Synopsis of the thesis on

Biochemical Changes in *Jatropha Curcas* Seeds on Storage.

Submitted to

The Maharaja Sayajirao University of Baroda

The Department of Biochemistry,
The Maharaja Sayajirao University of Baroda

For the degree of

Ph. D. (Doctor of Philosophy) in Biochemistry

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December**, 2017**

The rapid depletion of petroleum reserves, the fast dwindling sources of fossil fuel and the deleterious impact of such fossil fuel on the environment in terms of huge exhaust emission necessisate an hunt for alternative energy resources. Any alternative in this regard should be renewable, biodegradable, non-toxic and eco friendly so that no further damage is caused to the environment. Among all the varied sources of alternatives, 'Biodiesel' has attracted extensive attention as renewable, biodegradable and non-toxic fuel since the past decade (Stavarache *et al.,* 2007; Sarin *et al.,* 2007; Tiwari *et al.*, 2007). Various edible oils such as sunflower seed oil, soyabean oil, palm oil, palm kernel oil have been subjected to research for the viability of being used as feedstock for biodiesel. India being a developing nation, these edible oils produced is not even sufficient enough to be used for the purpose of food. Hence this compels us to look for an alternative system. Therefore inedible oil emerges out to be the best alternative source for biodiesel. Among the many inedible oils available, *Jatropha curcas* stands out to be the best feed stock for biodiesel production with its remarkable advantages (Veljkovic *et al.*, 2006).

Jatropha curcas (physic nut) of the Euphorbiaceae family, known to be an oil-rich, drought resistant shrub originated in central America and has become naturalized in many tropical and subtropical areas of the world. *Jatropha curcas* oil mainly consists of fatty acids such as palmitic acid (13%), stearic acid (2.53%), oleic acid (48.8%) and linoleic acid (34.6%) (Martínez-Herrera *et al.*, 2006). High percentage of unsaturated fatty acid seen in *Jatropha curcas* qualifies it to be feed stock for biodiesel.

Lipids are stored as oilbodies, which routinely consists of 94-98% lipids, 0.5 to 2% phospholipids and 0.5 to 3.5% proteins (Chen *et al*., 2004). Among these proteins, 80-90% belong to the class of proteins known as oleosins which covers the entire surface of the oil body and dictate its size and keep the oilbodies isolated from each other (Capuano *et al.,* 2007).

Due to its high fuel rate and great oxidation stability, J. *curcas* oil is more suitable for fuel purpose as compared with other vegetable oils and to be chosen as the best alternative for biodiesel (Sarin *et al.,* 2007).

Therefore the seeds of *Jatropha curcas* are of great importance. Thus quality of the post harvested seeds and its oil content and quality needs to be taken into consideration before being subjected to pressing. Therefore any study towards seed deterioration of *Jatropha curcas* would not only ensure better yield of oil more suited for biodiesel but also will help to maintain the seed quality.

Therefore ensuring crop quality and better yield, the information regarding seed behaviour and its deterioration during storage is of great significant. The purposes of seed storage are varied, starting from up keeping and sustaining the seed genetic resources at seed banks to distribution of quality seeds for species that are both regularly and irregularly used in plant and crops production. Seed storage is also a big boon to the entire seed trade (Santos and Paula, 2007). Seed storage becomes a greater challenge today in order to maintain the quality of the seeds. Even though optimal environment is created still seed deterioration is inevitable and this becomes a greater hurdle in the field of agriculture and germplasam conservation (Ying Li *et al.,* 2017). Seed ageing refers to deterioration which is irreversible, degenerative and unstoppable process leading to loss of seed quality, viability, vigour and eventually leading to seed death. Those that with stand deterioration to certain extend may end up in reduced germination and emergence of weak seedling. The deterioration of oil-rich seeds decreases not only the oil content but also reduces drastically the quality of the oil as well. Storing oilseeds is more difficult than storing cereal grains as they are more susceptible to quality deterioration. Storage of oilseeds requires a planned approach, careful management and a suitable storage system. For seeds like Jatropha, whose economic potential depends on its oil quality, it is important to know the changes that happen during the course of its storage.

High moisture content and high temperature during storage are the prime causes of seed deterioration (Kibinza *et al.,* 2006). The stored lipid within the seed gets deteriorated in three different ways: 1. Oxidation due to high temperature and moisture content, 2. Hydrolysis, where fat is degraded into fatty acids, 3. Contamination due to contaminants (Abdellah and Ishag, 2012). The lipid peroxidation is widely reported as one of the main causes of deterioration of seeds. Compared to starchy seeds, the oleaginous seeds, such as physic nut, are more challenging to store for a longer period. These seeds are rich in lipids and lipids are less stable molecules than starch (McDonald, 1999). Lipid peroxidation in lipid rich seeds is a progressive process involving a sequence of events such as: reduction of biosynthesis activity, changes on the membrane systems, reduction on germination speed and loss of seed viability (Delouche and Baskin, 1973). Lipid peroxidation at cellular level may lead to faulty protein synthesis, impairment of RNA and DNA degradation, loss of membrane integrity and reduced energy metabolism etc (Kibinza *et al*., 2006).

