011
Available online 23 July 2011

Keywords:
Hevea brasiliensis
Rubber seed oil
Oil acid levels
Storage
Mildew
Biodiesel

ABSTRACT

High acid levels, characteristic of rubber seed oil (RSO), limit RSO use in biodiesel production. The aims of this study were to determine the causes of these high acid levels by investigating what affects the storage of rubber seeds and RSO had on the acid levels. Two storage conditions/methods were evaluated, one representing a proposed storage method (SM 1), the other mimicking storage conditions characteristic to the Xishuangbanna region (SM 2). Furthermore, RSO storage was evaluated by testing RSO acid levels over a 2-month period, under standard storage conditions. Seeds from SM 2 displayed increased seed pile temperatures, higher levels of Mildew infection, lower seed oil content and higher acid levels. Low seed oil content and high acid values of SM 2 were resultant of the high Mildew infection and increased seed pile temperatures. In addition, a critical value of 90% relative humidity of seed piles was identified, above which Mildew infection increased sharply. Storage of crude RSO resulted in increased acid values. This data shows that in order to reduce high acid values, seed pile temperature, humidity and Mildew infections need to be kept to a minimum, as well as the storage time of the seeds and the RSO.

1. Introduction

Rubber seed oil (RSO), derived from the seeds of *Hevea brasiliensis* Müll. Arg., is one of a number of oils being explored for its potential use as an alternative to petroleum fuels. Biodiesel is typically produced from natural or renewable materials such as oils or vegetable fats. Research into biodiesel has revealed a range of vegetable oils, such as soybean oil, sunflower oil, palm oil, and peanut oil that are suitable, after refinement, to be used in diesel engines [1–4]. However, the drawback with the use of these oils as biodiesel is that, as edible oils, their market value is fairly high [5]. Thus not only is the production price of biodiesel from edible oils high, it also competes with food crops for land and resources [6–8]. As a result, alternative oils, such as RSO, are being explored for their potential use as biodiesels [8]. A recent review published by Azocar et al. (2010) [9] stated that Jatropha, Pongamia, and rubber seeds are the most eligible sources of oil for the production of biodiesel.

Rubber seeds contain a high amount of oil and RSO has been shown to have a number of different industrial and commercial uses, RSO can be used as an ingredient in soaps, alkyd resins, as a lubricant and as a foaming agent [10–13]. Although the oil content of rubber seeds varies amongst different regions, the average oil yield is reported to be between 40% and 50% of seed weight [6,7,11]. RSO has been proven suitable for use as a source of biodiesel, but like most vegetable oils, which contain high levels of free fatty acids, RSO must first be chemically modified through a refining process [7,14]. The high free fatty acid content of RSO is also represented by the high acid values (mg KOH/g) of the RSO [15]. These high acid values mean that the oil cannot be converted into biodiesel via the conventional alkaline catalyzed transestification process applied to other vegetable oils, as the free fatty acids react with the catalyst, forming soaps [16]. Thus, the oil needs to be refined before it can be converted into biodiesel via this technique. Other techniques of converting RSO into biodiesel are available, such as acid-estification, but these processes are expensive and time consuming [7,17,18].

Acid values for RSO in the current literature range from 15.03 to 81.6 mg KOH·g^{-1} [7,19–29]. Although the acid values range widely, studies of the acid values of Chinese RSO have revealed a much smaller range, from 31.7 to 32.6 mg KOH/g [22,30]. The variability

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doi:10.1016/j.energy.2011.06.054

amongst the acid values from different studies, countries, and years indicates that factors related to the storage of raw rubber seeds and the production processes in each locality may largely influence the acid values.

Yunnan Province, China, is currently a focal area for rubber production in Asia, thus resulting in a surplus of rubber seeds and the potential for rubber seed oil production. Xishuangbanna Prefecture, located in the south of Yunnan Province, is particularly suitable for rubber production and has seen an expansion in the size and number of rubber plantations since the mid-twentieth century. In this region, most rubber seeds are simply dried and used as feed for livestock, or local farm workers use a small amount for the production of vegetable oils. However, some rubber seed oil processing plants have opened and begun processing rubber seeds and producing oil.

