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# Effect of natural, accelerated and saturated salt accelerated aging on the *Jatropha curcas* L. seeds in optimizing the yield of seed oil as feedstock for biodiesel

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## ABSTRACT

Uninterrupted and good quality of feedstock is a key factor for biodiesel. *Jatropha curcas* L. has emerged as a favorite unconventional source of fuel. This paper aims to map the changes in *Jatropha curcas* L. oil, extracted from seeds subjected to natural aging (NA), accelerated aging (AA) and saturated salt accelerated aging (SSAA) are two methods employed to mimic natural aging. The results depicted that prolonged and intense aging decreased the oil yield and caused deterioration of the oil with a high free fatty acids content, saponification value, peroxide value, and low iodine value. Decrease in oleic acid content is the result of lipid peroxidation due to prolonged storage. However, *Jatropha curcas* L. seeds subjected to natural aging even up to one year could still serve as the best feedstock because of its resistance towards deteriorative aging effects.

## ARTICLE HISTORY

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## KEYWORDS

Aging/storage; feedstock; *Jatropha curcas* L.; lipid peroxidation; oxidative stability

## Introduction

The rapid depletion of petroleum reserves with the deleterious impact of such fossil fuel on the environment makes it imperative to look for alternative energy resources. 'Biodiesel' has attracted extensive attention as renewable, biodegradable and non-toxic fuel since the past decade (Stavarache et al. 2007; Tiwari, Kumar, and Raheman 2007). *Jatropha curcas* L. stands out to be the best feedstock for biodiesel production with its closeness to biodiesel characteristics (Veljkovic et al. 2006). Among all the other non-edible oils *Jatropha curcas* L. oil singles itself out to be the best suited due to its unique combination of high percentage of monounsaturated fatty acids (C16:1, C18:1), a low percentage of polyunsaturated fatty acids (C18:2, C18:3) and a small fraction of saturated fatty acids (C16:0, C18:0) (Wang et al. 2011). Semi-drying property of the *Jatropha curcas* L. oil is due to the high percentage of unsaturated fatty acids (77.9%) (El Kinawy 2009). Although the high content of monounsaturated fatty acids in *Jatropha curcas* L. seed oil is a boon for biodiesel conversion yet it can also make the seeds vulnerable to auto-oxidation when exposed to factors like unfavorable temperature and moisture.

Our laboratory has successfully reported an increase in seed yield from *Jatropha curcas* L. by exogenous phytohormones application (Makwana, Shukla, and Robin 2010). However, we observed that the seeds being rich in lipids were susceptible to decay during long-term storage. Undesirable event like contaminant formation of peroxides, aldehydes, acids, and alcohol takes place (Dunn Robert 2008; Yamane et al. 2007). Factors like hydrolysis and oxidation are the most deleterious process that make the oil highly deteriorative (Jain and Sharma 2010; Knothe 2005). This decomposed oil if extracted and used, it would yield a poor quality of biodiesel.

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Color versions of one or more of the figures in the article can be found online at [www.tandfonline.com/ueso](http://www.tandfonline.com/ueso).

Storage conditions like temperature, moisture content, and storage period play a crucial role in determining oil quality and quantity. A few studies on short-term storage stability of the oil (Sushma 2014) have been undertaken but study on long-term storage of 24 months of the *Jatropha curcas* L. seeds (instead of oil) has not been done before. This paper aims to map the changes in oil quantity and quality during natural aging, accelerated and saturated salt accelerated aging thus optimizing oil yield. Such study also leads to extraction of oil with sufficient quantity and suited quality of oil for the conversion of biodiesel before the dawn of deterioration and rancidity of oil. Analysis of fatty acids by gas chromatography and the parameters of oxidation stability of oil such as free fatty acids content (FFA), saponification value (SV), peroxide value (PV) and iodine value (IV) were done. Monitoring and mapping of physiological and biochemical changes during seed aging will also help to design a feasible storage method/model/measure for preservation and conservation of *Jatropha curcas* L. oil seeds.

## Materials and methods

### Seeds

The seeds of *Jatropha curcas* L. were collected from the areas of Dhaod, Gujarat, India. Seeds were initially sunlight dried for 48 h to ward off the initial high moisture content of the seeds. The seed lots were grouped into three categories: 1. Fresh seeds were treated as control. 2. Some seeds were given accelerated aging treatment. 3. Others were termed as seeds of natural aging (NA) since they were kept for storage in normal room temperature packed with polythene bags. These seeds were subjected to natural aging of one month (NA1m), three months (NA3m), six months (NA6m), nine months (NA9m), twelve months (NA12m), fifteen months (NA15m), eighteen months (NA18m), twenty-one months (NA21m) and twenty-four months (NA24m).

### Aging treatment

Accelerated aging (AA) and saturated salt accelerated aging (SSAA) are the techniques developed as alternative methods where seeds are exposed to higher humidity, temperature in order to accelerate the aging of seeds artificially (Powell and Matthews 1984). Many years of aging effect could be brought about in a few hours of accelerated aging. This overcomes the hurdle of seeds being kept for very longer duration to study the aging effect. Aging treatment comprises accelerated aging and saturated salt accelerated aging. For accelerated aging; seeds were exposed to controlled temperature (42–46° C) and high moisture (80–100%) and for saturated salt accelerated aging; seeds were exposed to controlled temperature (42–46°C) and to saturated salt (40%) for controlling the moisture content. The aging treatments were given for 12 h, 1day, 2days, 3days, 4days, 5days, 7days, 10days, 12days and 15days. These seeds were designated as AA12hr, AA1d, AA2d, AA3d, AA4d, AA5d, AA7d, AA10d, AA12d and AA15d for accelerated aging and SSAA12hr, SSAA1d, SSAA2d, SSAA3d, SSAA4d, SSAA5d, SSAA7d, SSAA10d, SSAA12d, and SSAA15d for saturated salt accelerated aging.

### Crude *Jatropha* oil extraction

The oil content (dry basis) of the seeds was determined by Soxhlet extraction method. Fifty grams of the seeds was milled to 1- mm particle size using standard methods (ASTM E11 2003) from each group of seed lots. The oil was extracted in using a Soxhlet extractor with petroleum ether as solvent and the milled seeds were wrapped in extraction thimbles. The solvent to solid ratio was 7:1, and the extraction was carried out for 8 h at a temperature of 75°C. The petroleum ether in the solvent-oil mixture was evaporated later. The oil was then collected and weighed. The percentage oil yield was calculated using the following expression:

$$Y = [M_o/M_m] * 100$$

Where Y is the oil yield (%),  $M_o$  is the weight of oil expressed (gm) and  $M_m$  is the weight of the sub-sample of milled *Jatropha curcas* L. seeds.

### **Qualitative analysis of the oil**

Different biochemical tests such as iodine value (IV), saponification value (SV), peroxide value (PV), acid value (AV), and percentage of free fatty acids (%FFA) were carried out as per standard methods and protocols prescribed by Cox and Pearson (1962); AOAC (1975); Thimmaiah (2006).

