CHAPTER – 6

INFLUENCE OF AGING OF *JATROPHA CURCAS* L. SEEDS ON THE OXIDATIVE STABILITY OF ITS OIL

6.1. INTRODUCTION

The rapid depletion of petroleum reserves with the deleterious impact of such fossil fuel on the environment make 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 et al., 2007). Uninterrupted and good quality feedstock is a key factor for biodiesel. *Jatropha curcas* L. has emerged as a favourite unconventional source of fuel. *J. curcas* 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 *J.curcas* 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 *J. curcas* 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 *J. curcas* seed oil is a boon for biodiesel conversion yet it can also make the seeds vulnerable to auto oxidation when exposed to factors like unfavourable temperature and moisture.

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

Fatty acid composition of raw material for biodiesel is important and decides its properties (Wang et al., 2012). Long chain saturated fatty acids (LCSF) and degree of unsaturation are crucial factors that contribute to the properties of biodiesel (Ramos et al., 2009). For the conversion to biodiesel, the oil extracted from *J. curcas* has to be transesterified. During this process of transesterification the compositions of fatty acids present in extracted oil do not change (Chuah et al., 2016) and oxidative stability of this synthesised biodiesel depends greatly on the composition of fatty acids present. Oxidative stability (OS), free fatty acids (FFA), cetane number (CN), iodine value (IV), peroxide value (PV), saponification value (SV) and cold filter plugging point (CFPP) which determine the quality of biodiesel depend upon the composition of fatty acids (Chuah et al., 2016). Determination of fatty acid compositions of monounsaturated fatty acids, polyunsaturated fatty acids and saturated fatty acids in raw material is essential in order to know the quality and suitability of the raw material before subjecting it to biodiesel conversion.

Oxidation is one of the major causes that affects the stability of the biodiesel by inducing ester polymerisation (Jain and Sharma, 2014). Often unsaturated fatty acids present in the biodiesel make it susceptible to oxidation (Rawat et al., 2014). It has been reported that oxidation stability present in parent oil is significantly higher than methyl esters formed from this parent oil through esterification. This decrease can be attributed to the loss of antioxidants occurring during the process of transesterification (Chuah et al., 2016). It is always preferable to choose a parent oil which has comparatively higher oxidation stability so that the loss which occur during esterification process can be minimised. Thus oil extracted from *J. curcas* needs to be analysed for fatty acid composition and other oxidative parameters such as free fatty acids (FFA), iodine value (IV), saponification value (SV) and peroxide value to determine the oxidative stability before the conversion of methyl esters. This would not only ensure the quality of parent oil but also yield biodiesel methyl esters with significant oxidation stability.

Biodiesel produced from the feed stock of *J. curcas* needs to meet the quality to ensure the optimum performance of engine. American Standards for Testing Materials (ASTM 6751-3) or the European Union Standard for biodiesel fuel (EN 14214) (Atadashi et al., 2010) and global standards such as in Germany (DIN 51606) and South Africa (SANS1935) (Wilson et al., 2005) are the few available international biodiesel standard specification. In order to ensure biodiesel produced out of *J. curcas* fulfils the specification to meet the international standard, feed stock with greatest quality must be chosen. Hence there is a need to study the storage effect of *J. curcas* to know the quality.

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 have been undertaken (Sushma, 2014) but there are no studies reported on the effect of long term storage (above 12 months) of the *J. curcas* seeds (instead of oil) on oil quality.

This chapter aims to map the changes in oil quantity and quality during natural aging, accelerated and saturated salt accelerated aging thus will help in optimizing oil yield.

6.2. RESULTS

6.2.1. Extraction of oil from *J. curcas* seeds

Oil was extracted using the standard method prescribed in ASTM E11 2003 (Chapter 2). The oil content was found to decrease significantly with the increase in the storage period from 12 months of natural aging (Figure 6.1) compared to control seeds. Also a similar kind of significant decrease was found in the oil content from the 3rd day of accelerated aging (Figure 6.2) and the 5th day of saturated salt accelerated aging (Figure 6.3) as compared to control seeds. As the accelerated aging and saturated salt aging were increased the oil content was significantly further reduced. This indicates that reduction of oil content is gradual and not rapid in natural aging.

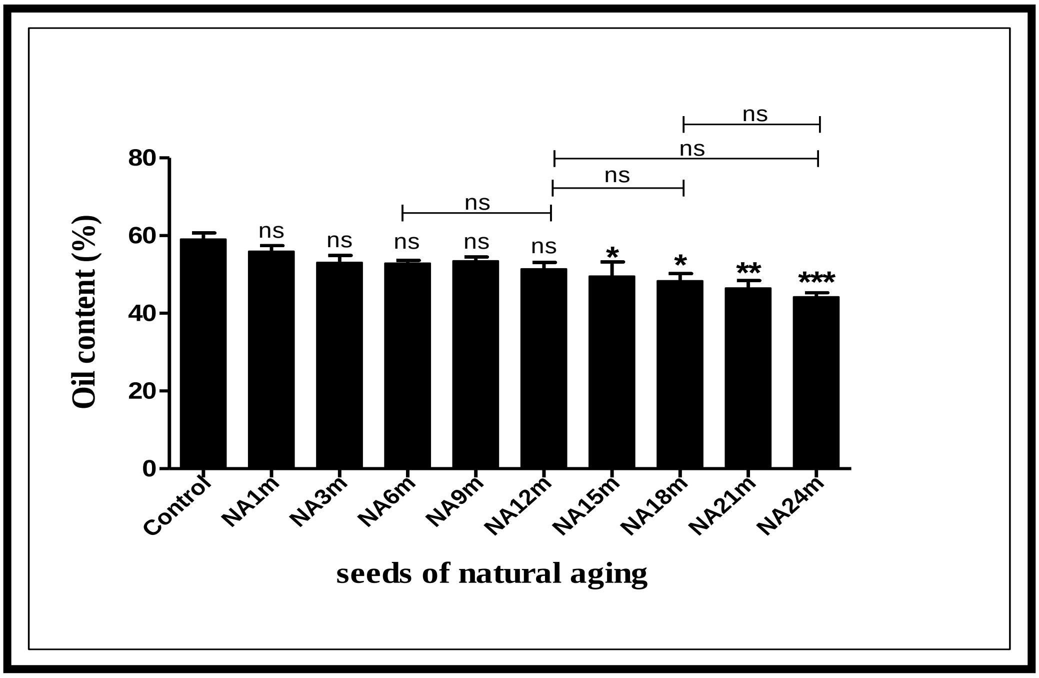

Figure 6.1: Oil content in control seeds and in seeds of natural aging of 1, 3, 6, 9, 12, 15, 18, 21 and 24 months. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, NA6m, NA12m and NA18m, n=3.**

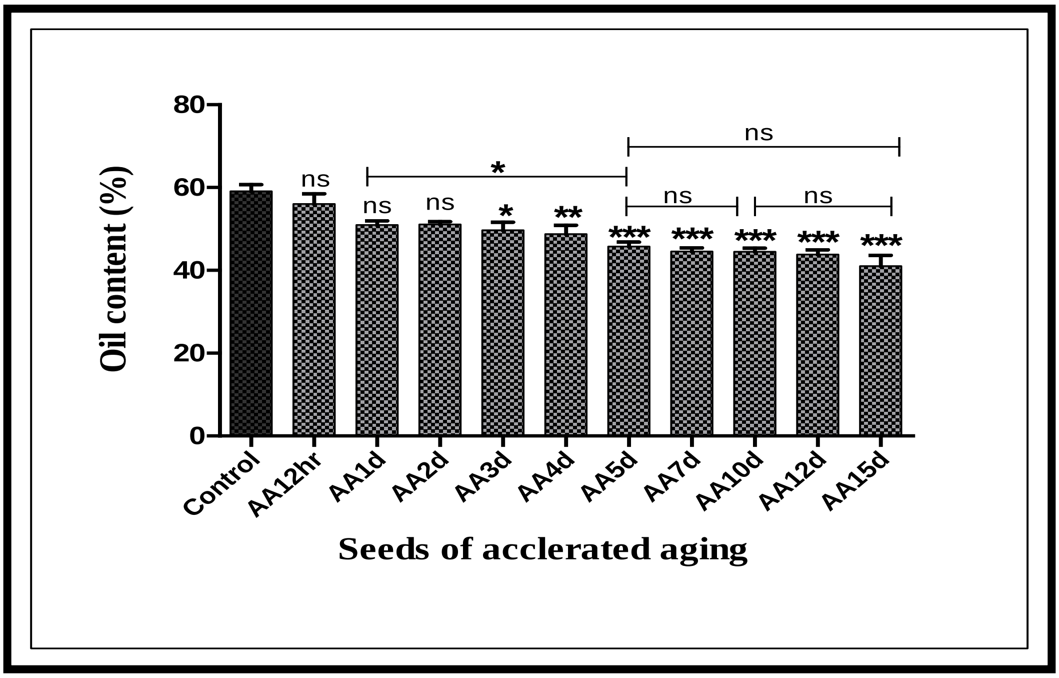

Figure 6.2: Oil content in control seeds and in seeds of accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, AA1d, AA5d and AA10d, n=3.**