This research was undertaken to explore options for increasing the utility of RSO, in light of the need for alternative fuel sources, such as biodiesel, in China [31]. We aim to achieve this by identifying factors in the storage and processing of rubber seeds and RSO that contribute to its high acid value, thereby minimizing the refining process of the oil, making the production of biodiesel more cost effective. It compares controlled storage and processing conditions with conditions typical to rubber seed oil plants in Xishuangbanna. The main objective being to identify the determinant phase in the production of RSO that is responsible for its high acidity. In order to achieve this the storage methods of the raw rubber seeds and the storage of crude rubber seed oil was investigated.

2. Materials and methods

2.1. Seed variety and storage conditions

Seeds from the rubber tree (*Hevea brasiliensis* Muell.Arg) were collected from a rubber plantation in the Xishuangbanna Prefecture, Yunnan Province, China. Seeds were collected from the *H. brasiliensis* variety RRIM600 one of the most widely planted varieties of *H. brasiliensis* in this area.

The seeds were separated into two groups and exposed to two different storage conditions/methods, representing controlled laboratory storage conditions (SM 1) and traditional storage conditions (SM 2). SM 1 consisted of seeds stored in crates, in a well-ventilated room (4 m × 5 m), in layers between 0.4 m and 0.5 m thick. Whereas SM 2 mimicked traditional storehouse conditions for the region and consisted of seeds being stored in piles of an average height of 3.5 m, in large storerooms (40 m × 15 m).

2.2. Humidity monitoring

The humidity levels of the indoor rubber seed storage units were monitored using three SMARTSENSOR AR847's (Hong Kong, China). The SMARTSENSOR has a 3% error in reading, therefore the three SMARTSENSORS were recalibrated between each measurement and were each calibrated to the same standards, thus minimizing error readings between the different sensors. Measurements were performed every day at 10 am, by inserting the humidity monitor to a depth of 0.5 m in the storehouse rubber seed piles (SM 2), and 0.2 m in the laboratory rubber seed layers (SM 1).

2.3. Seed moisture content

After the seed samples had been weighed, the seed casings were removed, thus allowing moisture inside the seeds to evaporate. The seeds were then placed in a stainless steel tray, loaded into a 110 °C constant temperature drying oven for 4 h, and then allowed to reach room temperature and again measured. Moisture content was then calculated using the following formula:

$$A = \frac{(W_1 - W_2)}{W_1} \times 100\%$$

Where A is seed moisture, W_1 is the initial weight of the seeds (before drying) and W_2 is the weight of the seeds post drying.

2.4. Mildew monitoring

After the moisture readings were taken, the seeds were then separated according to those that had been affected by mildew and those which remained unaffected. The percentage of mildew affected seeds was then calculated according to the following formula:

$$B = \frac{n_1}{(n_1 - n_2)} \times 100\%$$

Where B is the percentage of mildew affected seeds, n_1 represents the mildew affected rubber seeds and n_2 represents unaffected rubber seeds.

2.5. Kernel oil content measurement

The seeds were recombined, crushed, weighed and a Soxhlet's extraction [32] was performed using hexane to extract the oil and fat from the seeds. This method has been used by a number of other studies on RSO and is selected for the high percentage oil (99.5%) extracted from the seeds [27,28,33]. After extraction, the seeds were weighed again and the seed oil content was calculated using the following formula:

$$C = \left(\frac{W_4}{W_3}\right) \times 100\%$$

Where C represents the seed oil content, W_3 is the seed weight after being crushed and W_4 represents the seed weight after oil extraction.

2.6. Acid value measurement (mg KOH. g^{-1})

Acid values were determined according to the methods prescribed by ISO 660 (1996) [34]. Acid values were calculated using the following formula:

$$D = \frac{V \times c \times 56.11}{m}$$

Where D (mg KOH. g^{-1}) represents the sample acid value; V (ml) is the sample volume of Potassium hydroxide standard titrant solution consumed; c (mol/L) represents the Potassium hydroxide standard titrant solution potency and m (g) is the sample size.

2.7. Crude RSO source and storage conditions

Crude oil was purchased from a rubber seed oil processing plant in Xishuangbanna and used to determine the short-term effects of storage on the acid value of the seed oil. Three separate batches of oil were purchased for sampling, the first was obtained immediately after the production process, the next batch was purchased 1 month after the production process (1 month factory storage), and the third was purchased 2-months after the production process (2 months factory storage). Each crude oil sample weighed 20 kg and was stored in 25 L sealed plastic containers at room temperature in a low light environment.

