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Effect of Ripening Stage and Temperature on Free Fatty Acid Content of *Jatropha curcas* Oil During Storage

Sukuntaros Tadakittisarn*, Phornphimom Janchai, Pilanee Vaithanomsat and Waraporn Apiwatanapiwat

ABSTRACT

The lipid content of *J. curcas* L. black-fruit seed was not different from that of yellow-fruit seed for all accessions: Mukdahan, Satun and Surin. However, protein in the black-fruit seed from all accessions was significantly ($P < 0.05$) higher than that of the yellow-fruit seed. Samples of seed and pressed seed oil were stored at 4, 37 and 50 °C for free fatty acid (FFA) analysis. The moisture contents of Mukdahan accessions stored at 4, 37 and 50 °C were constant after 60 d at 12.0, 5.5 and 3.5%, respectively, for both the yellow- and black-fruit seed. The FFA contents of Surin black-fruit seed stored at 4, 37 and 50 °C increased from 0.49% to 0.77, 0.72 and 0.63% in 2 mth, respectively, while the yellow-fruit seed showed less of an increase at 37 and 50 °C and at 4 °C did not change. The FFA content of oil from the Mukdahan yellow-fruit seed did not change over 4 mth. The FFA content of black-fruit oil increased rapidly at all temperatures. However, when settling the oil before storage, the oil of the yellow fruit did not change at any temperature, while the oil of the black-fruit seed could be kept for 4 mth at 4 and 37 °C with the FFA content not exceeding 1%.

Keywords: *Jatropha curcas* L., storage stability, free fatty acid

INTRODUCTION

Recently, biodiesel has received increasing attention due to its lower level of pollution than petroleum diesel and as a renewable energy resource in place of fossil fuels. Mostly, biodiesel is prepared from vegetable oil such as soybean, rapeseed, sunflower and safflower among others (Lang *et al*., 2001). These oils are naturally edible. With no competitive food uses, *Jatropha curcas* L. which is a source of non-edible oils can provide an alternative for biodiesel production (Openshaw, 2000).

J. curcas is a multipurpose plant with many attributes and considerable potential. It is a tropical plant that can be grown in low to high rainfall areas and can be planted in reclaimed land as a hedge or commercial crop or both. The plant produces many useful products, especially the seed from which oil can be extracted; this oil has similar properties to palm oil. Because it can be used in place of kerosene and diesel and fuel wood, it has been promoted to make rural areas self sufficient in fuel for cooking, lighting and motive power (Openshaw, 2000).

Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: aapsrt@ku.ac.th

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J. curcas seeds contain about 40˘60% (w/w) oil, which is mainly used for the production of candles and soap, cosmetics, as a fuel and after transesterification as biodiesel (Banerji *et al*., 1985; Makker *et al*., 1997). The physico-chemical properties of *J. curcas* biodiesel are acceptable for use as biodiesel in diesel engines (Oliveira *et al*., 2009). The characteristics of ethyl esters from *J. curcas* oil meet the standards for biodiesel, suggesting its possible substitution for conventional diesel (Chatakanonda *et al*., 2005).

During storage and use, vegetable oil-derived industrial products such as biodiesel and biodegradable lubricants are subjected to oxidation of their unsaturated components. The materials arising during oxidation and subsequent degradation can seriously impair the quality and performance of such products. Therefore, oxidative stability is a substantial issue with these vegetable oil-derived products. Consequently, understanding the influence of various components of vegetable oils and storage parameters is necessary. The quantity of free fatty acid (FFA) provides important information about quality deterioration of crude *J. curcas* seed oil (CJCO) due to improper handling and inappropriate storage conditions. The FFA content of seed oil increased with increasing moisture content and time of storage (Molteberg *et al*.,1995). The FFA and moisture contents have substantial effects on the transesterification of glycerides in alcohol with a catalyst (Goodrum, 2002). The oil with high FFA content (greater than 1% w/w) quickly reacts with the alkaline catalyst to produce soaps that inhibit the separation of the ester and glycerin so that then the separation of the products is very difficult, resulting in low yields of biodiesel product. However, the biodiesel production from CJCO with a high content of FFA has been developed using a two-stage transesterification process to improve the biodiesel yield. However, it will take more time to develop this process.