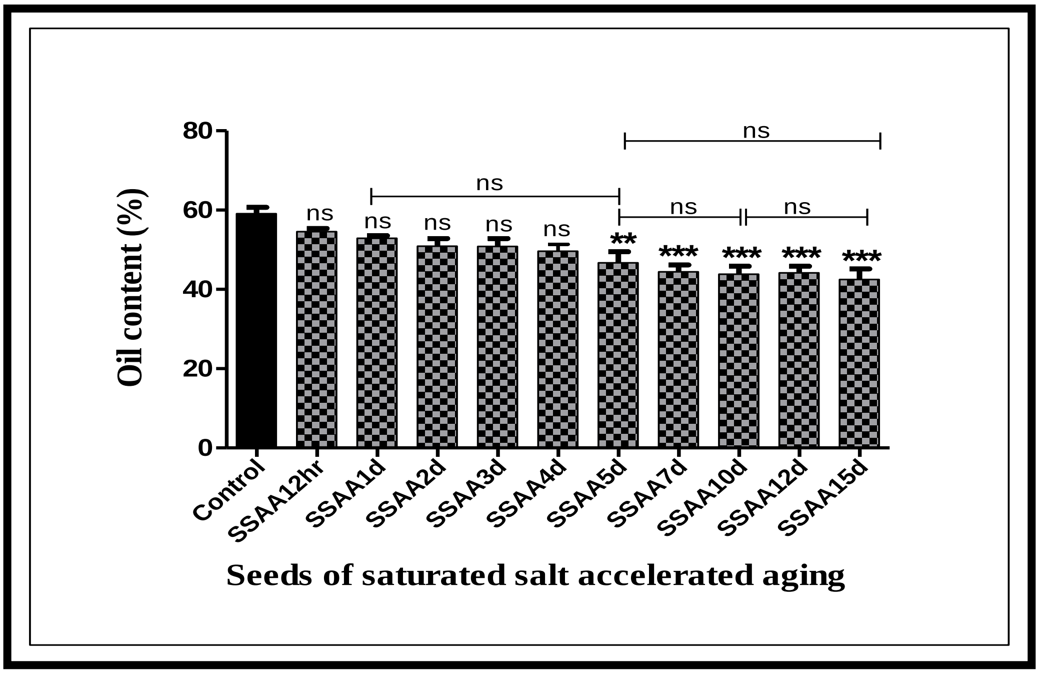

Figure 6.3: Oil content in control seeds and in seeds of saturated salt accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, SSAA1d, SSAA5d and SSAA10d, n=3.**

Seeds exposed to one day of accelerated aging and saturated salt accelerated aging had the same negative effect on oil reduction as found in six months of natural aging. Similarly percentage of oil content found in AA2d and SSAA2d is equivalent to percentage observed in NA12m and oil content noted in AA7d and SSAA7d is equal to NA24m (Table 6.1).

Table 6.1: Comparative analysis of oil content extracted from seeds of natural aging, accelerated aging and saturated salt accelerated aging.

Mean ± SD	Mean ± SD	Mean ± SD
NA6m =52.9 ± 1.27	AA1d = 52.1 ± 1.76 (ns)	SSAA1d = 52.9 ± 1.17 (ns)
NA12m = 51.4 ± 2.95	AA2d = 51.1 ± 1.23 (ns)	SSAA2d = 51.4 ± 3.31 (ns)
NA 24m = 44.25 ± 1.23	AA7d = 44.6 ± 1.42 (ns)	SSAA7d = 44.4 ± 2.93 (ns)

ns – Non – significant compared to NA6m, NA12m and NA24m respectively.

6.2.2. Determination of acid value/free fatty acids content in *J. curcas* oil

Acid value/free fatty acid was estimated and calculated as per methods and protocols reported by Cox and Pearson, (1962); AOAC (1975); Thimmaiah, (2006) (Chapter 2).

Table 6.2: Acid value of *Jatropha curcas* L. oil extracted from seeds of natural aging and accelerated aging and saturated salt accelerated aging.

Acid Value - mg KOH / gm			
Control	**Natural aging Mean ± SD**	**Accelerated aging Mean ± SD**	**Saturated salt accelerated aging Mean ± SD**
0.751+ 0.0611	NA1m - 0.762 ± 0.0629 NA3m - 0.791 ± 0.0602 NA6m - 0.85± 0.0536 NA9m - 0.888 ± 0.0867 NA12m - 0.939 ± 0.115 NA15m - 1.13 ± 0.099 NA18m - 1.40 ± 0.153 NA21m - 1.94 ± 0.324 NA24m - 2.18 ± 0.403	AA12h - 0.835 ± 0.109 AA1d - 0.868 ± 0.082 AA2d - 0.931 ± 0.087 AA3d - 0.981 ± 0.093 AA4d - 1.2 ± 0.179 AA5d - 1.29 ± 0.133 AA7d - 1.57 ± 0.288 AA10d - 2.15 ± 0.419 AA12d - 2.65 ± 0.368 AA15d - 3.11 ± 0.566	SSAA12h - 0.808 ± 0.071 SSAA1d - 0.826 ± 0.08 SSAA2d - 0.89 ± 0.092 SSAA3d - 0.932 ± 0.086 SSAA4d - 0.961 ± 0.078 SSAA5d - 1.08 ± 0.139 SSAA7d - 1.51 ± 0.073 SSAA10d - 1.83 ± 0.152 SSAA12d - 2.02 ± 0.269 SSAA15d - 2.54 ± 0.403

From the Acid value, the value of free fatty acids was calculated using the formula, % free fatty acids (FFA) = K x Acid Value (AV), Where, K = Constant (0.503) described in Cox and Pearson, (1962); AOAC (1975); Thimmaiah, (2006) (Chapter 2).

In this present study, free fatty acid content in the extracted oil of *J. curcas* seeds significantly increased from 15 months of natural aging as compared to control (Figure 6.4). Similarly increase of free fatty acid content was evident throughout the 24 months of natural aging. From the 4^{th} day of accelerated aging and the 5^{th} day of saturated salt accelerated aging onwards a similar kind of significant increase was found compared to control (Figure 6.5 and Figure 6.6). Prolonged natural aging, accelerated aging and saturated salt aging yielded a negative effect of increased free fatty acids.