### **Determination of acid value**

Oil sample of 0.1 gm was dissolved in 10 ml of neutral solvent. The mixture was titrated using 0.1 N KOH solution after an addition of few (2–3) drops of 1% phenolphthalein indicator. The values were substituted in the following formula to estimate the acid value: acid value (mg KOH/gm) =  $(A \times 0.1 \times 56.1)/W$ , where A = Titrated value (ml), 0.1 = Normality of KOH, W = Weight of sample (gm) and 56.1 gm/mol is the molecular weight of KOH.

### **Determination of free fatty acids**

Percentage of free fatty acids (%FFA) was determined using the following equation: % FA = K x acid value (AV), Where K = Constant (0.503).

### **Determination of saponification value**

0.5 gm of sample was taken and dissolved in 12.5 ml of 0.5 N alcoholic KOH solution. Using air condenser attached to the flask, the sample was boiled gently for about 30 min, which was then be cooled at room temperature. It was then titrated with 0.5 N HCl with 1% phenolphthalein indicator. Besides, a blank was also run to have precise comparison among duplicates and the mean results were recorded.

Saponification value (SV) (mg KOH) =  $(A \times 28.06/W)$ , Where A = Amount of HCL (ml) (Blank-Titrate value), W = Weight of sample (gm)

### **Determination of peroxide value**

1gm of oil sample was taken and dissolved in 15 ml of solvent mixture (2:1, glacial acid: chloroform). The tube was then placed in boiling water for 30 s to remove the liquid boils. This content was then transferred quickly to a conical flask containing 20 ml of 5% potassium iodide solution. The mixture was titrated against 0.05 N sodium thiosulfate, using starch as an indicator. A blank was also set at the same time.

Peroxide value (meq/kg sample) =  $(A \times N \times 1000/W)$ , Where A = Amount of  $\text{Na}_2\text{S}_2\text{O}_3$  (ml), N = Normality of  $\text{Na}_2\text{S}_2\text{O}_3$ , W = Weight of sample (gm).

### **Determination of iodine value**

0.5 gm of oil sample was taken and dissolved in 10 ml of chloroform. Hanus iodide solution of 12.5 ml was added to this mixture and was kept in dark for 30 min. Later, 15 ml of KI solution was added. The mixture was then titrated with 0.1N sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) solution until yellow solution turns almost colorless. Few drops of 1% starch solution (Indicator) was added and titrated until the blue color completely disappears. A blank should be set at the same time.

Iodine value (IV) =  $[(A \times N \times 0.1269 \times 100)/W]$  gm  $\text{I}_2/100$  gm of oil, Where A = Amount of  $\text{Na}_2\text{S}_2\text{O}_3$  (Blank-Titer), N = Normality of  $\text{Na}_2\text{S}_2\text{O}_3$ , W = Weight of sample (gm).

### **Fatty acid analysis of oil by gas chromatography**

Oil measuring 1–30 mg was taken and added small volume of 0.4 N sodium methylate. Cover of the mouth of the tube was sealed with aluminum foil and heated in water bath at 65°C and later vortexed. The appearance of homogenous solution indicated the composition of esterification. An aliquot (1–2 µl) was injected into preconditioned GC in which the maintained temperature of injection port is 280°C and column temperature 165°C. After all the components of the sample have come out of the column, the standard methyl esters were injected separately and noted the retention time of esters of different FAs. GC is equipped with SS packed column and flame ionization detector. The individual peaks of the samples were identified by comparing their relative position or retention time with the standards. GC automatically calculated the peak area of each acid sample and compared with that of standards (Kates 1986).

### **Statistical analysis**

The statistical significance of the results was determined by using one-way ANOVA method. The values were considered statistically significant at p-values of 0.05, 0.01 and 0.001 indicated by \*, \*\* and \*\*\*, respectively, with increasing order of significance using Tukey test of comparison. Means of 3 replicates  $\pm$  SD.

## **Results and discussion**

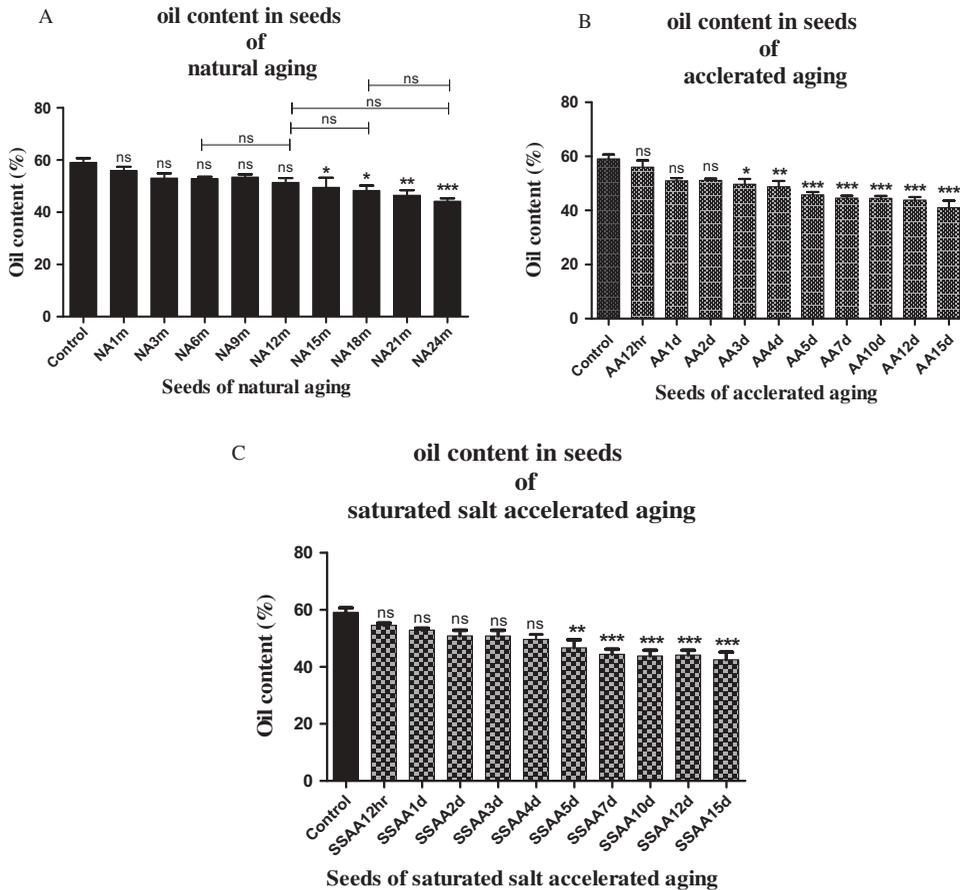
### ***Jatropha curcas* L. oil extraction**

The commercial success of the cultivation of *Jatropha curcas* L. depends mainly upon the oil yield. The oil content was found to decrease significantly ( $p \leq 0.05$  and  $p \leq 0.01$  and  $p \leq 0.001$ ) with the increase in the storage period from 12 months of natural aging (Figure 1a) compared to control seeds. Also a similar kind of significant decrease was found in the oil content from the 3<sup>rd</sup> day of accelerated aging (Figure 1b) ( $p \leq 0.05$  and  $p \leq 0.01$  and  $p \leq 0.001$ ) and the 5<sup>th</sup> day of saturated salt accelerated aging (Figure 1c) ( $p \leq 0.01$  and  $p \leq 0.001$ ) as compared to control seeds. As the accelerated aging and saturated salt aging were increased the oil content was significantly further reduced. Half yearly and yearly analysis of the oil yield shows no significant decrease in oil content (Figure 1a). This indicates that reduction of oil content is gradual and not rapid in natural aging.