The objective of the present study was to investigate the effects of the ripening stage of *J. curcas* fruit and storage temperature on the FFA content in *J. curcas* seed and the pressed oil of *J. curcas* seed in order to determine any storage time limitations.

MATERIALS AND METHODS

Materials

Seed samples of 3 accessions of *J. curcas* collected from various locations in Mukdahan, Satun and Surin provinces, Thailand were obtained from the National Corn and Sorghum Research Center. The seed samples obtained were in 2 different stages of maturity involving physiological maturity (yellow fruit) and harvest maturity (black fruit).

Methods

Sample preparation

The samples were separated into 2 groups; the seed was divided into 200 g portions, packed in paper bags and stored. The hydraulic pressed oil from *J. curcas* seed was kept in a cold room (4 °C) to settle the precipitate. Then the upper part of the oil samples was directly transferred to amber glass bottles (10 and 30 mL) and stored under the same conditions as the seed.

Storage conditions

The seeds and pressed oil with 14 treatments and 3 replications were stored for 2˘6 mth in an incubator at 37 and 50 °C while the control was kept at 4 °C (Figure 1). A random sample of each treatment and replicate was tested periodically at 0, 0.25, 0.5, 1, 1.25, 2, 4, 7, 10, 14, 28, 69, 120 and 180 d to determine the FFA content.

Determination of chemical composition

Proximate analysis of *J. curcas* seed for the moisture, fat, protein, fiber and ash contents was performed according to AOAC (1995).

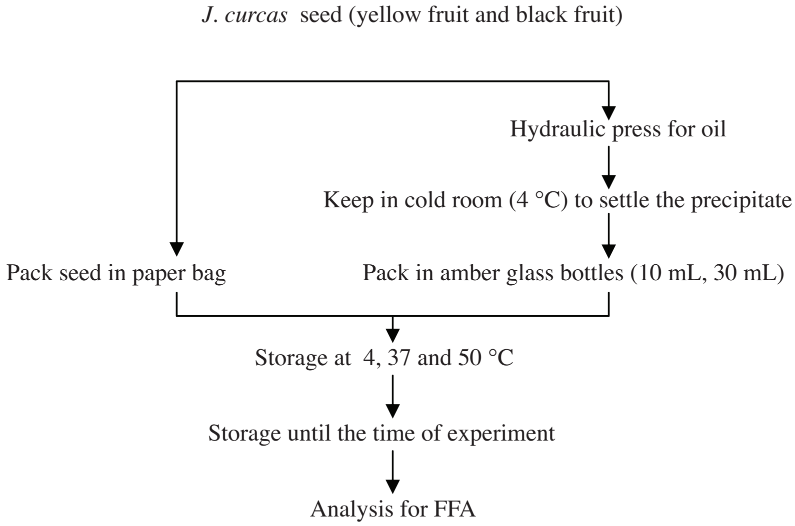

Figure 1 Preparation steps for FFA determination of *J. curcas* seed and oil from Mukdahan variety.

Determination of FFA in seed and oil

Seed extraction of the FFA from the *J. curcas* seed was performed using iso-propanol and hexane (Lam *et al*., 2001). The dehulled *J. curcas* seeds were ground with a blender for 2 min. The weighed ground sample (5˜15 g) was placed into a 250 mL Erlenmeyer flask. Iso-propanol (37.5 mL) and hexane (7.5 mL) were introduced to the flask and the mixture was mixed thoroughly in a fume hood. The extraction was performed by shaking at 220 rpm for 30 min. The filtrate was collected through a filter membrane (no. 4). The extraction was repeated three times. The pooled filtrate was titrated with 0.05 M NaOH as described in AOCS (1993).