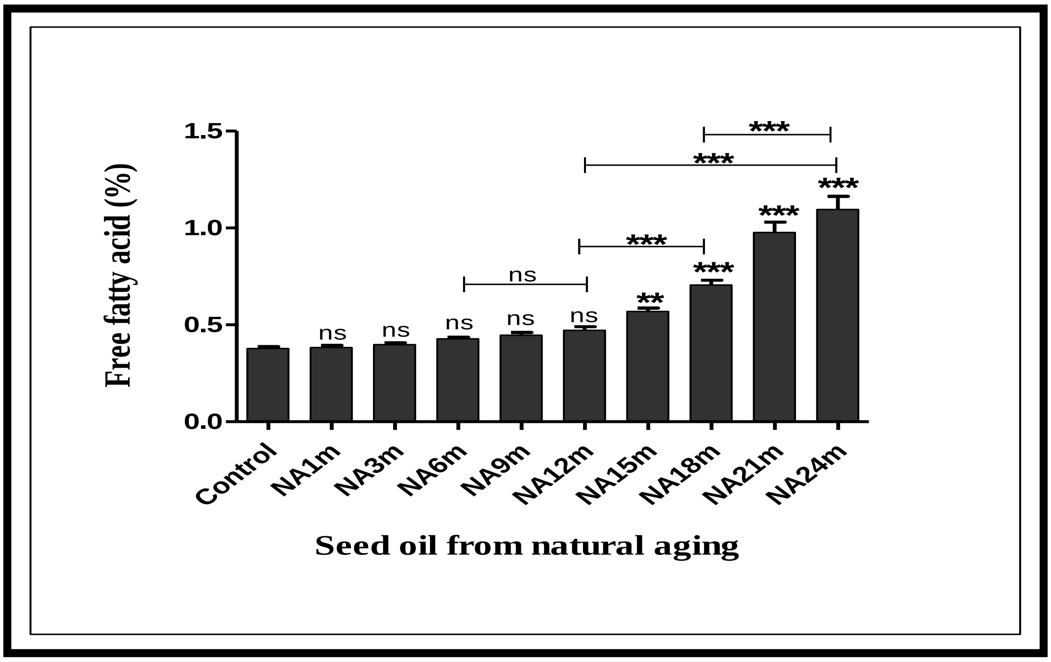

Figure 6.4: Free fatty acids content found in *J. curcas* oil extracted from control seeds and seeds of natural aging of 1, 3, 6, 9, 12, 15, 18, 21 and 24 months. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, NA6m, NA12m and NA18m, n=3.**

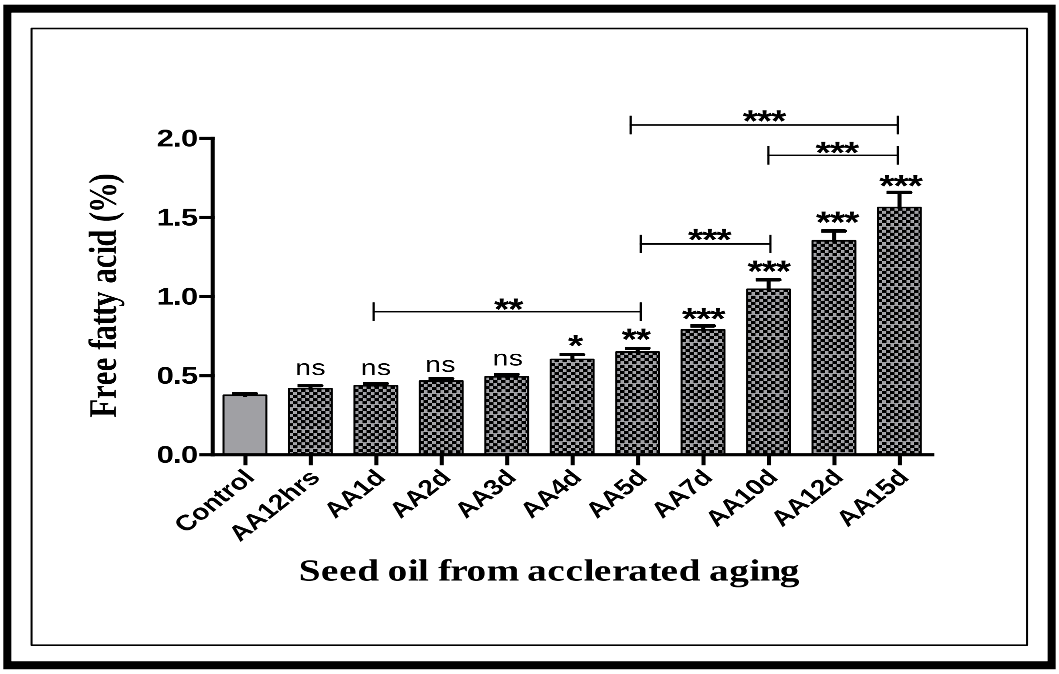

Figure 6.5: Free fatty acids content found in *J. curcas* oil extracted from control seeds and seeds of accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, AA1d, AA5d and AA10d, n=3.**

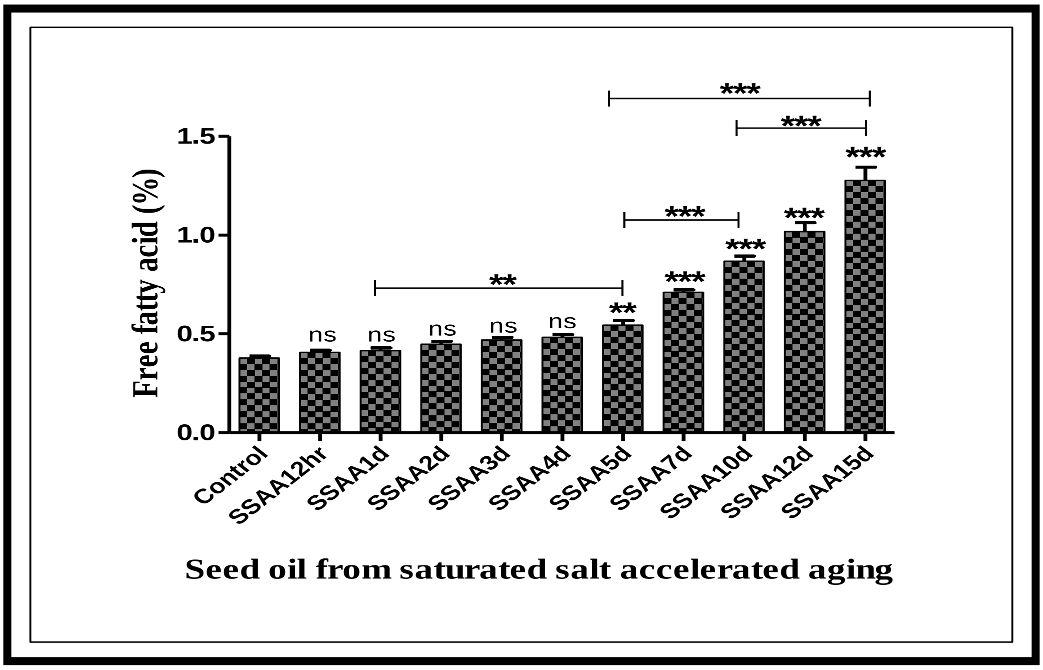

Figure 6.6: Free fatty acids content found in *J. curcas* oil extracted from control seeds and seeds of saturated salt accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, SSAA1d, SSAA5d and SSAA10d, n=3.**

Seeds that were exposed to extreme conditions of temperature and moisture like that of AA1d or SSAA1d, AA2d or SSAA3d and AA10d or SSAA12d resulted in possessing increased acid value as noted in 6 months, 12 months and 24 months of natural aging respectively (Table 6.3).

Table 6.3: Comparative analysis of acid value estimated in *J. curcas* oil extracted from seeds of natural aging, accelerated aging and saturated salt accelerated aging.

Mean ± SD	Mean ± SD	Mean ± SD
NA6m = 0.85± 0.0536	AA1d = 0.868 ± 0.0826 (ns)	SSAA1d = 0.89 ± 0.0926 (ns)
NA12m = 0.939 ± 0.115	AA2d = 0.931 ± 0.0878 (ns)	SSAA3d = 0.932 ± 0.086 (ns)
NA 24m = 2.18 ± 0.403	AA10d = 2.15 ± 0.419 (ns)	SSAA12d = 2.02 ± 0.269 (ns)

ns – Non – significant compared to NA6m, NA12m and NA24m respectively.

6.2.3. Determination of saponification value in *J. curcas* oil

Saponification value was estimated and calculated as per methods and protocols described by Cox and Pearson, (1962); AOAC (1975); Thimmaiah, (2006) (Chapter 2). Saponification value of *Jatropha curcas* L. seed oil revealed a gradual increase during storage. From NA15m to NA24m a significant increase was observed compared to control (Figure 6.7). A similar kind of increase was also marked in AA5d to AA15d and SSAA5d to SSAA15d (Figures 6.8 and 6.9).