2.8. Statistical analysis

SPSS Statistics 17.0 software was used for statistical data analysis. Tests for variable correlation were carried out between factors and an analysis of variance was carried out to determine differences the two different storage conditions/methods.

3. Results and discussion

3.1. Seed pile temperature, humidity and percentage mildew infection

There was a marked difference in the temperature of the seed piles under the two different storage conditions/methods (Fig. 1a), with the seeds stored under SM 2 having approximately a 2-fold increase in temperature for much of the storage time. This pattern is mirrored in the relative humidities of the respective seed piles, where, as with seed pile temperatures, the relative humidity of the seed piles under SM 2 were significantly higher than those of SM 1 (Fig. 1b). The drop in seed pile temperature and humidity after approximately 30 days (Fig. 1a,b) of storage is a response to the end of the "sweating" period of seed storage, whereby during the first 6 weeks of storage seeds can have a high respiratory rate before entering dormancy [35]. The decline in temperature was most noted in SM 2 due to the poor ventilation allowing a build up of heat, whereas the heat generated by the seed piles in SM 1 was allowed to dissipate. This increase in temperature and humidity resulted in an increase in the occurrence of Mildew infections (Fig. 1c), as evidenced by the correlations between seed pile temperature and Mildew and seed pile humidity and Mildew (Fig. 2a,b). Previous studies have shown that these conditions of high temperature and humidity can result in an increased number of Mildew infections affecting rubber seeds [36,37]. The relationship between seed pile temperature and the percentage Mildew infections (Fig. 2a) is linear, thus there is a constant influence of increasing temperature on the rate of Mildew infections, showing the sensitivity of Mildew to temperature changes. Whereas the relationship between seed pile humidity and Mildew infections is exponential (Fig. 2b), with a critical value of about 90% humidity. Below this critical value humidity has a weak effect on the percentage Mildew infections, however, above this value the influence of humidity is greatly enhanced.

The drastic differences in the temperature, humidity and Mildew infection levels when comparing the seeds from the two different storage conditions/methods indicates that SM 1 is a far more effective way of storing the rubber seeds. Furthermore, the storage time that the seeds were stored for greatly influenced the number of seeds infected with Mildew, after 60 days the seeds from SM 2 had approximately an 8-fold increase in the amount of seeds infected with Mildew in comparison to the seeds of SM 1 (Fig. 1c). This indicates the need for both the correct storage conditions of the seeds and also the need for an efficient production line, thereby minimizing the storage time of the rubber seeds.

3.2. Kernel oil content and acid values

Fig. 3a shows the differences between the kernel oil content of the two storage conditions/methods, with the seeds from SM 1 having a significantly higher oil content than those from SM 2. The influence of the different storage conditions/methods on seed oil content is the result of two factors: the increased seed pile temperature and the Mildew infections arising from seed storage. The influence of seed pile temperature on seed oil content is two fold, in that the Mildew infections have been linked with the rise in temperature, and, secondly, an increase in temperature also results