Samples of oil (5 g) were weighed and extracted for FFA using iso-propanol:hexane (75:15) in an Erlenmeyer flask, thoroughly mixed and then titrated with 0.05 M NaOH by the same method used for the seed FFA extraction.

Statistics

Duncan s multiple range test was used to evaluate the differences between mean values with the test for significance set at the 5% level ($P < 0.05$).

RESULTS AND DISCUSSION

The proximate compositions of the seed of the 3 *J. curcas* accessions are presented in Table 1. All *J. curcas* accessions contained a high lipid content ranging from 37.61 to 40.71% and the protein content varied from 13.85 to 19.51%. The lipid content in the seed from black fruit was not different from that in seed from yellow fruit. However, the protein in the black fruit from all accessions was significantly higher than that in yellow fruit. From this result, it can be concluded that *J. curcas* fruit was harvested at the yellow-fruit stage in order to reduce the time until harvest.

The moisture content of seeds from the *J. curcas* accession Mukdahan for both yellow- and black-fruit stored at 4, 37 and 50 °C is presented in Table 2. The seed from the yellow fruit originally contained high moisture content (26%) compared with black fruit (11.44%). Seed moisture content is dependent on the maturity of the seed; at physiological maturity, the seed moisture content was still too high and would have declined until reaching harvesting maturity (Ekpong, 2009). The moisture content of seed from yellow fruit rapidly reduced under storage at 50 °C and at a lower rate when stored at 37 °C and became constant within 4 and 10 d, respectively. The moisture content reached a constant level at different rates as the storage temperature differed. At temperatures of 4, 37 and 50 °C, the moisture content was constant at 12.0, 6.0 and 4.0%, respectively, for both yellow- and black-fruit seeds.

FFA contents of stored seed

The FFA content of *J. curcas* yellow- and black-fruit seed from the accession Surin stored at 4, 37 and 50 °C are presented in Figure 2. Over the 2 month period, the FFA was initially 0.49% for seed from black-fruit and this increased to 0.77,

Table 1 Proximate composition of seeds of 3 *J. curcas* accessions from Mukdahan, Satun and Surin provinces.

Accession	Moisture content (%)	Lipid (%)	Protein (%)	Fiber (%)	Ash (%)
Mukdahan (Yellow)	26.00 ± 1.46	39.79 ± 0.94^{bc}	13.85 ± 0.34^{a}	43.51 ± 1.54^{c}	3.04 ± 0.08^{a}
Mukdahan (Black)	11.44 ± 0.57	40.71 ± 2.07^{c}	17.17 ± 0.16^{bc}	40.47 ± 0.40^{b}	4.40 ± 0.06^{b}
Satun (Yellow)	6.47 ± 0.12	38.16 ± 0.69^{ab}	14.84 ± 0.37^{b}	41.83 ± 1.39^{c}	3.83 ± 0.06^{b}
Satun (Black)	6.75 ± 0.11	37.61 ± 0.56^{a}	18.71 ± 0.55^{d}	38.35 ± 0.03^{a}	4.69 ± 0.13^{d}
Surin (Yellow)	6.72 ± 0.10	38.16 ± 1.02^{ab}	17.31 ± 0.38^{c}	42.38 ± 1.03^{c}	3.85 ± 0.13^{b}
Surin (Black)	6.51 ± 0.10	38.27 ± 0.54^{ab}	19.51 ± 0.20^{e}	37.17 ± 0.61^{a}	4.69 ± 0.06^{d}

Means in the same column superscripted by different letters are significantly different ($P < 0.05$).
Values are means of triplicate determinations ± standard deviation.

Table 2 Moisture content of seeds of *J. curcas* accession Mukdahan stored at 4, 37 and 50 °C.

