CHAPTER-1
INTRODUCTION

1. INTRODUCTION

1.1. Increasing need of fossil fuel

Energy drives and sustains almost all human activities and is fundamental to human existence and development. It is used in households and other sectors such as agriculture, industry, transport, and services (Sokoto et al., 2013). Almost three fourths of the world's energy demand is met by fossil fuels, which are non-renewable energy sources. Global energy demand is increasing rapidly with the rise in population and modernization. According to the World population census projection, the population will reach 8 billion by 2025. Since the bulk of this growth will be in the developing countries like Brazil, India and China, the energy demand of these countries will also be accelerated (Matthews, 2007; Focacci, 2005). According to the Statistical Review of World Energy - 2017, energy consumption has increased by 129% and 203%, in India and China, respectively, while world-wide energy demand has increased by 41%. Energy shortage has a negative impact on individual life, social life and the economy of the country. Among the various energy sources, the demand for fossil energy is the most.

The demand for and consumption of petroleum products is increasing day by day. According to Petroleum Conservation Research Association, India, diesel consumption alone constitutes around 42 million metric tons (MMT) of the total primary commercial energy consumption of 380 MMT in India. There is a widening and alarming gap between petroleum oil production and consumption (Figure 1.1).

This calls for a serious reflection on the search for an alternative. The world marketed energy consumption had been significantly increasing and still projected to be on further rise in the coming decades (Figure 1.2). Countries like India and China need an exponential increase in global energy consumption in order to sustain their economic growth. A major portion of this energy consumption is from the use of fossil fuels across sectors like industry, transport and domestic.

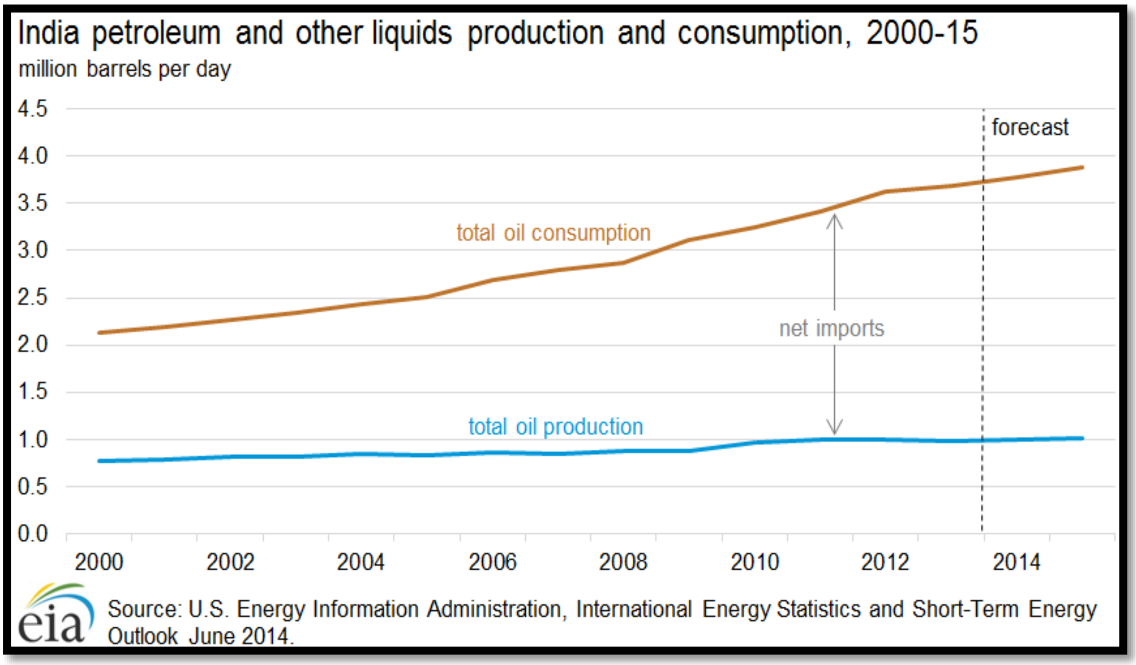


Figure 1.1: Widening gap between petroleum oil production and consumption (2000 – 2015).