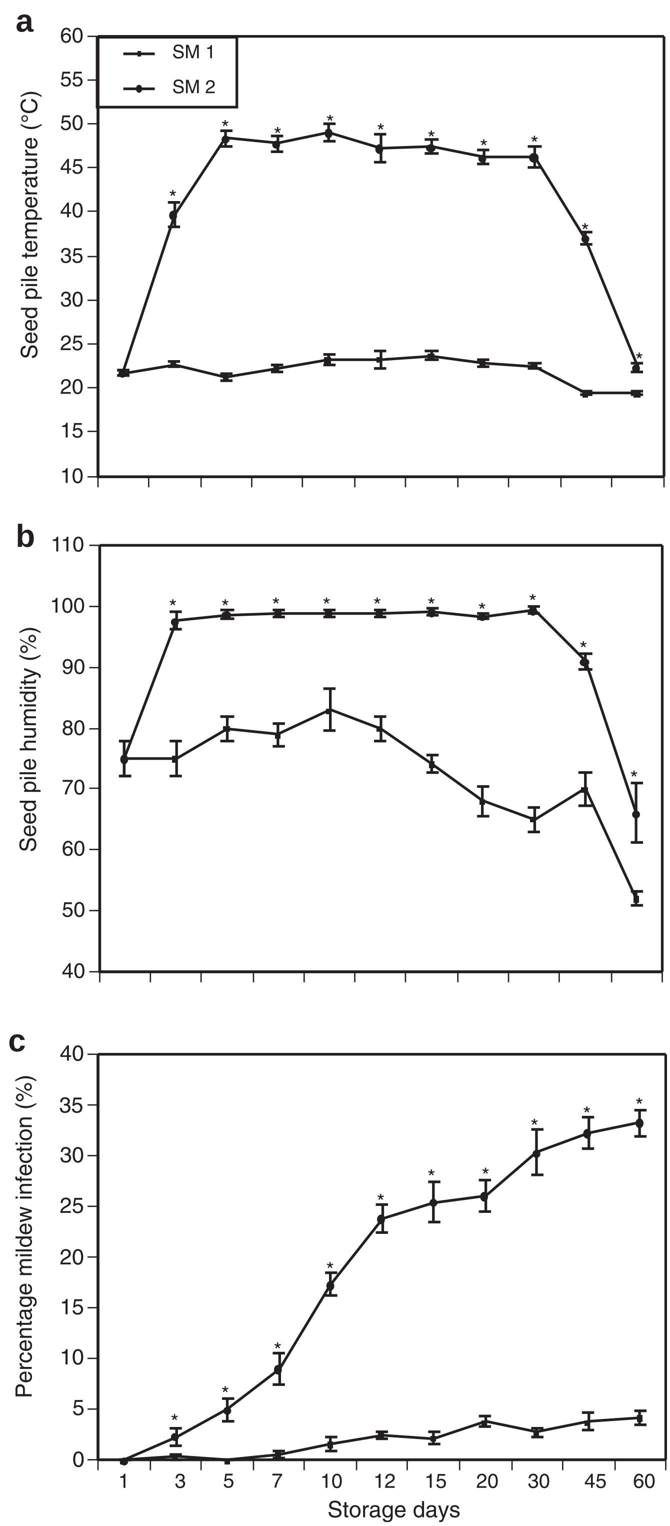

Fig. 1. The seed pile temperature (°C) (a), seed pile humidity (%) (b) and percentage Mildew infection of seeds (%) (c) of *Hevea brasiliensis* seeds, over a 2-month period. The seeds were stored according to two different storage methods, storage method 1 (SM 1) represents a proposed storage method whilst storage method 2 (SM 2) represents the storage conditions found in the Xishuangbanna district in China. The values represent the means ($n = 5$) and an "*" indicates significant differences ($p \leq 0.05$) between SM 1 and SM 2 for the respective days.