Storage duration (days)	MC at 4 °C (%)		MC at 37 °C (%)		MC at 50 °C (%)	
	SMY	SMB	SMY	SMB	SMY	SMB
0	26.00^{c}	11.44^{a}	26.00^{c}	11.44^{b}	26.00^{c}	11.44^{a}
0.125	28.04^{c}	13.90^{b}	25. 92^{c}	14.92^{c}	26. 56^{c}	14.70^{b}
0.25	27.33^{c}	13.30^{b}	27.99^{c}	14.52^{b}	25.72^{c}	11.87^{a}
0.5	26.77^{c}	14.02^{b}	26.69^{c}	10.85^{a}	25.69^{c}	12.79^{a}
1	27.47^{c}	13.40^{b}	24.12^{b}	12.58^{a}	21.48^{c}	9.44^{a}
1.25	27.60^{c}	14.45^{b}	24.66^{c}	10.45^{a}	17.93^{c}	9.10^{a}
2	25.81^{c}	12.82^{a}	19.94^{a}	9.07^{a}	12.29^{a}	5.31^{a}
4	26.60^{c}	13.05^{b}	17.33^{b}	6.98^{a}	5.54^{a}	4.12^{a}
7	20.90^{c}	13.06^{b}	9.08^{a}	5.68^{a}	5.41^{a}	3.94^{a}
9	18.28^{c}	10.80^{a}	7.39^{a}	6.86^{a}	4.99^{a}	5.28^{b}
14	17.08^{b}	12.09^{a}	5.89^{a}	5.61^{a}	3.81^{a}	3.69^{a}
28	15.06^{b}	12.06^{a}	5.78^{a}	5.53^{a}	3.62^{a}	3.65^{a}
60	12.90^{b}	11.57^{a}	5.78^{a}	5.53^{a}	3.72^{a}	3.69^{a}
120	11.39^{a}	11.64^{a}	5.28^{a}	5.51^{a}	3.24^{a}	3.13^{a}
178	11.39^{a}	11.64^{a}	5.28^{a}	5.51^{a}	3.24^{a}	3.13^{a}
225	11.39^{a}	11.64^{a}	5.28^{a}	5.51^{a}	3.24^{a}	3.13^{a}

Means in the same column superscripted by different letters are significantly different ($P < 0.05$).
Values are means of triplicate determinations. MC = moisture content
SMB = seed from Mukdahan black fruit; SMY = seed from Mukdahan yellow fruit

0.72 and 0.63% when stored at 4, 37 and 50 °C, respectively. The FFA level of black-fruit seed increased rapidly at 4, 37 and 50 °C, respectively. In contrast, the FFA content of yellow-fruit seed kept at 4 and 37 °C did not change; however, the FFA content at 50 °C increased a little (from 0.52 to 0.56%) after 2 mth. From the results, it could be concluded that after 2 mth, the black-fruit seed at 50 °C had a lower (5.66%) moisture content than that at 4 °C (12.0%) and consequently, the FFA content increased because the seed at this stage was more physiologically mature and ready to germinate and develop into the seedling stage. When the seed moisture increased, the seed was easily induced to germinate. This implied that lipase enzyme (triacylglycerol hydrolase, EC 3.1.1.3.) in the *J. curcas* catalyzed the breakdown of triacylglycerol into glycerol and FFA was most likely responsible for the activation of the triacylglycerol hydrolysis which then resulted in an increase in the FFA content (Beisson *et al.*, 2000). This did not happen with the yellow fruit as the physiological maturity at this stage was less than for the black fruit. This was confirmed by the result at 37 °C, with the FFA formation of black-fruit seed being higher than at 50 °C because the optimum temperature of lipase was at 37 °C. The lipase activity in the black-fruit seed should