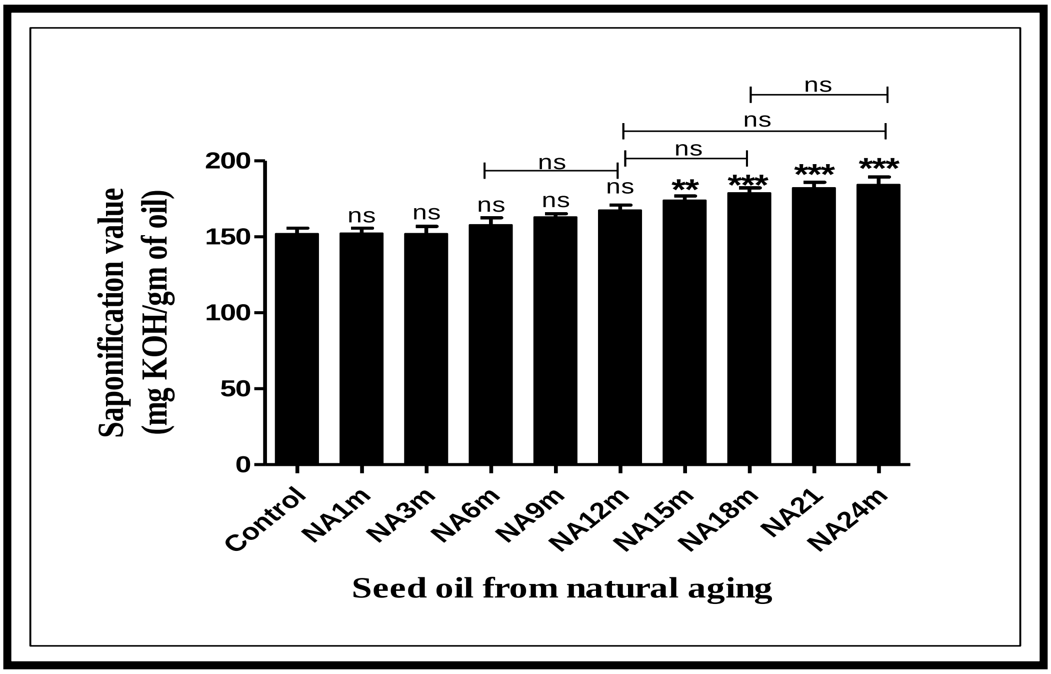

Figure 6.7: Saponification value found in *J. curcas* oil extracted from control seeds and seeds of natural aging of 1, 3, 6, 9, 12, 15, 18, 21 and 24 months. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, NA6m, NA12m and NA18m, n=3.**

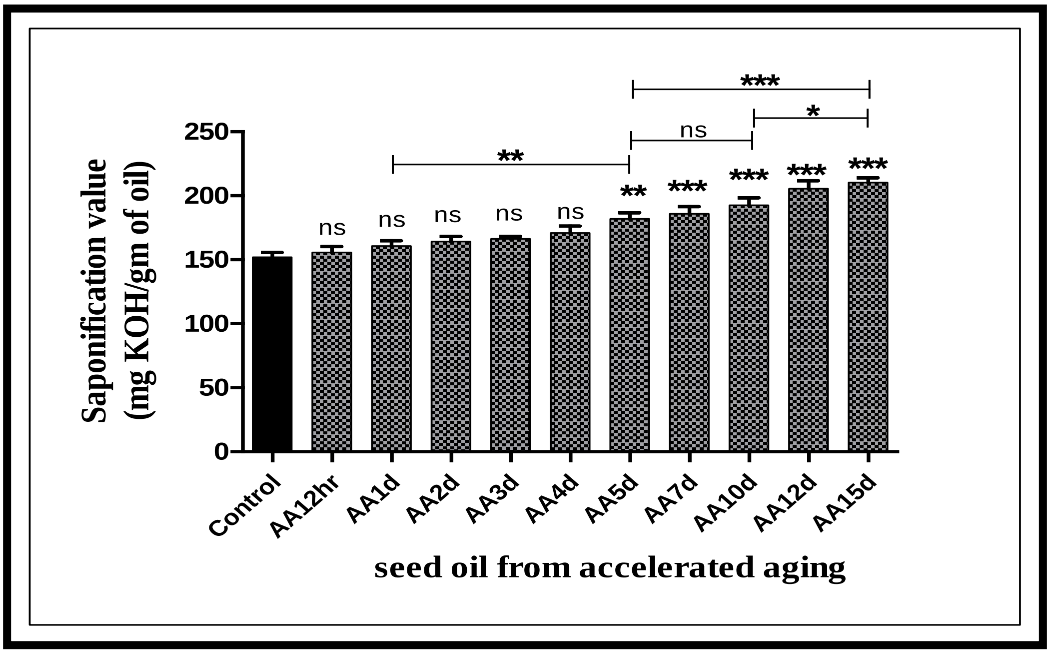

Figure 6.8: Saponification value found in *J. curcas* oil extracted from control seeds and seeds of accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, AA1d, AA5d and AA10d, n=3.**

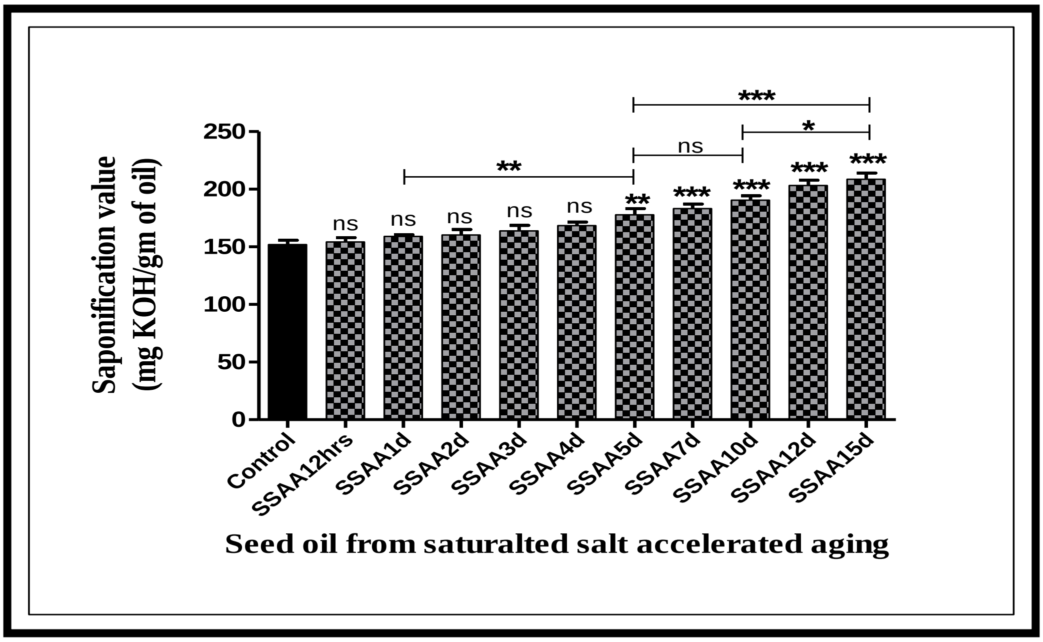

Figure 6.9: Saponification value found in *J. curcas* oil extracted from control seeds and seeds of saturated salt accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, SSAA1d, SSAA5d and SSAA10d, n=3.**

As regards the saponification value, 1 day of accelerated aging and 2 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 6.4).

Table 6.4: Comparative analysis of saponification value determined in *J. curcas* oil extracted from seeds of natural aging, accelerated aging and saturated salt accelerated aging.

Mean ± SD	Mean ± SD	Mean ± SD
NA6m =158 ± 4.76	AA1d = 160 ± 4.24 (ns)	SSAA2d = 160 ± 4.75 (ns)
NA12m = 168 ± 3.39	AA3d = 169 ± 5.41 (ns)	SSAA4d = 169 ± 3.03 (ns)
NA 24m = 184 ± 5.11	AA7d = 185 ± 5.63 (ns)	SSAA7d = 183 ± 4.13 (ns)

ns – Non – significant compared to NA6m, NA12m and NA24m respectively.