A major initiator of these deleterious changes is the group of molecules popularly known as the reactive oxygen species (ROS). Antioxidant system comprised of both enzmatic antioxidants and non enzymatic antioxidants. By the way of scavenging these free radicals are supposed to maintain a balance between ROS production and antioxidant system. Among the enzymatic antioxidant system there are number of enzymes that eleminates the excess amount of ROS. Bailly (2004) showed that Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX) and Glutathione reductase (GR) are among the main antioxidant enzymes in plants. Less harmful free radical H_2O_2 is formed from a more harmful free radical superoxide (O_2^-) by the catalytic action of SOD (Kliebenstein *et al.,* 1998). This less harmful free radical H_2O_2 formed is further eliminated by the action of CAT and APX. H_2O_2 is converted to water and oxygen by CAT (Willekens *et al.,* 1995). Scavenging H_2O_2 may also take place through the ascorbate–glutathione cycle that involves enzymes such as APX and GR. Using ascorbate as a reducing power APX reduces H_2O_2 to water, generating monodehydroascorbate and/or dehydroascorbate (Noctor and Foyer, 1998). This increased level of ROS triggers the carbonylation of proteins (Rajjou et al., 2008), which eventually gets degraded resulting in the loss of functionality. The uncontrolled production and accumulation of ROS during seed aging causes an oxidative stress in oil seeds. This oxidative stress manifests itself in diverged deleterious effects such as lipid peroxidation, break down of antioxidant defense system, loss of cell integrity and eventually seed deterioration (Bailly, 2004). The mechanism behind these biochemical changes that occur during storage can be understood by determining the physiological and biochemical changes that occur in seeds.

The knowledge of the behavior of the seeds during storage is important in trying to increase their longevity and to avoid significant losses in their physiological quality. Systematic and detailed studies with respect to biochemical changes that occur in *Jatropha curcas* seeds during storage have not been reported so far. Studies on effects of storage of *Jatropha curcas* seeds on lipids, electrolyte leakage, oil content and free radical scavenging potentiality of the antioxidant systems will affirm the biochemical changes and deterioration that take place during storage. Also estimating the acivity of the antioxidant enzymes will give us the knoweldge about the seed viability and quality. Besides studying the oil content of the seed, microscopic studies of the oil bodies will reveal the direct effect of seed deterioration more vividly. Extraction and estimation of the novel ampipathic protein oleosin and isolation of genetic DNA will not only further enhance the knowedge of the storage effect in Jatropha curcas but will give way to desgin newer methods/models to maintain the seeds and seed oil quality. In order to study the effect of ageing on seed, seeds need to be stored for a longer period of time. To overcome this problem Accelerated ageing & Saturated salt accelerated ageing techniques have been developed as alternative methods where seeds are exposed to higher temperature, humidity and saturated salt in order to accelerate the aging of seeds artificially (Powell and Mathews, 1984 & Mendonca et al., 2008). Hence, the present study aims to investigate the physiological and biochemical changes in *Jatropha curcas* seeds on storage.

The other benefits of study are:-

- Will help to identify time points of seed deterioration
- Will help to identify modifiable targets to increase shelf life of seeds
- helps to prevent loss and maintain high yield of best quality oil from *Jatropha curcas* for the production of Biodiesel.

Objectives of the study:

1. **To study the Biochemical changes in *Jatropha curcas* seeds with storage:**
2. **To study the physiological changes in *Jatropha curcas* seeds with storage on:**
3. **To study the effect of storage on quantity & quality of *Jatropha curcas* oil**
4. **To study the changes in the activity of antioxidant enzymes with storage.**
5. **To study the effect of storage on Non – enzymatic antioxidants.**
6. **To study the effect of storage on size and structure of oil bodies and the oil body protein–Oleosin in *Jatropha curcas* seeds.**
7. **To study the effect of storage on the integrity of cell and cell death.**

Grouping of seeds:

1. ***Control seeds(CS) –*** freshly harvested seeds.
2. ***Normal aging (NA):*** **-** Seeds were stored in normal condition from 1 months to 24 months.
3. ***Accelerated Aging (AA):*** – Seeds were exposed to controlled high temperature and moisture to speed up the ageing process artificially (from 12hrs to 15 days).