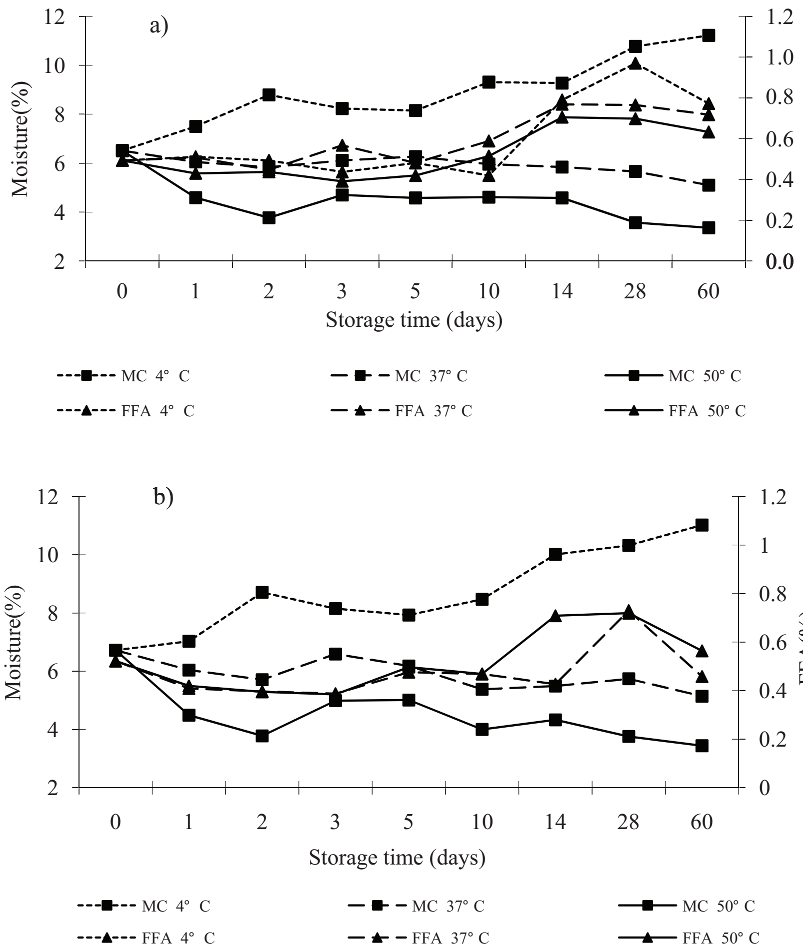

Figure 2 Moisture and FFA content of seed of *J. curcas* accession Surin stored at 4, 37 and 50 °C: a) black fruit; and b) yellow fruit. (MC = moisture content).

be inhibited at 50 °C. The higher level of FFA in the yellow-fruit seed at 50 °C than at the other temperatures may have been caused by oxidation due to the high temperature and not due to any lipase action.

FFA content of stored oil

The pressed oil of *J. curcas* accession Mukdahan was directly transferred to brown transparent glass bottles and stored for 6 mth. The FFA content in the oil for both yellow and black fruit stored at 4, 37 and 50 °C are presented in Table 3. The FFA content of the oil from yellow fruit started with a low FFA content (0.17%) compared with the black-fruit oil with a high FFA content (0.90%) that was close to the limit of 1% FFA that could be converted into biodiesel by transesterification using an alkaline catalyst (Tiwari *et al*., 2007). The FFA of the yellow-fruit oil changed less at any temperatures over 4 mth. However, the FFA content of the oil from black fruit increased more rapidly from 0.90% to 3.37, 1.85 and 1.72% at 37, 50 and 4 °C, respectively, after 6 mth. This indicated that the oil from yellow fruit of accession Mukdahan could be stored at any of the 3 temperatures (4, 37 or 50 °C) for at least 6 mth. In contrast, the black-fruit oil could not be stored at any temperature as the FFA content was higher than 1%. This result was consistent with seed results and indicated that the black-fruit seed was more physiologically mature than the yellow seed and ready to germinate and develop into the seedling stage. When the seed was damaged by pressing to extract the oil, the lipase enzyme was induced and activated to hydrolyze triacylglycerol to FFA as an energy supply for seed germination. Lipase has been detected in dormant seeds of caster bean (Huang, 1978) and also in germinated and dormant seed of *J. curcas* (Abigor *et al*., 2002; Staubmann *et al*., 2002). Attia *et al.* (1995) reported that fully ripe, dry chickpea (*Cicer arietinum* L.) seeds had higher lipase activity than green, tender seed. This was supported by the present results where the *J. curcas* oil from black