6.2.4. Determination of peroxide value in *J. curcas* oil

Peroxide value was estimated and calculated as per methods and protocols prescribed by Cox and Pearson, (1962); AOAC (1975); Thimmaiah, (2006) (Chapter 2). Compared to control a significant increase in peroxide value was found from NA15m to NA24m (Figure 6.10), AA4d to AA15d (Figure 6.11) and SSAA7d to SSAA15d (Figure 6.12).

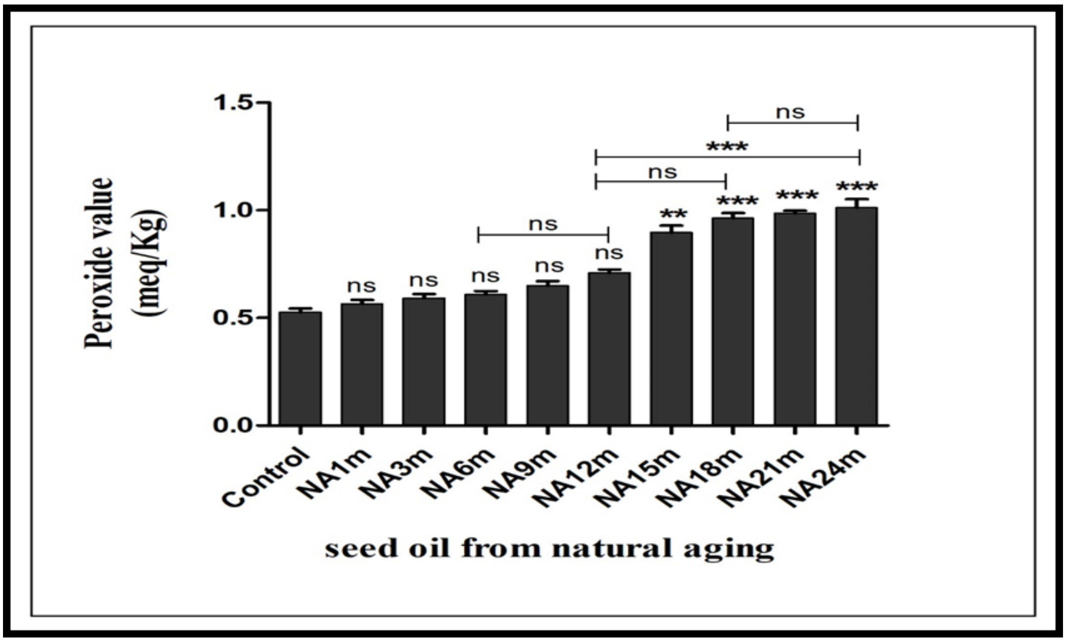

Figure 6.10: Peroxide value found in *J. curcas* oil extracted from control seeds and seeds of natural aging of 1, 3, 6, 9, 12, 15, 18, 21 and 24 months. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, NA6m, NA12m and NA18m, n=3.**

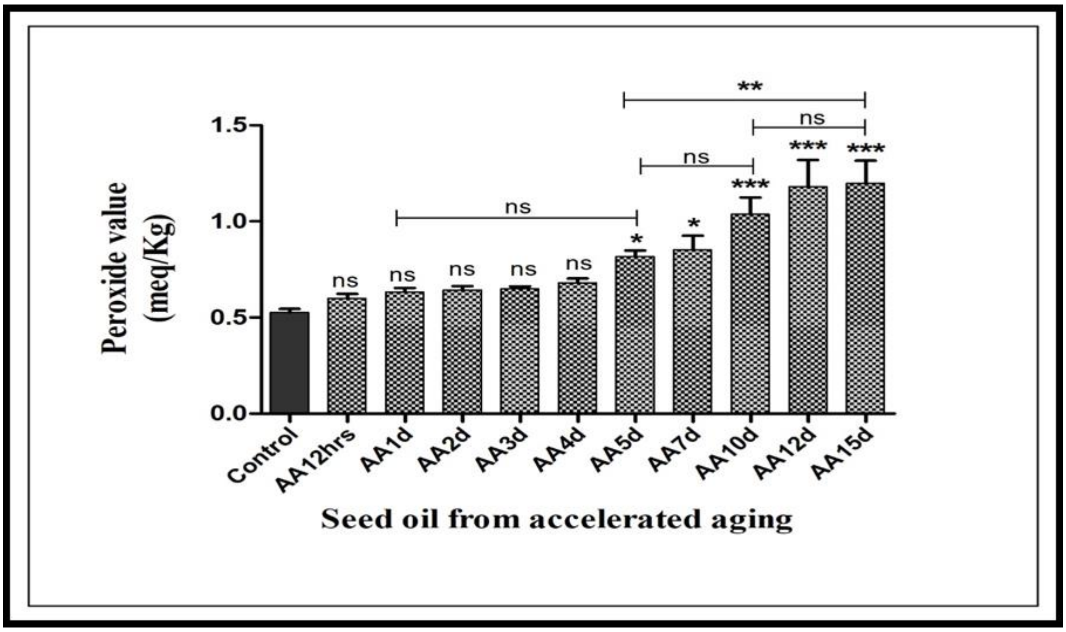

Figure 6.11: Peroxide value found in *J. curcas* oil extracted from control seeds and seeds of accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, AA1d, AA5d and AA10d, n=3.**

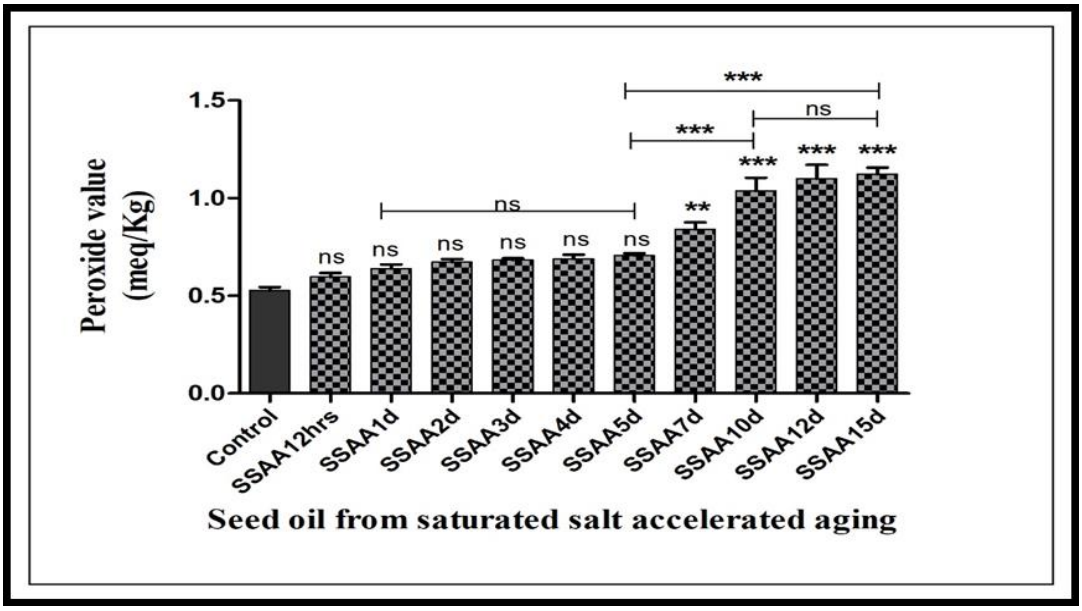

Figure 6.12: Peroxide value found in *J. curcas* oil extracted from control seeds and seeds of saturated salt accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, SSAA1d, SSAA5d and SSAA10d, n=3.**

The peroxide value found in AA1d and SSAA2d was the same as that of NA6m (Table 6.5). 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, 4days of AA or SSAA and 10days of AA or SSAA brought about a peroxide value which is equivalent to 12 months and 24 months of natural aging respectively (Table 6.5).

Table 6.5: Comparative analysis of peroxide value determined in *J. curcas* oil extracted from seeds of natural aging, accelerated aging and saturated salt accelerated aging.