4. ***<u>Saturated salt Accelerated aging (SSAA):</u>*** – Seeds were exposed to controlled temperature and saturated salt to speed up the ageing process artificially. (from 12hrs to 15 days).

<u>Results:-</u>

1. **To study the <u>Biochemical changes</u> in *Jatropha curcas* seeds with storage on:**

1.1. MDA estimation: - Significant increase in MDA level from 3rd month of natural aging onwards was seen. In case of AA & SSAA, significant increase was seen from 12hours onwards. MDA content in seeds after 1day of AA & 2days of SSAA is same as in seeds kept for 6 months of natural aging. MDA content in seed after 5 days of AA and 7 days of SSAA is same as value for MDA content in seed kept for 12 months under natural aging. The significant increase in MDA level indicates the onset of Lipid peroxidation.

1.2. Electrical conductivity: - Significant increase in electrolyte leakage was seen in NA6m onwards and in all the groups of AA & SSAA. This indicates the membrane damage in deteriorated seeds.

1.3. DPPH radical scavenging activity test: - In case of NA, the significant increase in DPPH free radical scavenging capacity was observed from 12th month of NA onwards. In both AA and SSAA there was a significant decrease in DPPH scavenging activity seen from 12th day of AA and SSAA compared to control. DPPH scavenging capacity found in AA1d & SSAA1d seeds is same as value in seeds kept for 6 months under natural aging. DPPH scavenging capacity found in AA5d & SSAA7d seeds is same as value in seeds kept for 12 months under natural aging. **Significant increase in naturally aged seeds indicates the ability of the seed to scavenge the free radicals indicating the active antioxidant systems. Significant decrease in treated seeds indicates the inability of the seed to scavenge the free radicals denoting the seed deterioration.**

2. **To study the <u>physiological changes</u> in *Jatropha curcas* seeds with storage on:**

Germination percentage and radicle length: - In our present study, the significant increase in germination percentage is seen in NA3m, NA6m suggests the complete breakdown of dormancy and more of seed vigour. Germinating capacity gets decreased in 9th month of NA compared to NA6m. From 12th month of NA onwards there is no germination seen. In AA germination is seen in 12 hr of AA and in 1d of AA but it is non-significant compared to control. But significant decrease in germination is seen in AA2d and AA3d. But germination gets diminished there after. Significant increase in radical length is seen up to 6m of NA. Significant decrease in radical length is seen up to AA3d and SSAA3 compared to NA3m. **Significant increase in germination denotes seed vigour and viability. Significant decrease and no germination denote the low quality seed and deteriorated seeds.**

3. **To study the effect of storage on quantity & quality of *JC* oil**

3.1. Quantity of Oil: - There is a significant decrease in oil content from 12th month of NA onwards & 1day of AA & 7days of SSAA onwards. Significant decrease in oil content found in seeds after 1day of AA and SSAA is same as value in seeds kept for 6 months of natural aging. Significant decrease in oil content found in seeds after 4 days of AA and 5 days of SSAA is same as value in seeds kept for 12 months of natural aging.

3.2. Quality of the oil:-

3.2.1. Free Fatty acid level: - Free fatty acid content gets increased in 9^{th} month of NA onwards as well from 3days of AA & SSAA onwards. This indicates a possible hydrolysis of TAG. Significant increase in free fatty acid found in seed after 1 day of AA and 2days of SSAA is same as value in seed kept for 6 months under natural aging. Significant increase in free fatty acid found in seed after 4 day of AA and 5 days of SSAA is same as value in seed kept for 12 months under natural aging. **Significant increase in free fatty acid indicates a possible hydrolysis of TAG or oxidation.**

3.2.2. Iodine value: - Decrease in iodine value is seen from 12^{th} month of NA onwards and 5days of AA & 3days of SSAA onwards. Significant decrease in Iodine Value found in seed after 1 day of AA and SSAA is same as value in seeds kept for 6 months under natural aging. Significant decrease in Iodine Value found in seed after 4 days of AA and 5 days of SSAA is same as value in seed kept for 12 months under natural aging. **Decreased IV indicates high level of saturation of fatty acids occurring in aged seeds due to seed deterioration.**

3.2.3. Peroxide Value: - There is increase in peroxide value in the 15^{th} month of NA onwards & in 5days of AA & 7days of SSAA. Significant increase in peroxide Value found in seed after 1 day of AA and SSAA is same as value in seed kept for 6 months under natural aging. Significant increase in peroxide Value found in seed after 4 days of AA and 5days of SSAA is same as value in seed kept for 12 months under natural aging. **Increase in PV indicates occurrence of peroxidation during storage.**

3.2.4. Saponification value: - Significant increase of SV is seen from 15th month of natural aging onwards & AA5d & SSAA5 onwards. Significant increase in saponification value found in seed after 1 day of AA and SSAA is same as value in seed kept for 6 months under natural aging. Significant increase in saponification Value found in seed after 4 days of AA and SSAA is same as value in seed kept for 12 months under natural aging. **Increased SV indicates the higher portion of lower molecular weight of free fatty acids as aging occurs.**

3.3. Fatty acids profile:- Palmitic acid and palmitoleic acid get increased right from 1 month of natural aging and from 12 hrs of AA and SSAA. Oleic acid gets decreased from 12th month of natural aging and from 10days of AA and 12 days of SSAA. Strearic acid remains unchanged.