Table 3 FFA content of *J. curcas* oil from accession Mukdahan stored at 4, 37 and 50 °C for 6 mth.

Storage duration (day)	FFA at 4 °C (%)		FFA at 37 °C (%)		FFA at 50 °C (%)	
	OMB	OMY	OMB	OMY	OMB	OMY
0	0.90^{a}	0.17^{a}	0.90^{a}	0.17^{abc}	0.90^{a}	0.17^{a}
0.25	1.02^{a}	0.17^{a}	nd	0.17^{abc}	0.94^{a}	0.16^{a}
0.50	1.03^{a}	0.18^{a}	1.00^{a}	0.16^{a}	1.28^{b}	0.17^{a}
1	1.03^{a}	0.17^{ab}	0.97^{a}	0.18^{abc}	1.25^{b}	0.17^{abc}
1.25	1.07^{a}	0.17^{ab}	1.08^{a}	0.20^{d}	1.11^{ab}	0.18^{bcd}
2	1.05^{a}	0.17^{ab}	1.17^{ab}	0.17^{abc}	1.50^{cd}	0.19^{d}
4	1.30^{bc}	0.16^{a}	1.28^{b}	0.16^{a}	1.23^{b}	0.16^{a}
7	1.09^{ab}	0.23^{e}	1.57^{cd}	0.26^{e}	1.32^{bc}	0.23^{e}
10	1.00^{a}	0.17^{abc}	1.60^{d}	0.18^{bcd}	1.72^{d}	0.18^{bcd}
14	1.22^{b}	0.18^{bc}	1.74^{d}	0.20^{d}	1.71^{d}	0.20^{d}
28	1.31^{bc}	0.19^{bc}	2.38^{f}	0.19^{d}	1.65^{d}	0.19^{d}
69	1.43^{bc}	0.20^{d}	2.77^{f}	0.22^{e}	2.05^{e}	0.21^{d}
120	1.65^{d}	0.20^{d}	3.06^{f}	0.22^{e}	2.66^{f}	0.26^{e}
180	1.72^{de}	nd	3.36^{f}	nd	1.85^{e}	nd

Means in the same column superscripted by different letters are significantly different ($P < 0.05$).

Values are means of triplicate determinations. nd = not determined.

OMB = Oil from Mukdahan black fruit; OMY = Oil from Mukdahan yellow fruit.

fruit had higher FFA content than that from yellow fruit. The FFA content in the oil of black fruit increased more rapidly at 37 °C because this temperature is the optimum for lipase activity (Abigor *et al*., 2002; Lam and Proctor, 2003).

A preliminary study that had produced pressed oil which was then settled at 4 °C for 1 mth (so that the upper part without water and suspended solid contamination could be separated) was used to study the impact of further storage. The results in Table 4 show that the FFA content of oil from yellow fruit did not increase at any temperature. The FFA content of oil from black fruit increased from 0.90% to 0.99 and 1.17% after 4 mth at 37 and 50 °C, respectively, while at 4 °C for the same storage period, the FFA content did not change. The FFA increased less compared with oil that had not been settled first (Table 3). This could have been due to lipase in the suspended solids being excluded from the settled oil. Lipase catalyzed the breakdown of triacylglycerol into glycerol and FFA at the water oil interface (Dellamora *et al*., 1997; Beisson *et al*., 2000; Saxena *et al*., 2003). As a result, the suspended solids accelerated the increase of the FFA content at high temperature (Sustainable Energy Ireland, 2004). Unlike, the last experiment (Table 3) that showed that the FFA content of black-fruit oil was highest at 37 °C, with the settled oil, black fruit contained the highest level of FFA at 50 °C. This result confirmed that FFA was the result of lipase activity, as after using settled oil (that had excluded some lipase in the removed suspended solids), the FFA content was highest at 50 °C instead of 37 °C because of the higher temperature.