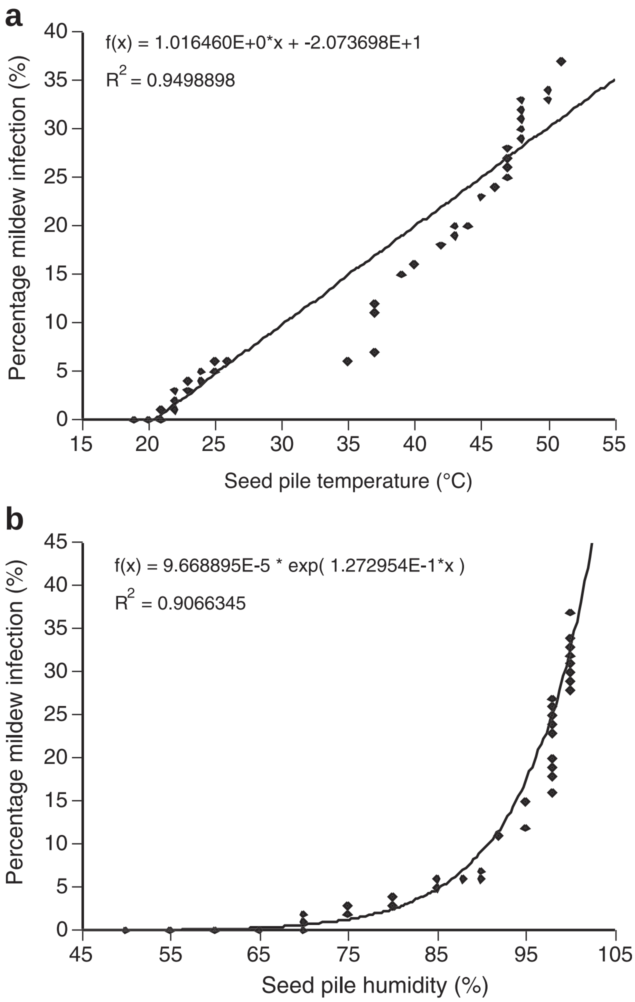

Fig. 2. The effect of seed pile temperature on the percentage Mildew infection (a) and the effect of seed pile humidity on the percentage Mildew infection (b) of *Hevea brasiliensis* seeds, over a 2-month period. The seeds were stored according to two different storage methods, storage method 1 (SM 1) represents a proposed storage method whilst storage method 2 (SM 2) represents the storage conditions found in the Xishuangbanna district in China.

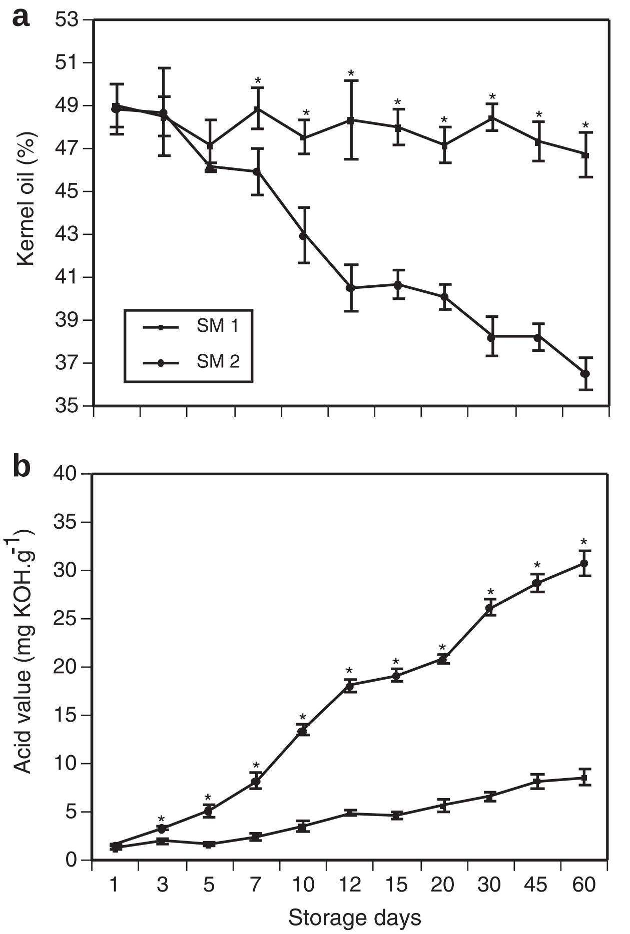

Fig. 3. The seed kernel oil content (%) (a) and rubber seed oil acid value (mg KOH.g) of *Hevea brasiliensis* seeds, over a 2-month period. The seeds were stored according to two different storage methods, storage method 1 (SM 1) represents a proposed storage method whilst storage method 2 (SM 2) represents the storage conditions found in the Xishuangbanna district in China. The values represent the means ($n = 5$) and an "*" indicates significant differences ($p \leq 0.05$) between SM 1 and SM 2 for the respective days.

in an increase in the conversion of oils into free fatty acids by the seed, thus lowering the oil content [35]. The influence of Mildew on seed oil content is evidenced by the negative correlation ($r^2 = 0.816$) between kernel oil content and the percentage Mildew infection (Fig. 4a). This is confirmed by the sharp drop in oil content of the seeds in SM 2 with increasing storage time (Fig. 3a), again noting the increase in Mildew infections of these seeds the longer they were stored under these conditions (Fig. 1c). The increased seed pile temperatures and high percentage Mildew infection of the seeds stored in SM 2 resulted in a 22% decline in total seed oil. The kernel oil content of the seeds from SM 1 (Fig. 3a) were within the accepted range of 40–50% for rubber seeds, yet those of SM 2 dropped below this threshold to approximately 37% [5,6,8,38]. The decline in the oil content of the seeds resulting from Mildew infection is in agreement with previous work showing that Mildew converts seed oils and fats into free fatty acids, through the process of lipolysis, thus raising the free fatty acid levels (acid values) of the seeds [39,40]. This is confirmed by previous studies showing that fungi known to infect rubber seeds have lipolytic abilities [41–43].