Mean ± SD	Mean ± SD	Mean ± SD
NA6m = 0.610 ± 0.026	AA1d = 0.633 ± 0.035 (ns)	SSAA2d = 0.623 ± 0.025 (ns)
NA12m = 0.710 ± 0.027	AA4d = 0.680 ± 0.041 (ns)	SSAA4d = 0.707 ± 0.208 (ns)
NA 24m = 1.01 ± 0.067	AA10d = 1.03 ± 0.151 (ns)	SSAA10d = 1.04 ± 0.151 (ns)

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

6.2.5. Determination of iodine value in *J. curcas* oil

It is a parameter to check the degree of unsaturation of fatty acids in triacylglycerol. Iodine value was estimated and calculated as per methods and protocols prescribed by Cox and Pearson, (1962); AOAC (1975); Thimmaiah, (2006) (Chapter 2). A significant decrease of iodine values found from NA15m to NA24m (Figure 6.13), AA5d to AA15d (Figure 6.14) and SSAA5d to SSAA15d (Figure 6.15) compared to control indicates the degree of unsaturation of fatty acids in these groups.

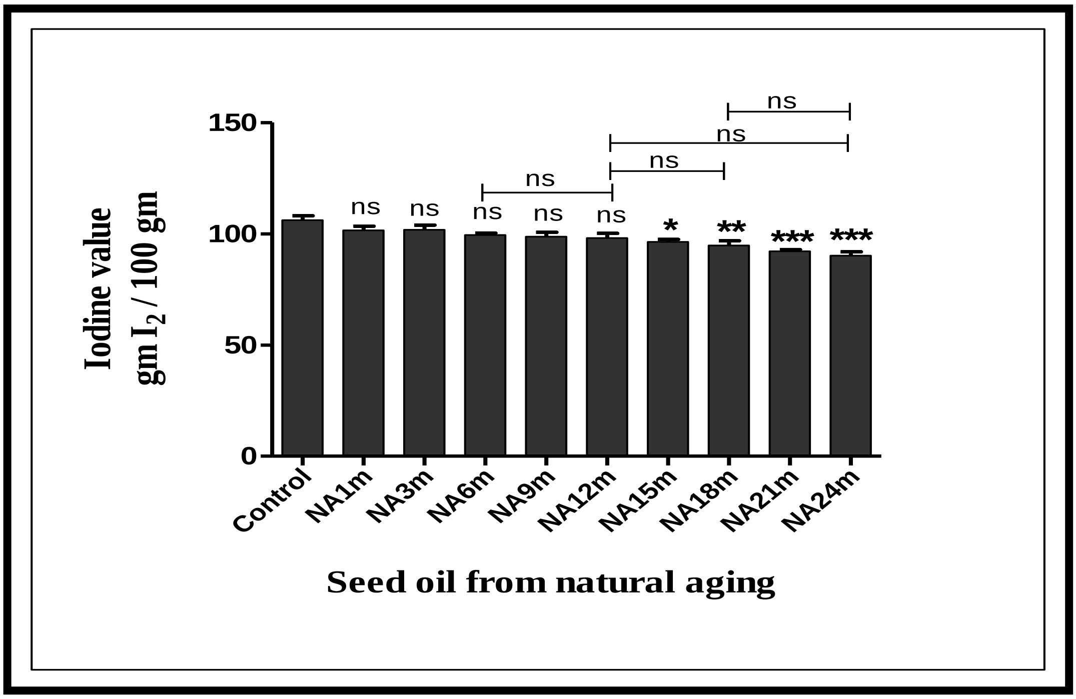

Figure 6.13: Iodine value found in *J. curcas* oil extracted from control seeds and seeds of natural aging of 1, 3, 6, 9, 12, 15, 18, 21 and 24 months. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, NA6m, NA12m and NA18m, n=3.**

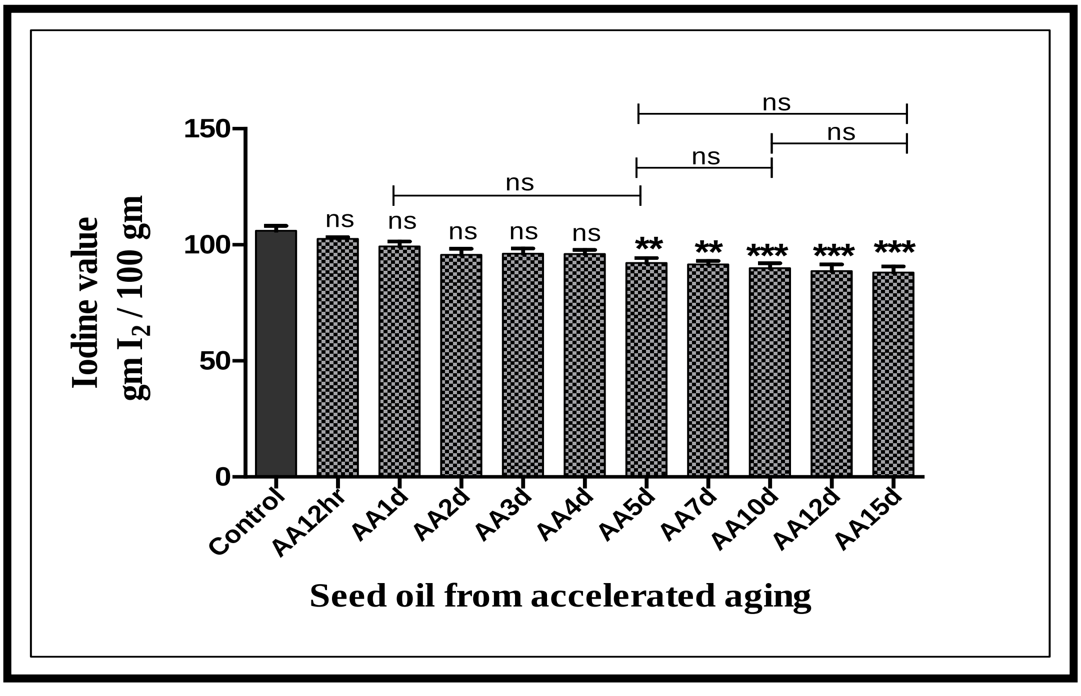

Figure 6.14: Iodine value found in *J. curcas* oil extracted from control seeds and seeds of accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, AA1d, AA5d and AA10d, n=3.**

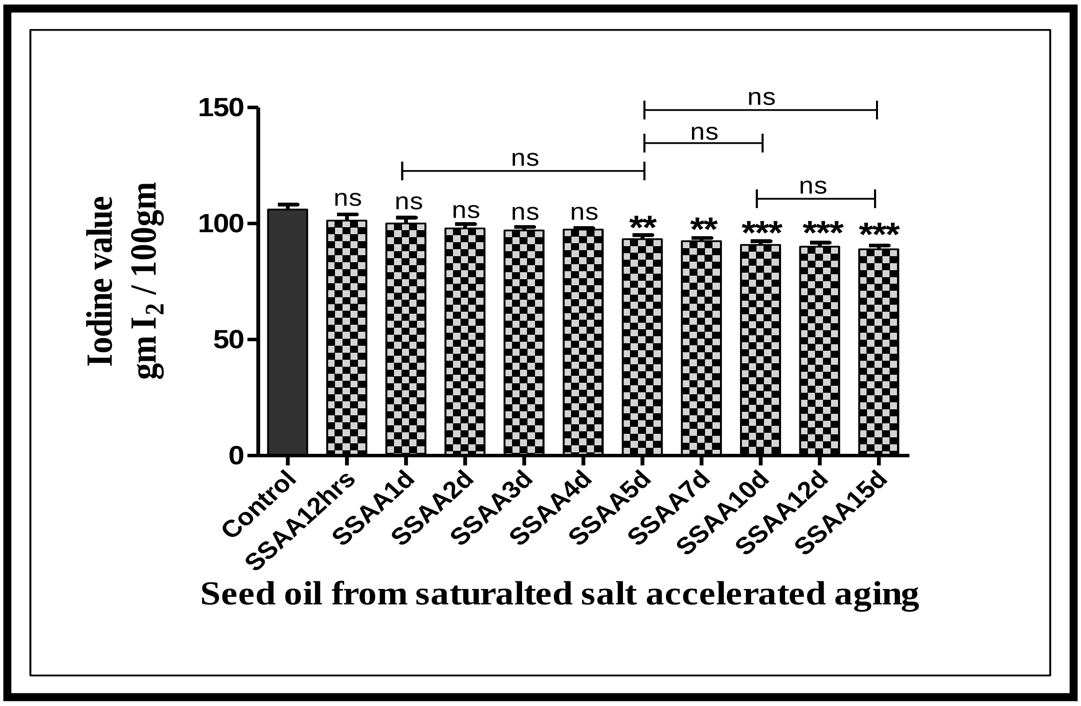

Figure 6.15: Iodine value found in *J. curcas* oil extracted from control seeds and seeds of saturated salt accelerated aging of 12hours, 1, 2, 3, 4, 5, 7, 10, 12 and 15 days. Values are mean ± SE; *, **, * indicates significantly different at P<0.05, P<0.01 and P<0.001 as compared to the control, SSAA1d, SSAA5d and SSAA10d, n=3.**

Seeds that were exposed to 1day of AA and 2 days of SSAA resulted in having iodine vale same as NA6m. Similarly seeds that underwent 10 days of AA and SSAA also have the iodine vale same as NA24m (Table 6.6).