CONCLUSION

The lipid content of *J. curcas* harvested as mature black fruit was not different from that in physiologically mature yellow fruit from all accessions. The moisture content of *J. curcas* accession Mukdahan stored at 4, 37 and 50 °C was constant at 12.0, 5.5 and 3.5%, respectively, for seed from both yellow and black fruit. The FFA formation in seed of *J. curcas* black fruit stored at

Table 4 FFA content of *J. curcas* oil from accession Mukdahan settled at 4 °C for 1 mth before storage at 4, 37 and 50 °C for 4 mth.

Storage duration	FFA at 4 °C (%)		FFA at 37 °C (%)		FFA at 50 °C (%)	
(days)	OMB	OMY	OMB	OMY	OMB	OMY
0	0.90^{a}	0.34^{a}	0.9^{0a}	0.34^{a}	0.90^{a}	0.34^{a}
0.38	0.93^{a}	0.44^{a}	0.85^{a}	0.52^{a}	0.87^{a}	0.39^{a}
1	0.85^{a}	0.38^{a}	0.89^{a}	0.36^{a}	0.88^{a}	0.71^{b}
2	0.84^{a}	0.34^{a}	0.86^{a}	0.41^{a}	0.89^{a}	0.70^{b}
4	0.86^{a}	0.37^{a}	0.87^{a}	0.36^{a}	0.87^{a}	0.54^{a}
7	0.89^{a}	0.36^{a}	0.87^{a}	0.46^{a}	0.90^{a}	0.70^{b}
10	0.87^{a}	0.41^{a}	1.05^{b}	0.44^{a}	1.06^{b}	0.73^{b}
14	0.90^{a}	0.29^{a}	0.94^{a}	0.58^{b}	0.90^{a}	0.45^{a}
28	0.85^{a}	0.87^{b}	0.89^{a}	0.87^{b}	0.90^{a}	0.93^{b}
42	0.94^{a}	0.33^{a}	0.92^{a}	0.93^{b}	1.00^{b}	0.45^{a}
60	0.87^{a}	0.37^{a}	0.90^{a}	0.63^{b}	1.01^{b}	0.39^{a}
120	0.86^{a}	0.47^{a}	0.99^{b}	0.68^{b}	1.17^{b}	0.52^{a}

Means in the same column superscripted by different letters are significantly different ($P < 0.05$).
Values are means of triplicate determinations.
OMB = Oil from Mukdahan black fruit; OMY = Oil from Mukdahan yellow fruit.

4, 37 and 50 °C increased more than that in yellow-fruit seed within 2 mth. In addition, the FFA level in the pressed oil from yellow fruit of accession Mukdahan changed less at all temperatures over 4 mth, while the pressed oil from the black fruit increased more rapidly. The pressed oil from black fruit of accession Mukdahan could not be stored at any temperature, as the FFA content was higher than 1%. For better storage, the oil should be kept at 4 °C for 1 mth having first removed any water and suspended solid contamination, as this oil could be stored for 4 mth at 4 and 37 °C without the FFA content increasing to more than 1%. As a result of the study, it was recommended that the harvesting stage for *J. curcas* fruit should be at the yellow-fruit stage in order to reduce the time until harvest and to increase the storage stability of the oil. It was also suggested that other parameters such as lipase activity and suspended solids should be determined along with the FFA content, in order to clarify the mechanism of deterioration of *J. curcas* seed and oil.

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