The acid values of the seed oil derived from seeds under SM 1 and SM 2 increased with storage time, and those of SM 2 rising to levels approximately 4 times higher than those of SM 1, after 60 days (Fig. 3b). Acid values of RSO, determined in other studies, appear to vary wildly, both within a region and across different regions. The studies carried out by Khan and Yusup and Yusup and Khan [27,33] found that Malaysian RSO had an acid value of 63.9 mg KOH.g^{-1}, yet in another study by Abdullah and Salimon [28] Malaysian RSO had an acid value of only 15.03 mg KOH/g, both studies extracted the oil using the same method. Similarly, the values for RSO derived from Nigerian rubber seeds ranged from 19.2 mg KOH.g^{-1} to 43.62 [11,12,28]. Thus it is clear that there is little consistency in the acid values of RSO and that the determining factors are yet to be elucidated. It may well be that factors pertaining to the storage conditions of the seeds are key in influencing the acid values of the RSO derived from the seeds. The large variation between the acid values of the RSO from SM 1 (8.6 mg KOH.g^{-1}) and SM 2 (30.8 mg KOH.g^{-1}) (Fig. 3b) provide evidence supporting this reasoning.

The variation in acid values between SM 1 and SM 2 is the result of the same factors causing a decline in the kernel oil content, Mildew infection and seed pile temperature. The positive correlation ($r^2 = 0.939$) between RSO acid values and the percentage Mildew infection (Fig. 4b) show how the acid values are influenced by the presence of Mildew. This is in agreement with previous studies determining the influence of Mildew on seed acid values [39,40] and the influence of seed pile temperature has already been

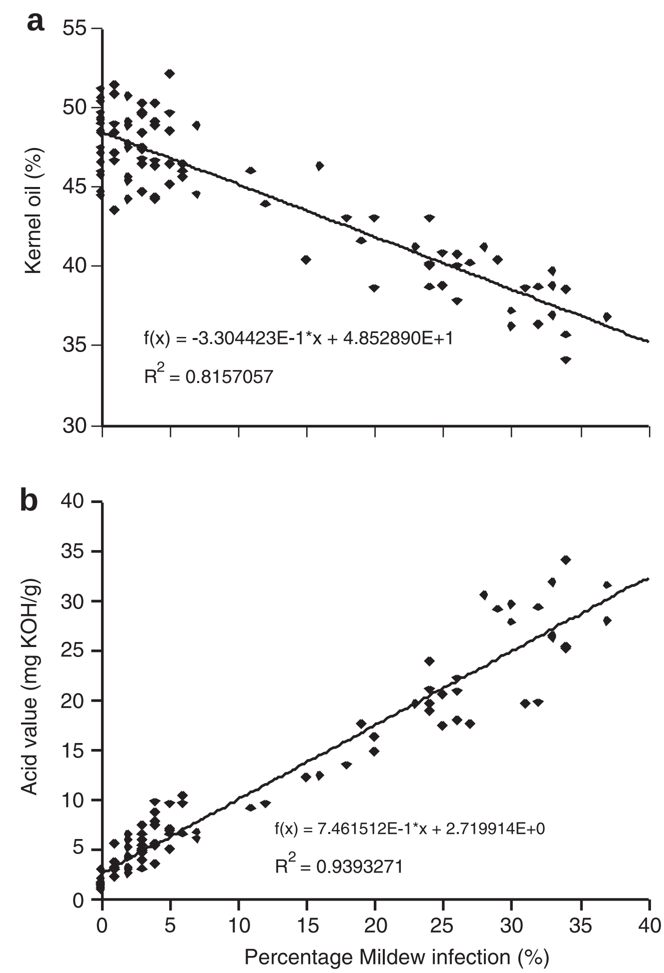

Fig. 4. The effect of Mildew infection on the kernel oil content (a) and the effect of Mildew infection on the rubber seed oil acid values (b) of *Hevea brasiliensis* seeds, over a 2-month period. The seeds were stored according to two different storage methods, storage method 1 (SM 1) represents a proposed storage method whilst storage method 2 (SM 2) represents the storage conditions found in the Xishuangbanna district in China.

established, in that high temperatures result in a faster conversion of seed oil into free fatty acids [35]. Whilst the acid values of the RSO derived from SM 2 were much higher than those of SM 1, they were still within the average range determined for China [22,30,44].