Table 6.6: Comparative analysis of iodine value determined in *J. curcas* oil extracted from seeds of natural aging, 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	--------------------	--------------------
NA 24m = 90.2 ± 2.07	AA10d = 90.1 ± 3.74 (ns)	SSAA10d = 90.8 ± 2.82 (ns)

ns – Non-significant compared to NA6m and NA24m.

6.2.6. Estimation of fatty acid methyl esters in *J. curcas* oil

Fatty acids methyl esters were estimated as per the method prescribed by Kates, (1986) (chapter 2). In this present study, marked significant changes were found in oleic acid and palmitic acid methyl esters (Tables 6.7 and 6.8) but stearic acid and palmitoleic acid methyl esters (Tables 6.9 and 6.10) remained unchanged during 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 *J. curcas* oil. As the natural aging was prolonged the oleic acid methyl esters content was significantly reduced in NA18m, NA21m and NA24m compared to control (Table 6.7). A similar kind of significant decrease was also found from AA7d to AA15d and from SSAA10d to SSAA15d compared to control (Table 6.7). On the other hand palmitic acid methyl esters which is a saturated fatty acids was significantly increased from NA18m to NA24m, AA7d to AA15d and SSAA10d to SSAA15d compared to control (Table 6.8).

Table 6.7: Oleic acid content found in *Jatropha curcas* L. seed oil of natural aging, accelerated aging and saturated salt accelerated aging.

OLEIC ACID CONTENT IN %						
Control	**Natural aging Mean ± SD**	**Accelerated aging Mean ± SD**		**Saturated salt accelerated aging Mean ± SD**		
43.4 + 1.14	NA1m - 42.7 ± 0.501 (ns)	AA12h	- 41.9 ± 0.5969 (ns)	SSAA12h	- 41.8 ± 0.816 (ns)	
	NA3m - 43.4 ± 1.12 (ns)	AA1d	- 41.9 ± 0.335 (ns)	SSAA1d	- 41.4 ± 0.563 (ns)	
	NA6m - 42.8 ± 0.414 (ns)	AA2d	- 41.8 ± 0.286 (ns)	SSAA2d	- 41.2 ± 1.06 (ns)	
	NA9m - 42.7 ± 1.4 (ns)	AA3d	- 41.7 ± 0.512 (ns)	SSAA3d	- 41.4 ± 1.11 (ns)	
	NA12m - 42.1 ± 0.835 (ns)	AA4d	- 41.5 ± 0.695 (ns)	SSAA4d	- 41.1 ± 0.509 (ns)	
	NA15m - 42.3 ± 0.915 (ns)	AA5d	- 41.6 ± 0.482 (ns)	SSAA5d	- 41.1 ± 0.824 (ns)	
	NA18m - 41.21 ± 0.798 *	AA7d	- 40.4 ± 0.447 *	SSAA7d	- 41.0 ± 0.447 (ns)	
	NA21m - 41.16 ± 0.838 *	AA10d	- 40.2 ± 0.684 *	SSAA10d	- 40.4 ± 0.646 *	
	NA24m - 41.0 ± 0.509 *	AA12d	- 40.3 ± 0.646 *	SSAA12d	- 40.2 ± 0.649 *	
		AA15d	- 39.8 ± 2.3 **	SSAA15d	- 40.1 ± 0.494 *	

*** and ** and *** indicates significantly different compared to control. ns – Non-significant, n=3.**

Table 6.8: Palmitic acid content found in *Jatropha curcas* L. seed oil of natural aging, accelerated aging and saturated salt accelerated aging.

PALMITIC ACID CONTENT IN %			
Control	**Natural aging Mean ± SD**	**Accelerated aging Mean ± SD**	**Saturated salt accelerated aging Mean ± SD**
14.1 ± 0.526	NA1m - 14.5 ± 0.734 (ns)	AA12h - 14.9 ± 0.300 (ns)	SSAA12h - 14.6 ± 0.594 (ns)
	NA3m - 14.8 ± 0.105 (ns)	AA1d - 15.0 ± 0.122 (ns)	SSAA1d - 14.6 ± 0.531 (ns)
	NA6m - 14.7 ± 0.055 (ns)	AA2d - 14.9 ± 0.181 (ns)	SSAA2d - 14.1 ± 0.933 (ns)
	NA9m - 14.9 ± 0.125 (ns)	AA3d - 14.6 ± 0.355 (ns)	SSAA3d - 15.0 ± 0.297 (ns)
	NA12m - 14.9 ± 0.172 (ns)	AA4d - 14.8 ± 0.146 (ns)	SSAA4d - 15.0 ± 0.266 (ns)
	NA15m - 14.6 ± 0.336 (ns)	AA5d - 14.8 ± 1.020 (ns)	SSAA5d - 15.1 ± 0.130 (ns)
	NA18m - 15.2 ± 0.296 *	AA7d - 15.2 ± 0.504 *	SSAA7d - 15.1 ± 0.085 (ns)
	NA21m - 15.2 ± 0.121 *	AA10d - 15.2 ± 0.260 *	SSAA10d - 15.4 ± 0.144 *
	NA24m - 15.1 ± 0.144 *	AA12d - 15.3 ± 0.160 *	SSAA12d - 15.4 ± 0.486 *
		AA15d - 15.5 ± 0.495 **	SSAA15d - 15.5 ± 0.361 **

Table 6.9: Stearic acid content found in *Jatropha curcas* L. seed oil of natural aging, accelerated aging and saturated salt accelerated aging.

STEARIC ACID CONTENT IN %			
Control	**Natural aging Mean ± SD**	**Accelerated aging Mean ± SD**	**Saturated salt accelerated aging Mean ± SD**
6.96 ± 0.0451	NA1m - 6.8 ± 0.586 (ns)	AA12h - 7.24 ± 0.101 (ns)	SSAA12h - 7.35 ± 0.489 (ns)
	NA3m - 6.8 ± 0.295 (ns)	AA1d - 6.88 ± 0.101 (ns)	SSAA1d - 6.78 ± 0.276 (ns)
	NA6m - 6.91 ± 0.015 (ns)	AA2d - 6.87 ± 0.060 (ns)	SSAA2d - 6.84 ± 0.276 (ns)
	NA9m - 6.72 ± 0.330 (ns)	AA3d - 6.94 ± 0.040 (ns)	SSAA3d - 6.90 ± 0.126 (ns)
	NA12m - 7.32 ± 0.711 (ns)	AA4d - 7.03 ± 0.384 (ns)	SSAA4d - 6.95 ± 0.151 (ns)
	NA15m - 7.9 ± 0.746 (ns)	AA5d - 6.89 ± 0.114 (ns)	SSAA5d - 6.97 ± 0.137 (ns)
	NA18m - 7.52 ± 0.360 (ns)	AA7d - 6.72 ± 0.295 (ns)	SSAA7d - 6. 69 ± 0.275 (ns)
	NA21m - 7.3 ± 0.549 (ns)	AA10d - 6.98 ± 0.085 (ns)	SSAA10d - 6.98 ± 0.155 (ns)
	NA24m - 8.22 ± 0.959 (ns)	AA12d - 6.91 ± 0.120 (ns)	SSAA12d - 7.07 ± 0.202 (ns)
		AA15d - 6.86 ± 0.208 (ns)	SSAA15d - 6.80 ± 0.704 (ns)

Table 6.10: Palmitoleic acid content found in *Jatropha curcas* L. seed oil of natural aging, accelerated aging and saturated salt accelerated aging.