The results observed in this study are in conformity with Sisman and Delibas (2004) who showed that a three-month storage of sunflower seeds resulted in a decrease in the oil content significantly. Suriyong (2007) also reported that the oxidation process - a reaction between unsaturated fatty acids and oxygen - is the causative agent of seed deterioration manifested in a significant decrease in the oil content. During storage and germination, the lipids are utilized as a source of energy by the seed itself (Ghasemnezhad and Honermeier 2009). This could be another reason for the seed oil reduction during long storage duration. Through the process of lipid peroxidation, the free radicals formed would act upon the lipids thus reducing the amount of total lipids (Sushma 2014). From the results, it can be deduced that *Jatropha curcas* L. is one of the potential putative plants for feedstock for biodiesel as it retains efficiently its maximum oil quantity even up to 12 months of natural aging. Seeds exposed to one day of accelerated aging and saturated salt accelerated aging was sufficient enough to bring about the same negative effect on oil reduction as found in six months of natural aging (Table 1). With respect to oil content analysis NA12m and NA24m are equivalents of AA2d and SSAA2d and AA7d and SSA7d, respectively (Table 1).

### **Free fatty acids content**

In our present study, free fatty acid content in the extracted oil of *Jatropha curcas* L. seeds gets significantly increased from 15 months of natural aging ( $p \leq 0.01$  and  $p \leq 0.001$ ) compared to



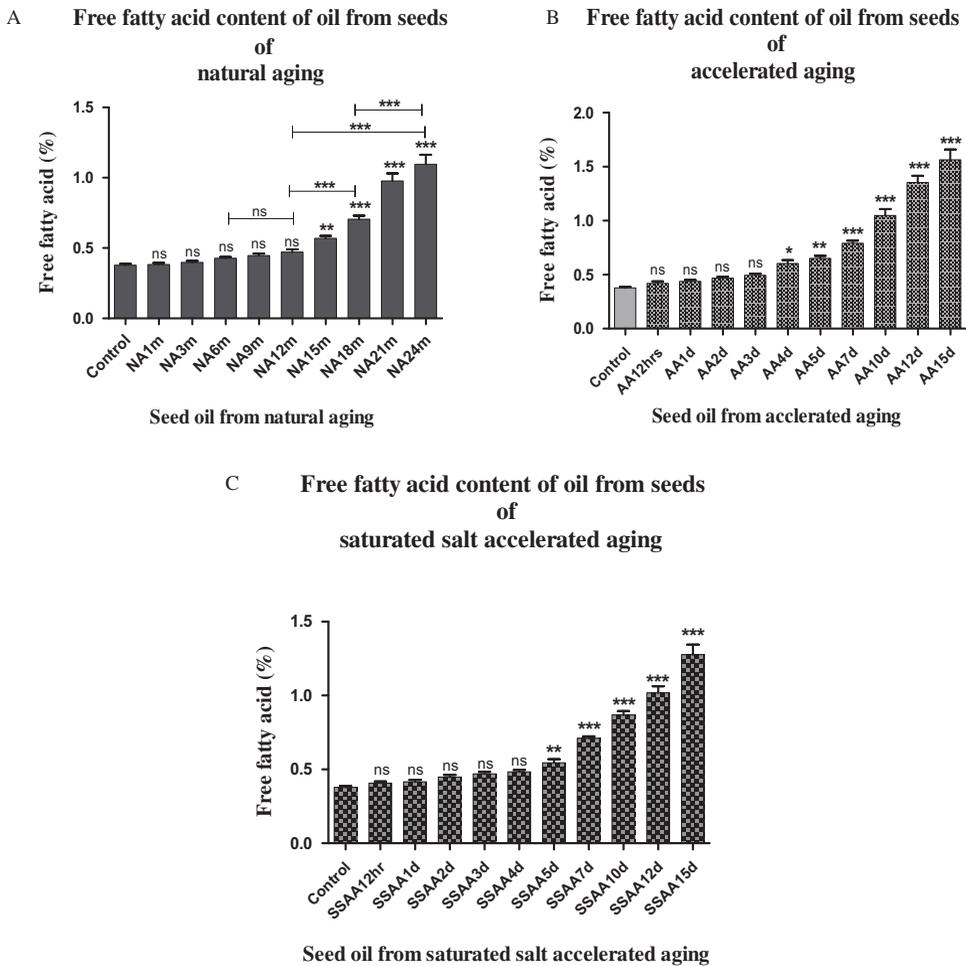
**Figure 1.** Levels of Oil content in control seeds, in seeds of natural aging of 1,3,6,9,12,15,18,21,24 months (Figure 1a), in seeds of accelerated aging of 12 h & 1day,2,3,4,5,7,10,12,15 days (Figure 1b), & in seeds of saturated salt accelerated aging of 12 h & 1day,2,3,4,5,7,10,12,15 days (Figure 1c). Values are means indicated either by \* or \*\* or \*\*\* represent statistically significant differences between the means of control group compared to each group of natural aging, accelerated aging and saturated accelerated salt aging.

**Table 1.** Values of oil content found in seeds of 6, 12 & 24 months of natural aging that correspond (~) to the values of oil content found in seeds of accelerated aging and saturated salt accelerated aging.

Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
NA6m = $52.9 \pm 1.27$	AA1d = $52.1 \pm 1.76$ (ns)	SSAA1d = $52.9 \pm 1.17$ (ns)
NA12m = $51.4 \pm 2.95$	AA2d = $51.1 \pm 1.23$ (ns)	SSAA2d = $51.4 \pm 3.31$ (ns)
NA24m = $44.25 \pm 1.23$	AA7d = $44.6 \pm 1.42$ (ns)	SSAA7d = $44.4 \pm 2.93$ (ns)

ns - non-significant compared to NA6m, NA12m, and NA24m.

control (Figure 2a). From the 4<sup>th</sup> day of accelerated aging and the 5<sup>th</sup> day of saturated salt accelerated aging onward a similar kind of significant increase ( $p \leq 0.05$  and  $p \leq 0.01$  and  $p \leq 0.001$ ) was found compared to control (Figure 2b–c). In half-yearly analysis, compared to 12 months of natural aging significantly increased ( $p \leq 0.001$ ) free fatty acids content was found in oil extracted from the seeds of 18 months of natural aging. A similar kind of increase ( $p \leq 0.001$ ) in free fatty acids was observed in oil from the seeds of 24 months of natural aging compared to 18 months of natural aging. In yearly analysis, first 12 months natural aging showed no significant increase in free fatty acids compared to control. But significant increase found in oil from the seeds of 24 months of natural aging compared to oil from the seeds of 12 months of natural aging indicates the severity of



**Figure 2.** Levels of free fatty acid in oil from control seeds, in oil from natural aging seeds of 1,3,6,9,12,15,18,21,24 months (Figure 1a), in oil from accelerated aging seeds of 12 h & 1 day, 2,3,4,5,7,10,12,15 days (Figure 1b), & in oil from saturated salt accelerated aging seeds of 12 h & 1 day, 2,3,4,5,7,10,12,15 days (Figure 1c). Values are means indicated either by \* or \*\* or \*\*\* represent statistically significant differences between the means of control group compared to each group of natural aging, accelerated aging and saturated accelerated salt aging.

deterioration (Figure 2a). In comparison of natural aging with that of accelerated and saturated salt aging, seeds get exposed to extreme conditions of temperature and moisture like that of AA1d or SSAA1d, AA2d or SSAA3d and AA10d or SSAA12d it brings about the same deteriorative effects as found in 6 months, 12 months and 24 months of natural aging, respectively (Table 3).