In order to successfully convert RSO into biodiesel an acid value of about 4 mg KOH. g^{-1} is required, studies have shown that even an increase in the acid levels to 6 mg KOH. g^{-1} will prevent the transestification process [7,17,18]. The RSO acid values for the seeds from SM 1 were in this range for the first ten days of storage and those from SM 2 remained within this range for the first three days of storage. The results from this section place emphasis on the need to store rubber seeds correctly and the need to minimize the rate of Mildew infection. By reducing the kernel oil content of the rubber seeds and by increasing the acid value of the RSO, the negative effects of seed pile temperature and Mildew reduce the profit margins and lower the feasibility of converting RSO into biodiesel. By extracting RSO from the seeds within the first ten days of storage (SM 1), the problematic high levels of acid values could be avoided, thereby greatly enhancing the biodiesel production process.

3.3. Crude RSO oil acid values

The short-term effects of crude RSO oil storage can be seen in Fig. 5, the first batch of crude RSO oil had the lowest acid values of

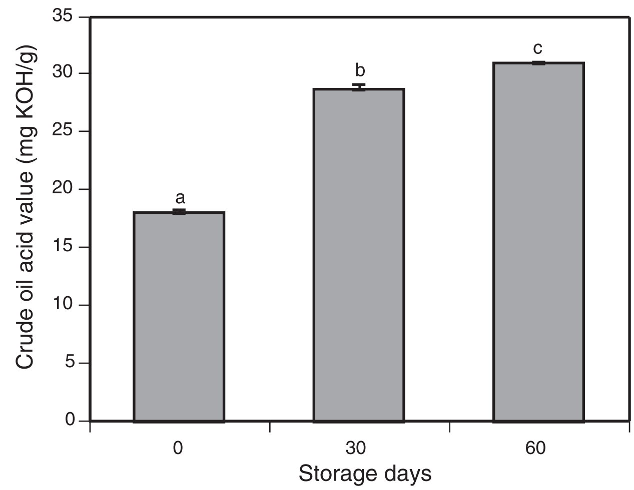

Fig. 5. The effect of storage time on the acid values of the crude rubber seed oil derived from the seeds of *Hevea brasiliensis*, over a 2-month period. The crude oil was obtained from a rubber seed oil processing plant in the Xishuangbanna district in China. The values represent the means ($n = 5$) and different letters indicate significant differences ($p \leq 0.05$) between storage times.

the three batches (18.1 mg KOH.g^{-1}), with the second batch (1 month old) having the second highest (28.8 mg KOH.g^{-1}) and the third batch (2 months) having the highest acid values (31.0 mg KOH.g^{-1}), indicating the effect of storage time on the acid values of crude RSO oil extracts. The acid values for the RSO from the first batch are, once again, below those of previous studies [12,22,30,44] however, the longer the oil is stored, the higher the values become, achieving a 58% increase in acid levels after 60 days of storage time. Thus, it is clear that the most cost effective way to produce biodiesel from RSO is a matter of efficiency. RSO should be converted into biodiesel as soon after production as possible, avoiding long storage periods that result in high acid levels.

4. Conclusion

The data provided clearly shows how the RSO was negatively affected by seed pile temperature, seed pile humidity and by Mildew infection. Increased temperatures and high Mildew infection levels resulted in lower kernel oil content of the rubber seeds and increased the acid values of the derived RSO, thus decreasing the feasibility of using this oil for biodiesel production. In addition, a critical value of 90% relative humidity was identified, with humidity rising above this value resulting in dramatic increases in Mildew infections.

The value of storing the seeds under optimal conditions was emphasized when comparing the oil content and RSO acid values of SM 1 and SM 2. Under the conditions described for SM 1, it was possible to extract RSO with an acid value lower enough for biodiesel production within the first ten days of storage, where after the acid values began to increase to levels beyond this. It is also evident that long-term storage of crude RSO has a negative effect on the acid values of the oil. Thus, the need for an efficient production line, thus minimizing storage time is emphasized. Furthermore, the use of fungicides on the seeds before storage can reduce the levels of Mildew infection.

Acknowledgments

Thanks to Juliet Lu and Nicholas Orr for their technical input and translational work.

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