4. To study the changes in antioxidant enzymes activity with storage.

4.1. Catalase activity (CAT):- In case of NA, the CAT increased activity was observed in 12 months and 15 months of natural aging & from2 days to 5 days of AA & from 1day to 4 days of SSAA. CAT activity found in seed after 1 day of AA and SSAA is same as value in seed kept for 6 months under natural aging. CAT activity found in seed after 5 days of AA and SSAA is same as value in seed kept for 12 months under natural aging. Decrease in CAT activity found in 5days of AA and SSAA onwards compare to 3 days of AA and SSAA indicates the lower capacity of CAT to scavenge the free radicals due to sever seed deterioration.

4.2. Super Oxide dismutase (SOD): - SOD activity gets increased significantly in 9 and 12 months of natural aging & 3rd and 4th day of AA & 2nd and 3rd SSAA. SOD activity found in seed after 1 day of AA & SSAA is same as value in seed kept for 6 months under natural aging. SOD activity found in seed after 5 days of AA & 3days of SSAA is same as value in seed kept for 12 months under natural aging. Decrease in SOD activity found in 5days of AA and 4 days of SSAA onwards compare to 3 days of AA and SSAA indicates the lower capacity of SOD to scavenge the free radicals due to sever seed deterioration

4.3. Peroxidase (POD):- Significant increase in POD activity is seen from 9 months to 18 months of natural aging & in 12 hrs to 5 days of AA & 12hrs to 10d of SSAA. POD activity found in seed after 1 days of AA and SSAA is same as value in seed kept for 6 months under natural aging. POD activity found in seed after 4 days of AA & 3days of SSAA is same as value in seed kept for 12 months under natural aging. Decrease in POD activity found in 5days of AA & SSAA onwards compare to 3 days of AA and SSAA indicates the lower capacity of POD to scavenge the free radicals due to sever seed deterioration.

5. To study the effect of storage on Non – enzymatic antioxidants.

5.1. Ascorbic acid (Vit. C) :- Decrease in Ascorbic acid is found in all the groups of natural aging and as well in all the groups of AA and SSAA. Ascorbic acid level found in seed after 1 days of AA & SSAA is same as value in seed kept for 12 months under natural aging.

5.2. Tocopherol (Vit E):- Work is in progress.

6. To study the effect of storage on size and structure of oil bodies & on the membrane protein–<u>Oleosin</u> in *JC* seeds.

6.1. Size and Structure of oil bodies. (Microscopic studies): - Up to 9 months of natural aging and 2days of AA & SSAA the oil bodies appeared normal and distinct from each other. The initial stage of oil body shrinkage in size seems to occur in 12 months of normal aging and in 12 hrs, 1day & 2days of AA and SSAA. Occurrence of vivid oil body shrinkage in size and appearance of reduced oil body structure compared to control was seen in 3day to 7days of AA and SSAA. Whereas in 10 days to 15days of AA & SSAA there is more prominent occurrence shrinkage of oil bodies in size is observed. Distorted oil body structure and complete reduction of size is also vividly observed. **<u>Work in progress</u>**:- in 15months, 18 months, 21 months and 24 months of natural aging.

6.2. Oleosin Content: - Our results showed that large amount of Oleosins present in naturally aged seeds up to 6m months. This increased content is correlated to the greater stability through distinct, prominent and unaffected oil bodies by microscopic studies. Decrease in Oleosin content in 9 months to 24 months of natural aging can be attributed to MDA increase in these groups where the deleterious effect free radicals reduces the content of Oleosins in these group of seeds. **<u>Work in progress</u>**: - seeds of all the groups of AA & SSAA.

7. To study the effect of storage on the integrity of cell and cell death.

7.1. DNA integrity: - DNA integrity was maintained up to 12 months of natural aging and up to 3 days of AA and SSAA. DNA streaking found in 15 months onwards of NA and 5 days of AA & SSAA seems to suggest a possible loss of integrity due to DNA oxidation and DNA disintegration which is correlated to increase in MDA content in these groups of seeds.

7.2. DAPI & PI staining: - Work <u>is in progress</u>

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