The acid value of any particular natural oil subjected towards biodiesel preparation is determined by the degree of unsaturation and free fatty acids (Ajayebi, Gnansounou, and Raman 2013). Oil exposed to open air and sunlight for a longer period of time would alter and increase the free fatty acid concentration. If the extracted oil contains the free fatty acid level more than 1% (acid value = 1.98 mg KOH/gm) (Tables 2 and 3) makes the oil unfit for biodiesel conversion because it would not only yield low quality of biodiesel but would lead to soap formation (Berchmans and Hirata 2008). The longer the fatty acid chains the greater the cetane number and oxidative stability of the oil (Wang et al. 2011). Therefore, if the quality of the feedstock for biodiesel is ensured of the free fatty acids level below 1%, it would increase the oxidative stability of biodiesel prepared out of these feedstocks. The oil with free fatty acids below 1% indicates the virginity of being unaffected by lipid

**Table 2.** Acid value of *Jatropha curcas* L. oil extracted from seeds of natural aging and accelerated aging and saturated salt accelerated aging.

Control	Acid value - mg KOH/gm		
	Natural aging Mean $\pm$ SD	Accelerated aging Mean $\pm$ SD	Saturated salt accelerated aging Mean $\pm$ SD
0.751 $\pm$ 0.0611	NA1m - 0.762 $\pm$ 0.0629	AA12hrs - 0.835 $\pm$ 0.109	SSAA12hrs - 0.808 $\pm$ 0.071
	NA3m - 0.791 $\pm$ 0.0602	AA1d - 0.868 $\pm$ 0.0826	SSAA1d - 0.826 $\pm$ 0.082
	NA6m - 0.85 $\pm$ 0.0536	AA2d - 0.931 $\pm$ 0.0878	SSAA2d - 0.89 $\pm$ 0.0926
	NA9m - 0.888 $\pm$ 0.0867	AA3d - 0.981 $\pm$ 0.0932	SSAA3d - 0.932 $\pm$ 0.086
	NA12m - 0.939 $\pm$ 0.115	AA4d - 1.2 $\pm$ 0.179	SSAA4d - 0.961 $\pm$ 0.078
	NA15m - 1.13 $\pm$ 0.099	AA5d - 1.29 $\pm$ 0.133	SSAA5d - 1.08 $\pm$ 0.139
	NA18m - 1.40 $\pm$ 0.153	AA7d - 1.57 $\pm$ 0.288	SSAA7d - 1.51 $\pm$ 0.073
	NA21m - 1.94 $\pm$ 0.324	AA10d - 2.15 $\pm$ 0.419	SSAA10d - 1.83 $\pm$ 0.152
	NA24m - 2.18 $\pm$ 0.403	AA12d - 2.65 $\pm$ 0.368	SSAA12d - 2.02 $\pm$ 0.269
		AA15d - 3.11 $\pm$ 0.566	SSAA15d - 2.54 $\pm$ 0.403

**Table 3.** Acid value of *Jatropha curcas* L. oil from seeds of 6, 12 & 24 months of natural aging that correspond (~) to Acid values of *Jatropha curcas* L. oil from seeds of accelerated aging and saturated salt accelerated aging.

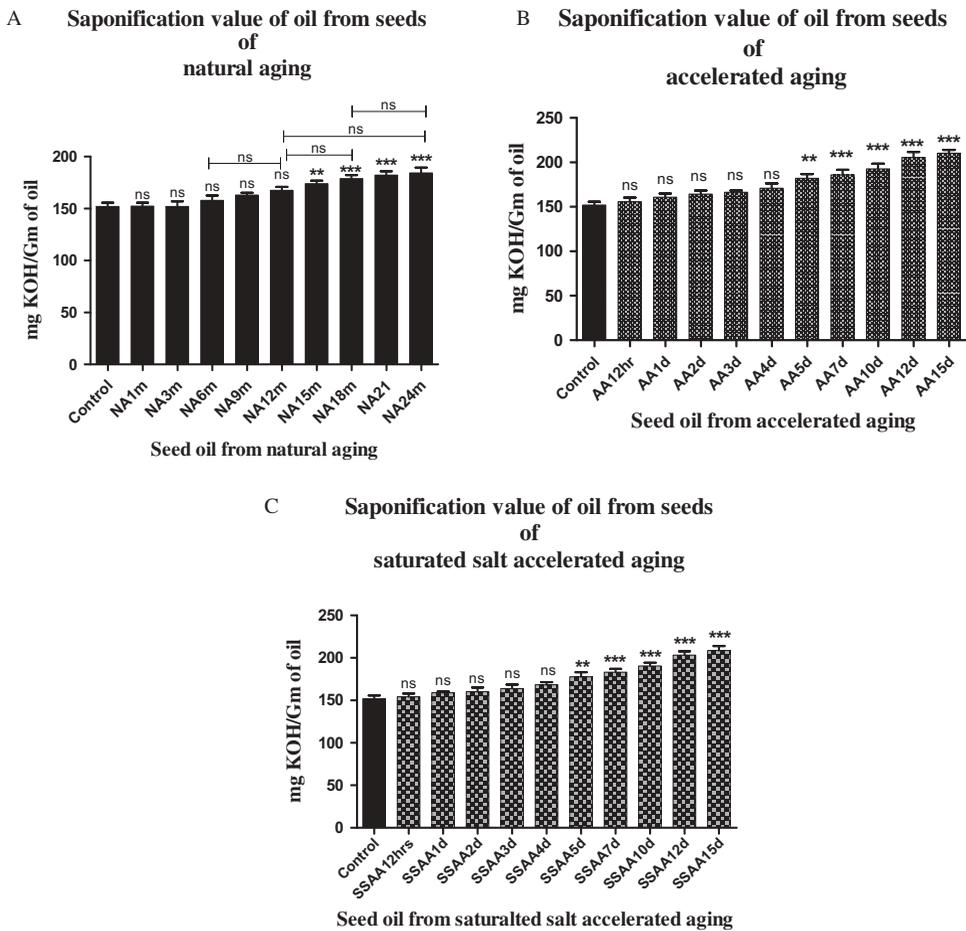
Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
NA6m = 0.85 $\pm$ 0.0536	AA1d = 0.868 $\pm$ 0.0826 (ns)	SSAA1d = 0.89 $\pm$ 0.0926 (ns)
NA12m = 0.939 $\pm$ 0.115	AA2d = 0.931 $\pm$ 0.0878 (ns)	SSAA3d = 0.932 $\pm$ 0.086 (ns)
NA24m = 2.18 $\pm$ 0.403	AA10d = 2.15 $\pm$ 0.419 (ns)	SSAA12d = 2.02 $\pm$ 0.269 (ns)

ns - non-significant compared to NA6m, NA12m, and NA24m, respectively.