PALMITOLEIC ACID CONTENT IN %			
Control	**Natural aging Mean ± SD**	**Accelerated aging Mean ± SD**	**Saturated salt accelerated aging Mean ± SD**
0.907 ± 0.046	NA1m - 0.823 ± 0.056 (ns)	AA12h - 0.890 ± 0.010 (ns)	SSAA12h - 0.887 ± 0.005 (ns)
	NA3m - 0.910 ± 0.200 (ns)	AA1d - 0.880 ± 0.200 (ns)	SSAA1d - 0.907 ± 0.026 (ns)
	NA6m - 0.910 ± 0.017 (ns)	AA2d - 0.903 ± 0.030 (ns)	SSAA2d - 0.887 ± 0.051 (ns)
	NA9m - 0.867 ± 0.032 (ns)	AA3d - 0.913 ± 0.015 (ns)	SSAA3d - 0.870 ± 0.050 (ns)
	NA12m - 0.863 ± 0.055 (ns)	AA4d - 0.900 ± 0.010 (ns)	SSAA4d - 0.883 ± 0.040 (ns)
	NA15m - 0.927 ± 0.015 (ns)	AA5d - 0.903 ± 0.023 (ns)	SSAA5d - 0.877 ± 0.030 (ns)
	NA18m - 0.970 ± 0.062 (ns)	AA7d - 0.910 ± 0.036 (ns)	SSAA7d - 0.873 ± 0.040 (ns)
	NA21m - 0.977 ± 0.025 (ns)	AA10d - 0.890 ± 0.030 (ns)	SSAA10d - 0.890 ± 0.041 (ns)
	NA24m - 0.997 ± 0.011 (ns)	AA12d - 0.917 ± 0.015 (ns)	SSAA12d - 0.867 ± 0.064 (ns)
		AA15d - 0.923 ± 0.023 (ns)	SSAA15d - 0.913 ± 0.032 (ns)

*** and ** and *** indicated significantly different compared to control. ns – Non-significant, n=3.**

6.3. DISCUSSION

6.3.1. Extraction of oil from *J. curcas* seeds.

The commercial success of the cultivation of *Jatropha curcas* L. depends mainly upon the oil yield. The results observed in this study are in conformity with reports published in oil seeds. Sisman and Delibas, (2004) showed that a three month storage of sunflower seeds resulted in a decrease in the oil content significantly. Gupta and Rao, (2008) also observed reduction in oil content when *Jatropha* seeds were stored at ambient temperature for a short storage period of 80 days. Similar observations were made by Savi´c et al., (2009) with stored rapeseed for 9 months at 10˚C and Harhar et al., (2010) with argon kernels. Fotouo-M et al., (2016) observed that when *Moringa oleifera* L. seeds were stored for 24 months it lead to a significant decrease of oil content within the seeds.

According to Sathya et al., (2006) hydrolysis and oxidation plays a significant negative role by reducing oil content in the stored oil seeds. 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 *J. curcas* 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.

6.3.2. Estimation of free fatty acid content (FFA) in *J. curcas* oil.

The acid value (AV) of any natural oil is determined by the degree of unsaturation and free fatty acids (Ajayebi et al., 2013). Oil/oil seeds 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) it 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; Sahoo and Das, 2009). 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 to have 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 that the virginity is unaffected by lipid peroxidation and rancidity. Such oil is characterized by long chain fatty acids with the right amount of monounsaturated fatty acids. Selection of feed stock with FFA below 1% would also cut down cost on catalyst and solvents to be used for additional stages of transesterification if the oil contains FFA above 1% (Karmakar et al., 2010).

Findings of this study reveals that seeds exposed to 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 *J. curcas* seeds to withstand high temperature and humidity in storage condition in spite of being oleaginous in nature. In the case of 10 days, 12 days and 15 days of accelerated aging and 12 days 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%. A level which will be achieved much beyond 24 months of storage at natural condition. There is a formation of gum and sludge when oil with high FFA undergoes oxidation (Savi´c et al., 2009; Karmakar et al., 2010). The high acid value can damage the engine parts (Verma et al., 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 *J. curcas* seeds as best feedstock that can withstand extreme conditions of temperature and moisture in tropical regions like India. The present study 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 formation of reactive oxygen species (ROS) due to temperature. Rape seeds when they were stored for 9 months at 10°C level of FFA content was increased (Savi´c et al., 2009). Gupta and Rao, (2008) reported that an increase of FFA is evident in *J. curcas* seeds stored for 72 days at ambient temperature. Such sudden rise in FFA in stored oil seeds is due to lipases and other enzymes that act upon triglycerides to convert them into free fatty acids, diglycerides and monoglycerides through the process of hydrolysis (Gulla and Waghray, 2011). These released free fatty acids further undergo oxidation to cause rancidity (Lin et al., 2010). The results of this study is also in agreement with the earlier reports done on *Moringa oleifera* L. Seeds. *Moringa oleifera*

L. seeds kept for 24 months of storage resulted in increase of FFA from 12 months of storage onwards (Fotouo-M., 2016).

6.3.3. Determination of saponification value (SV) in *J. curcas* oil

The high proportion of lower fatty acids in any oil is indicated by a higher saponification value for saponification value is 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. *J. curcas* 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).

6.3.4. Determination of peroxide value (PV) in *J. curcas* oil

During 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. Highly reactive free radicals are the intermediary products of lipid peroxidation. These intermediates react with oxygen to form hydroperoxides (Iqbal et al., 2002). Hydroxyperoxides 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 et al., (2007) showed that in order to safe guard any biodiesel from oxidation and to increase its stability its peroxide value must always be kept low. Current 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 biodiesel formation (Yaakob et al., 2014).

6.3.5. Determination of iodine value (IV) in *J. curcas* oil

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). 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). This study showed 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).

6.3.6. Estimation of fatty acids methyl esters (FAME) content in *J. curcas* oil

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). The result of the present study on *J. curcas* seed oil is in conformity with an earlier report (Senou et al ., 2017) on oleic acid methyl ester - a monounsaturated fatty acid as the highest constituent of total fatty acids present in *J. curcas* oil. Decrease in oleic acid content during prolonged storage and artificial aging in this present study indicates a possible oxidation of the double bonds present in oleic acids. Reduction of oleic acid can also be understood in line with the production of free fatty acids and conversion to other types of fatty acids. Prolonged storage and extreme temperature and humidity during artificial aging could possibly cleave oleic acids from their respective triacylglycerol moiety through hydrolysis, producing the free forms of oleic acids and these free forms of oleic acids eventually undergo oxidation leading to either MDA formation or other aldehydes or ketones and eventually to rancidity (Ghasemnezhad and Honermeier, 2007). On the other hand, significant increase in palmitic acid content can be attributed to the hydrolysis of fatty acids during prolonged storage period, accelerated aging and saturated salt accelerated aging. Ghasemnezhad and Honermeier, (2007) concluded that increase of palmitic acid can also come from the conversion of oleic acids to saturated fatty acids like palmitic acids in sunflower seeds during storage. Changes in fatty acid profile were observed in olive oil as well due to unfavourable storage condition (Morello et al., 2004). Lin and Lee, (2010) investigated the oxidative stability of biodiesel extracted from fish oil and concluded that a significant decrease in the methyl esters content and greater degradation rate of same occur in a high temperature prevailing condition. The current study reveals that seeds of natural aging up to 15 months, accelerated aging up to 5 days and saturated salt accelerated aging up to 7 days contain oil with unaltered fatty acid~~s~~ profile indicating the greater oxidation stability present within seed oil. Increased FFA and PV found in seeds of natural aging extended beyond the point 15 months, 5 days of accelerated aging and 7 days of saturated salt accelerated aging further consolidates findings of the present study that prolonged storage brings in negative deteriorative effects on fatty acids of *J. curcas* seed oil.