peroxidation and rancidity. Such oil is characterized by long chain fatty acids with the needed amount of monounsaturated fatty acids. Our study reveals that seeds belonging to the natural aging of up to 18 months can be easily chosen as feedstock since they contain free fatty acids level below 1%. This proves the capacity of *Jatropha curcas* L. seeds withstanding high temperature and humidity in storage condition. In the case of 10 days, 12 days and 15 days of accelerated aging and 12 and 15 days of saturated salt accelerated aging, there is occurrence of severe lipid peroxidation disqualifying the seed for feedstock since it contain free fatty acids above 1%. The high acid value can damage the engine parts (Verma, Verma, and Gaur 2015). Seven days of accelerated aging and 10 days of saturated salt accelerated aging did not raise the free fatty acids level above 1%. This once again further qualifies the *Jatropha curcas* L. seeds as best feedstock that can withstand extreme conditions of temperature and moisture in tropical regions like India. Our results are in agreement with Berchmans and Hirata (2008) who reported that free fatty acids have been found to increase due to hydrolysis of triglycerides in the presence of moisture and oxidation through the formation of reactive oxygen species (ROS) due to temperature.

### Saponification value

Saponification value of *Jatropha curcas* L. seed oil revealed a gradual increase during storage. From NA15m to NA24m a significant increase ( $p \leq 0.01$  and  $p \leq 0.001$ ) was observed (Figure 3a). A similar kind of increase was also found in AA5d to AA15d and SSAA5d to SSAA15d ( $p \leq 0.01$  and  $p \leq 0.001$ ) (Figure 3b-c). A half-yearly analysis of the saponification value shows no significant increase from 6 months to 12 months, from 12 months to 18 months, and from 18 months to 24 months of natural aging. Yearly analysis too showed no significant change (Figure 3a). As regards the saponification value concerned, 1 day of accelerated aging and 3 days of saturated salt accelerated aging brought about the same effect as found in 6 months of natural aging. Similarly, 3 and 7 days of AA and 4 and 7 days of SSAA were corresponding to 12 and 24 months of natural aging, respectively (Table 4). The high proportion of lower fatty acids in any oil is indicated by a higher saponification value for saponification value is



**Figure 3.** Saponification value found in oil from control seeds, in oil from natural aging seeds of 1,3,6,9,12,15,18,21,24 months (Figure 1a), in oil from accelerated aging seeds of 12 h & 1 day,2,3,4,5,7,10,12,15 days (Figure 1b), & in oil from saturated salt accelerated aging seeds of 12 h & 1 day,2,3,4,5,7,10,12,15 days (Figure 1c). Values are means indicated either by \* or \*\* or \*\*\* represent statistically significant differences between the means of control group compared to each group of natural aging, accelerated aging and saturated accelerated salt aging.

**Table 4.** Saponification value found in oil of *Jatropha curcas* L. seeds of 6, 12 & 24 months of natural aging that correspond (-) to saponification values found in oil of *Jatropha curcas* L. seeds of accelerated aging and saturated salt accelerated aging.

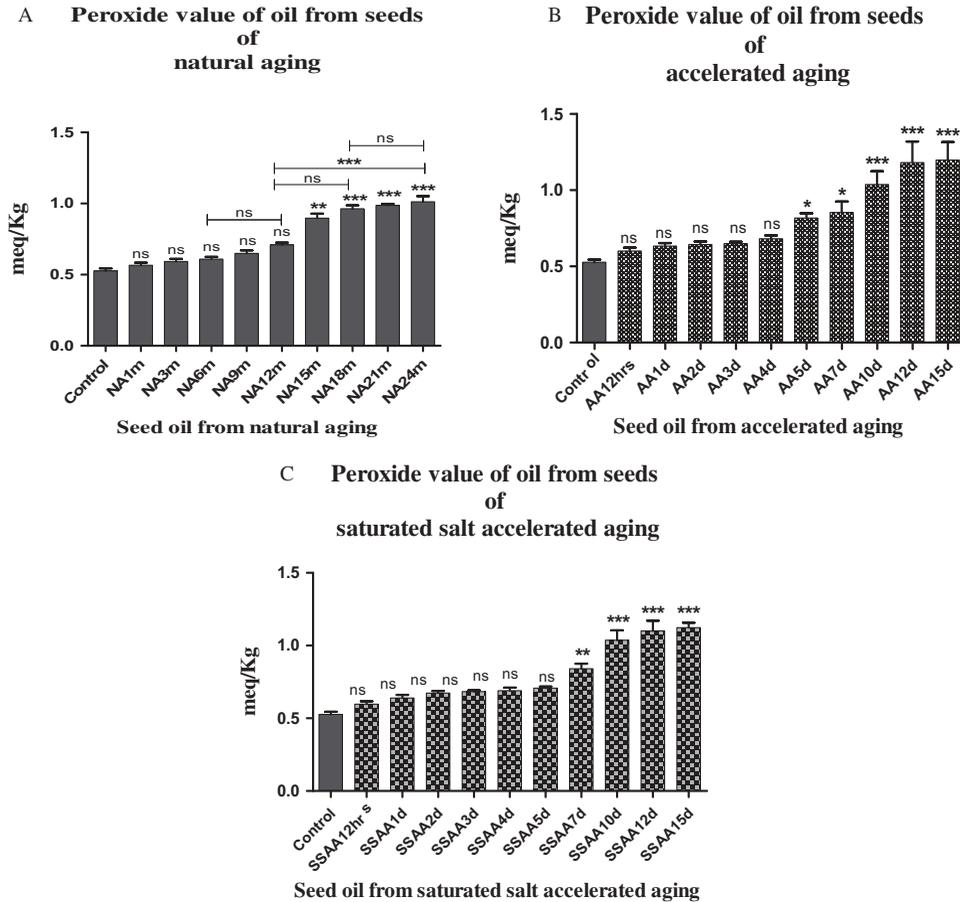
Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
NA6m = 158 $\pm$ 4.76	AA1d = 160 $\pm$ 4.24 (ns)	SSAA2d = 160 $\pm$ 4.75 (ns)
NA12m = 168 $\pm$ 3.39	AA3d = 169 $\pm$ 5.41 (ns)	SSAA4d = 169 $\pm$ 3.03 (ns)
NA24m = 184 $\pm$ 5.11	AA7d = 185 $\pm$ 5.63 (ns)	SSAA7d = 183 $\pm$ 4.13 (ns)

ns – non-significant compared to NA6m, NA12m, and NA24m.

inversely proportional to the average molecular weight of total fatty acids present (Muhammad et al. 2011). In the present study, the increase in the saponification value might be due to aging treatment which brought about the oxidation leading to bond breakage of long chain fatty acids in the oil. *Jatropha curcas* L. oil with high saponification value is not a very good candidate for biodiesel production as it would lead to soap formation (Abdulhamid et al. 2013). Similar kind of results was also observed in cotton seeds during storage and accelerated aging (Iqbal et al. 2002).

### Peroxide value (PV)

During any storage of oil or lipids, measuring the peroxide values becomes an indicator of oxidation and rancidity. The higher the peroxide value the more oxidized the oil is. Compared to control a significant increase in peroxide value was found from NA15m to NA24m, AA4d to AA15d and SSAA7d to SSAA15d ( $p \leq 0.05$  and  $p \leq 0.01$  and  $p \leq 0.001$ ) (Figure 4a–c). A half-yearly analysis indicates no significant increase from fresh seeds to 6 months, 6 months to 12 months, 12 months to 18 months and 18 months to 24 months of natural aging. In the yearly analysis, there is no significant increase from fresh seeds to 12 months but there is a significant increase ( $p \leq 0.001$ ) from 12 months to 24 months of natural aging (Figure 4a). This indicates how rapid the onset of formation of peroxides is as the storage period gets prolonged beyond 12 months of natural aging. The peroxide value found in AA1d and SSAA2d was the same as that of NA6m (Table 5). This indicates that 1 or 2 days of accelerated aging or saturated salt accelerated aging brings about the same negative effect in peroxide value as seen in 6 months of natural aging. Similarly, 4 days of AA or SSAA and 10 days of AA or SSAA brought about a peroxide value which is equivalent to 12 months and 24 months of natural aging, respectively (Table 5). Highly reactive free radicals are the intermediary products of lipid peroxidation. These intermediates react with oxygen to form



**Figure 4.** Peroxide value found in oil from control seeds, in oil from natural aging seeds of 1,3,6,9,12,15,18,21,24 months (Figure 1a), in oil from accelerated aging seeds of 12 h & 1 day, 2,3,4,5,7,10,12,15 days (Figure 1b), & in oil from saturated salt accelerated aging seeds of 12 h & 1 day, 2,3,4,5,7,10,12,15 days (Figure 1c). Values are means indicated either by \* or \*\* or \*\*\* represent statistically significant differences between the means of control group compared to each group of natural aging, accelerated aging and saturated accelerated salt aging.

**Table 5.** Peroxide value found in oil of *Jatropha curcas* L. seeds of 6, 12 & 24 months of natural aging that correspond (~) to peroxide values found in oil of *Jatropha curcas* L. seeds of accelerated aging and saturated salt accelerated aging.

Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
NA6m = 0.610 $\pm$ 0.026	AA1d = 0.633 $\pm$ 0.035 (ns)	SSAA2d = 0.623 $\pm$ 0.025 (ns)
NA12m = 0.710 $\pm$ 0.027	AA4d = 0.680 $\pm$ 0.041 (ns)	SSAA4d = 0.707 $\pm$ 0.208 (ns)
NA24m = 1.01 $\pm$ 0.067	AA10d = 1.03 $\pm$ 0.151 (ns)	SSAA10d = 1.04 $\pm$ 0.151 (ns)

ns – non-significant compared to NA6m, NA12m, and NA24m.

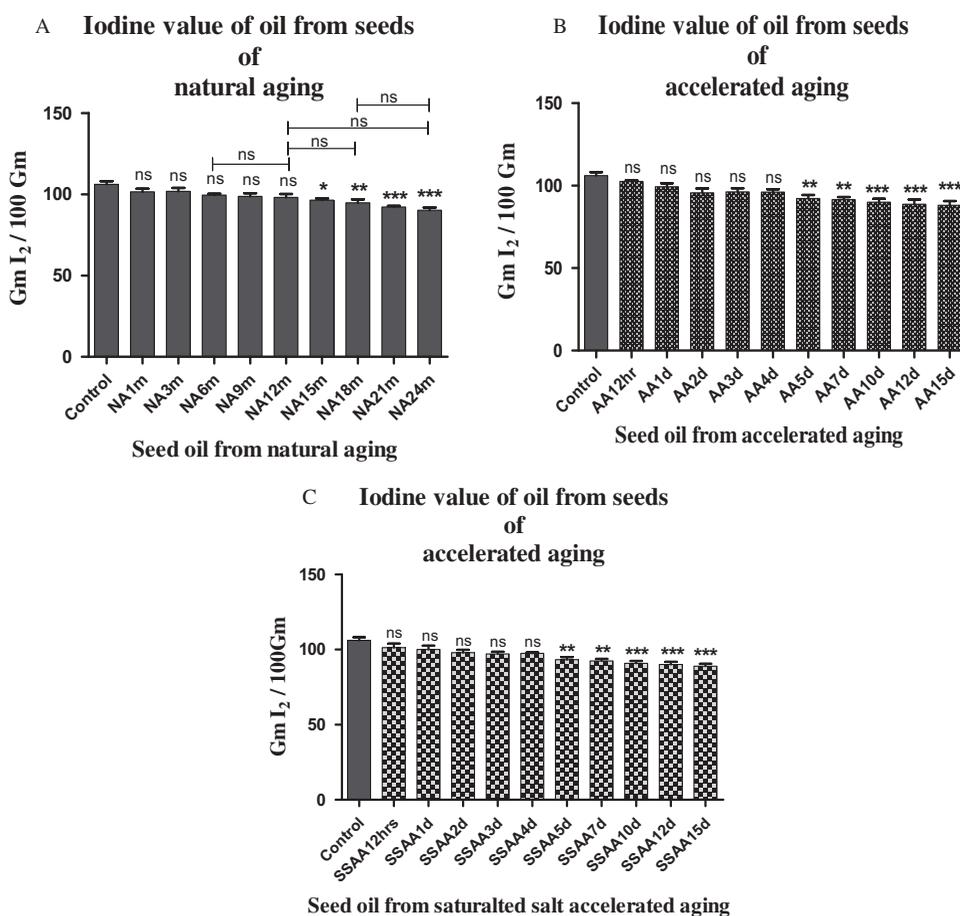
hydroperoxides (Iqbal et al. 2002). Hydroperoxides as primary products of lipid peroxidation (Yaakob et al. 2014) cause denaturation of nucleic acids and proteins and inactivation of enzymes (Iqbal et al. 2002). Increase in peroxide value brings in other deleterious effects such as increase in cetane number which lowers the ignition time (Van et al. 1996), corrosion and damage of the engine and hardening of the rubber components (Monyem and Van Gerpen 2001). Bouaid, Martinez, and Aracil (2007) showed that in order to safeguard any biodiesel from oxidation and to increase its stability its peroxide value must always be kept low. Our results are in conformity with the earlier reports on the stability of biodiesel which showed that 180 days of storage of oil leads to an increase of peroxide value (Das et al. 2009). Oil extracted up to 12 months of natural aging, up to 4 days of accelerated aging and 5 days of saturated salt accelerated aging serves as the best feedstock for biodiesel conversion since its peroxide value is kept low. Increase in storage period or accelerated aging elevates the peroxide value and lowers the oxidation stability of the oil making it unsuitable for subjection of biodiesel (Yaakob et al. 2014).

### **Iodine value**

It is a parameter to check the degree of unsaturation of fatty acids in triacylglycerol. Higher iodine value indicates higher unsaturation of the fatty acids in a given fat sample (Knothe 2002). A significant decrease of iodine values found from NA15m to NA24m, AA5d to AA15d and SSAA5d to SSAA15d compared to control indicates the degree of unsaturation of fatty acids in these groups ( $p \leq 0.05$  and  $p \leq 0.01$  and  $p \leq 0.001$ ) (Figure 5a–c). Both half-yearly (NA6m compared with control, NA12m with NA6m, NA18 with NA12m and NA24m with NA18m) and yearly analysis (NA12m compared with control and NA24m with NA12m) show no significant change in iodine value, indicating that unsaturation is not rapid but progressive (Figure 5a). If seeds get exposed to 1 day of AA, 2 days of SSAA and 10 days of AA and SSAA it will bring about a decrease in the iodine value as it is found in NA6m and NA24m, respectively (Table 6). Oil that contains higher unsaturated acid will have higher iodine number. Such oil even though has better cold flow properties but has a negative impact on oil stability (Akminul Islam et al. 2013). In our studies, significant decrease in iodine value can't be attributed to aging or accelerated aging. The hydrogens present are allylic and bis-allylic to the double bonds can't be determined by iodine value which is a factor to determine the oxidation stability of the biodiesel. Oxidation stability is determined by the position and number of bis-allylic methylene moieties present adjacent to double bonds (Knothe 2002).

### **Fatty acid methyl ester (FAME) content**

Studies on saturated fatty acid methyl esters and unsaturated fatty acid methyl esters reveal the stability of the oil. In storage, due to the process of auto-oxidation, the fatty acid methyl esters content gets decreased as the unsaturated fatty acid esters get oxidized (Lin and Lee 2010). In our present study, marked significant changes were found in oleic acid and palmitic acid methyl esters (Tables 7 and 8) but stearic acid and palmitoleic acid methyl esters (Tables 9 and 10) remained unchanged due to storage and accelerated or saturated salt accelerated aging. Oleic acid methyl ester which is a monounsaturated fatty acid is the highest constituent of total fatty acids present in



**Figure 5.** Iodine value found in oil from control seeds, in oil from natural aging seeds of 1,3,6,9,12,15,18,21,24 months (Figure 1a), in oil from accelerated aging seeds of 12 h & 1 day,2,3,4,5,7,10,12,15 days (Figure 1b), & in oil from saturated salt accelerated aging seeds of 12 h & 1 day,2,3,4,5,7,10,12,15 days (Figure 1c). Values are means indicated either by \* or \*\* or \*\*\* represent statistically significant differences between the means of control group compared to each group of natural aging, accelerated aging and saturated accelerated salt aging.

**Table 6.** Iodine value found in oil of *Jatropha curcas* L. seeds of 6, 12 & 24 months of natural aging that correspond (~) to iodine values found in oil of *Jatropha curcas* L. seeds of accelerated aging and saturated salt accelerated aging.

Mean ± SD	Mean ± SD	Mean ± SD
NA6m = 99.4 ± 3.39	AA1d = 99.3 ± 3.56 (ns)	SSAA2d = 97.9 ± 3.28 (ns)
NA12m = 98.1 ± 3.67		
NA24m = 90.2 ± 2.07	AA10d = 90.1 ± 3.74 (ns)	SSAA10d = 90.8 ± 2.82 (ns)

ns – non-significant compared to NA6m, NA12m, and NA24m.

*Jatropha curcas* L. oil. As the natural aging got prolonged the oleic acid methyl esters content got significantly reduced in NA18m, NA21m and NA24m compared to control ( $p \leq 0.05$ ) (Table 7). A similar kind of significant decrease was also found from AA7d to AA15d and from SSAA10d to SSAA15d compared to control ( $p \leq 0.05$  and  $p \leq 0.01$ ) (Table 7). This indicates a possible oxidation of the double bonds present in oleic acids due to prolonged storage and extreme condition of heat and moisture in accelerated aging and saturated salt accelerated aging. Compared to control significant increase in palmitic acid (saturated fatty acid) methyl ester content was found in natural aging, accelerated aging and saturated salt accelerated aging ( $p \leq 0.05$  and  $p \leq 0.01$ ) (Table 8). This

**Table 7.** Oleic acid content found in *Jatropha curcas* L. seed oil of natural aging, accelerated aging and saturated salt accelerated aging.

Control	Oleic acid content in %		
	Natural aging Mean $\pm$ SD	Accelerated aging Mean $\pm$ SD	Saturated salt accelerated aging Mean $\pm$ SD
43.4 $\pm$ 1.14	NA1m – 42.7 $\pm$ 0.501 (ns)	AA12hrs – 41.9 $\pm$ 0.5969 (ns)	SSAA12hrs – 41.8 $\pm$ 0.816 (ns)
	NA3m – 43.4 $\pm$ 1.12 (ns)	AA1d - 41.9 $\pm$ 0.335 (ns)	SSAA1d - 41.4 $\pm$ 0.563 (ns)
	NA6m – 42.8 $\pm$ 0.414 (ns)	AA2d - 41.8 $\pm$ 0.286 (ns)	SSAA2d - 41.2 $\pm$ 1.06 (ns)
	NA9m – 42.7 $\pm$ 1.4 (ns)	AA3d - 41.7 $\pm$ 0.512 (ns)	SSAA3d - 41.4 $\pm$ 1.11 (ns)
	NA12m – 42.1 $\pm$ 0.835 (ns)	AA4d - 41.5 $\pm$ 0.695 (ns)	SSAA4d - 41.1 $\pm$ 0.509 (ns)
	NA15m – 42.3 $\pm$ 0.915 (ns)	AA5d - 41.6 $\pm$ 0.482 (ns)	SSAA5d - 41.1 $\pm$ 0.824 (ns)
	NA18m – 41.21 $\pm$ 0.798 *	AA7d - 40.4 $\pm$ 0.447 *	SSAA7d - 41.0 $\pm$ 0.447 (ns)
	NA21m – 41.16 $\pm$ 0.838 *	AA10d - 40.2 $\pm$ 0.684 *	SSAA10d - 40.4 $\pm$ 0.646 *
	NA24m – 41.0 $\pm$ 0.509 *	AA12d - 40.3 $\pm$ 0.646 *	SSAA12d - 40.2 $\pm$ 0.649 *
		AA15d - 39.8 $\pm$ 2.3 **	SSAA15d - 40.1 $\pm$ 0.494 *

\* & \*\* & \*\*\* indicate significantly different compared to control. ns – non-significant.

**Table 8.** Palmitic acid content found in *Jatropha curcas* L. seed oil of natural aging, accelerated aging and saturated salt accelerated aging.

Control	Palmitic acid content in %		
	Natural aging Mean $\pm$ SD	Accelerated aging Mean $\pm$ SD	Saturated salt accelerated aging Mean $\pm$ SD
14.1 $\pm$ 0.526	NA1m – 14.5 $\pm$ 0.734 (ns)	AA12hrs – 14.9 $\pm$ 0.300 (ns)	SSAA12hrs – 14.6 $\pm$ 0.594 (ns)
	NA3m – 14.8 $\pm$ 0.105 (ns)	AA1d - 15.0 $\pm$ 0.122 (ns)	SSAA1d - 14.6 $\pm$ 0.531 (ns)
	NA6m – 14.7 $\pm$ 0.055 (ns)	AA2d - 14.9 $\pm$ 0.181 (ns)	SSAA2d - 14.1 $\pm$ 0.933 (ns)
	NA9m – 14.9 $\pm$ 0.125 (ns)	AA3d - 14.6 $\pm$ 0.355 (ns)	SSAA3d - 15.0 $\pm$ 0.297 (ns)
	NA12m – 14.9 $\pm$ 0.172 (ns)	AA4d - 14.8 $\pm$ 0.146 (ns)	SSAA4d - 15.0 $\pm$ 0.266 (ns)
	NA15m – 14.6 $\pm$ 0.336 (ns)	AA5d - 14.8 $\pm$ 1.020 (ns)	SSAA5d - 15.1 $\pm$ 0.130 (ns)
	NA18m – 15.2 $\pm$ 0.296 *	AA7d - 15.2 $\pm$ 0.504 *	SSAA7d - 15.1 $\pm$ 0.085 (ns)
	NA21m – 15.2 $\pm$ 0.121 *	AA10d - 15.2 $\pm$ 0.260 *	SSAA10d - 15.4 $\pm$ 0.144 *
	NA24m – 15.1 $\pm$ 0.144 *	AA12d - 15.3 $\pm$ 0.160 *	SSAA12d - 15.4 $\pm$ 0.486 *
		AA15d - 15.5 $\pm$ 0.495 **	SSAA15d - 15.5 $\pm$ 0.361 **

\* & \*\* & \*\*\* indicate significantly different compared to control. ns – non-significant.

**Table 9.** Stearic acid content found in *Jatropha curcas* L. seed oil of natural aging, accelerated aging and saturated salt accelerated aging.

Control	Stearic acid content in %		
	Natural aging Mean $\pm$ SD	Accelerated aging Mean $\pm$ SD	Saturated salt accelerated aging Mean $\pm$ SD
6.96 $\pm$ 0.0451	NA1m – 6.8 $\pm$ 0.586 (ns)	AA12hrs – 7.24 $\pm$ 0.101 (ns)	SSAA12hrs – 7.35 $\pm$ 0.489 (ns)
	NA3m – 6.8 $\pm$ 0.295 (ns)	AA1d - 6.88 $\pm$ 0.101 (ns)	SSAA1d - 6.78 $\pm$ 0.276 (ns)
	NA6m – 6.91 $\pm$ 0.015 (ns)	AA2d - 6.87 $\pm$ 0.060 (ns)	SSAA2d - 6.84 $\pm$ 0.276 (ns)
	NA9m – 6.72 $\pm$ 0.330 (ns)	AA3d - 6.94 $\pm$ 0.040 (ns)	SSAA3d - 6.90 $\pm$ 0.126 (ns)
	NA12m – 7.32 $\pm$ 0.711 (ns)	AA4d - 7.03 $\pm$ 0.384 (ns)	SSAA4d - 6.95 $\pm$ 0.151 (ns)
	NA15m – 7.9 $\pm$ 0.746 (ns)	AA5d - 6.89 $\pm$ 0.114 (ns)	SSAA5d - 6.97 $\pm$ 0.137 (ns)
	NA18m – 7.52 $\pm$ 0.360 (ns)	AA7d - 6.72 $\pm$ 0.295 (ns)	SSAA7d - 6.69 $\pm$ 0.275 (ns)
	NA21m – 7.3 $\pm$ 0.549 (ns)	AA10d - 6.98 $\pm$ 0.085 (ns)	SSAA10d - 6.98 $\pm$ 0.155 (ns)
	NA24m – 8.22 $\pm$ 0.959 (ns)	AA12d - 6.91 $\pm$ 0.120 (ns)	SSAA12d - 7.07 $\pm$ 0.202 (ns)
		AA15d - 6.86 $\pm$ 0.208 (ns)	SSAA15d - 6.80 $\pm$ 0.704 (ns)

\* & \*\* & \*\*\* indicate significantly different compared to control. ns – non-significant.

can be attributed to the hydrolysis of fatty acids due to prolonged storage period and due to high moisture content in both accelerated aging and saturated salt accelerated aging. Lin and Lee (2010) investigated the oxidative stability of biodiesel extracted from fish oil and concluded that a

**Table 10.** Palmitoleic acid content found in *Jatropha curcas* L. seed oil of natural aging, accelerated aging and saturated salt accelerated aging.

Control	Palmitoleic acid content in %		
	Natural aging Mean $\pm$ SD	Accelerated aging Mean $\pm$ SD	Saturated salt accelerated aging Mean $\pm$ SD
0.907 $\pm$ 0.046	NA1m – 0.823 $\pm$ 0.056 (ns)	AA12hrs – 0.890 $\pm$ 0.010 (ns)	SSAA12hrs – 0.887 $\pm$ 0.005 (ns)
	NA3m – 0.910 $\pm$ 0.200 (ns)	AA1d - 0.880 $\pm$ 0.200 (ns)	SSAA1d - 0.907 $\pm$ 0.026 (ns)
	NA6m – 0.910 $\pm$ 0.017 (ns)	AA2d - 0.903 $\pm$ 0.030 (ns)	SSAA2d - 0.887 $\pm$ 0.051 (ns)
	NA9m – 0.867 $\pm$ 0.032 (ns)	AA3d - 0.913 $\pm$ 0.015 (ns)	SSAA3d - 0.870 $\pm$ 0.050 (ns)
	NA12m – 0.863 $\pm$ 0.055 (ns)	AA4d - 0.900 $\pm$ 0.010 (ns)	SSAA4d - 0.883 $\pm$ 0.040 (ns)
	NA15m – 0.927 $\pm$ 0.015 (ns)	AA5d - 0.903 $\pm$ 0.023 (ns)	SSAA5d - 0.877 $\pm$ 0.030 (ns)
	NA18m – 0.970 $\pm$ 0.062 (ns)	AA7d - 0.910 $\pm$ 0.036 (ns)	SSAA7d - 0.873 $\pm$ 0.040 (ns)
	NA21m – 0.977 $\pm$ 0.025 (ns)	AA10d - 0.890 $\pm$ 0.030 (ns)	SSAA10d - 0.890 $\pm$ 0.041 (ns)
	NA24m – 0.997 $\pm$ 0.011 (ns)	AA12d - 0.917 $\pm$ 0.015 (ns)	SSAA12d - 0.867 $\pm$ 0.064 (ns)
		AA15d - 0.923 $\pm$ 0.023 (ns)	SSAA15d - 0.913 $\pm$ 0.032 (ns)

\* & \*\* & \*\*\* indicate significantly different compared to control. ns – non-significant.

significant decrease in the methyl esters content and greater degradation rate of same occur in a high-temperature prevailing condition. Our study reveals that oil extracted from seeds of *Jatropha curcas* L. still holds the greater stability up to 15 months of natural aging, 5 days of accelerated aging and 7 days of saturated salt accelerated aging based on fatty acid methyl esters analysis.

## Conclusion

In this present investigation of seed storage of *Jatropha curcas* L., there is a significant reduction in content of oil in seeds and oleic acid methyl esters of oil when seeds were stored beyond the point of one year. However, elevated levels of free fatty acids, saponification value and peroxide value of the oil were recorded as well after one year of storage. In a tropical country like India, *Jatropha curcas* L. seeds emerge out to be one of the best feedstocks well suited for biodiesel as they maintain optimum oil quantity and quality even up to one year of storage withstanding high temperature and moisture. Technique of saturated salt accelerated aging revealed that control of moisture will delay the process of seed deterioration.

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