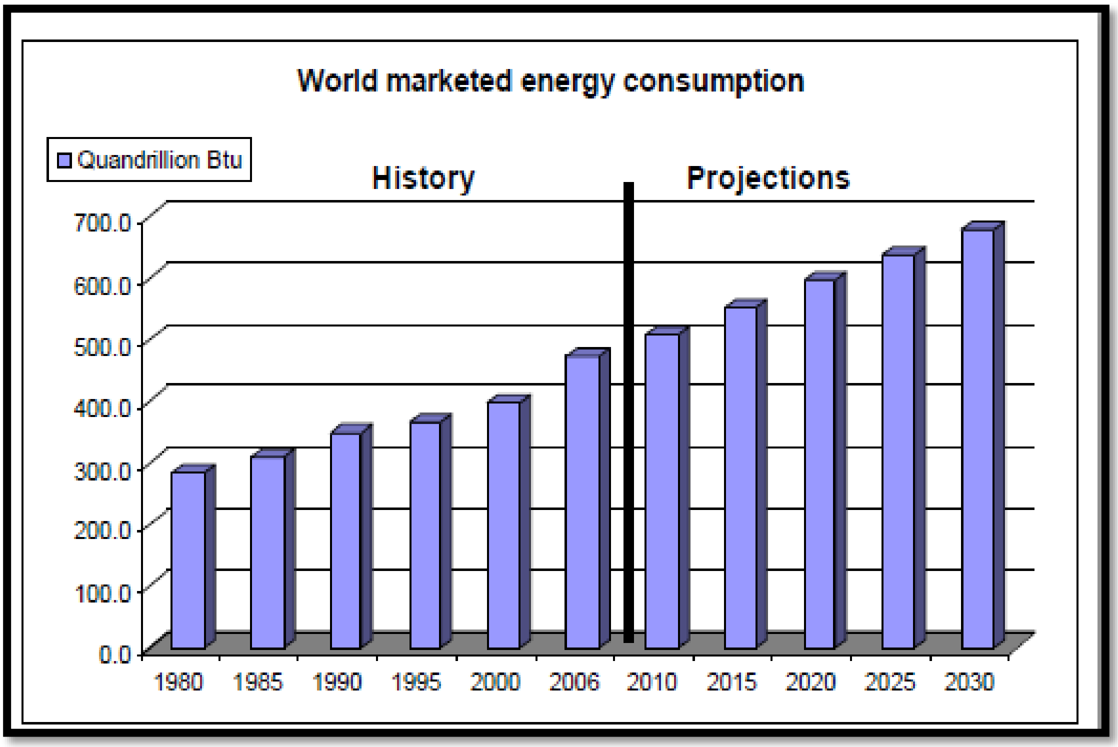


Figure 1.2: World energy consumption (1980-2030) (Adapted from www.eia.doe.gov).

Fossil fuels, which are the major source of energy supply, are a non-renewable and limited resource, and constitute 90% of the world's energy. They include petroleum, natural gas, oil and coal (Sokoto et al., 2013). Since the need for petro-based products exceeds the available source of fossil fuel, India has to depend on the import of crude oil. Only 24% of the total oil demand is met by the local production sector. Increased import of crude oil becomes necessary to meet the hiked demand (US EIA, 2013). The rapid depletion of petroleum reserves, the fast-dwindling sources of fossil fuel and the deleterious impact of fossil fuel use on the environment call for an urgent need for alternative energy resources. Apart from this we also need to look at the flip side of use of fossil fuel.

1.2. Hazards of fossil energy

The accelerated import and use of fossil fuel has only put humanity at risk with its adverse impact on human health and ecology. Excessive use of fossil fuels has caused an alarming increase in the emission of greenhouse gases (GHS), erratic behaviour of the present climate system, global warming and harm to human health.

1.2.1. Carbon emission

Global warming, climate change and various illnesses are the result of an increase of the chief global pollutant CO_2 (Al-Mulali et al., 2015). CO_2 accounts for 75% of the greenhouse gases. This extremely elevated level of CO_2 does not vanish but keeps circulating from one zone to another in the biosphere for thousands of years (Environmental Protection Agency, 2016). At the global level, energy-related CO_2 emission grew alarmingly by 1.4% in 2017, an increase of 460 million tonnes (Mt), attaining a historical high of 32.5 giga tonnes (Gt) (Figure 1.3). This increase is equivalent to CO_2 emissions arising from an additional 170 million automobiles coming into use due to the fast-growing global economy. Lower fuel prices and inadequate energy efficiency efforts across the globe are the additional causes for the increase of automobiles and increased fossil fuel utility (International Energy Agency, 2018).

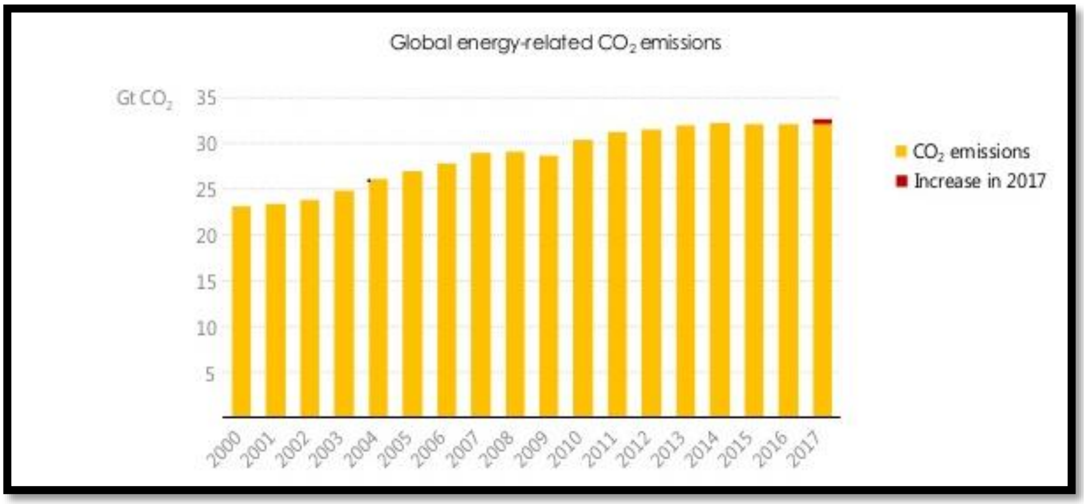


Figure 1.3: Global energy-related CO_2 emissions, 2000-2017 in Global energy and CO_2 status report – 2017 by International Energy Agency, 2018.

Rise in global GDP and energy demand impact and increase the CO_2 emission globally (Figure 1.4). Asian economies contribute towards a two-third increase of CO_2 emission. This contribution is mainly from countries like China and India. The European Union too accounts for a 1.5% growth in carbon emission, adding almost 50 Mt CO_2. The Southeast Asian economies also contribute to this increase of carbon emission (International Energy Agency, 2018). The United States of America stands next to China in the emission of carbon, increasing the global CO_2 level (Union of Concerned Scientists).

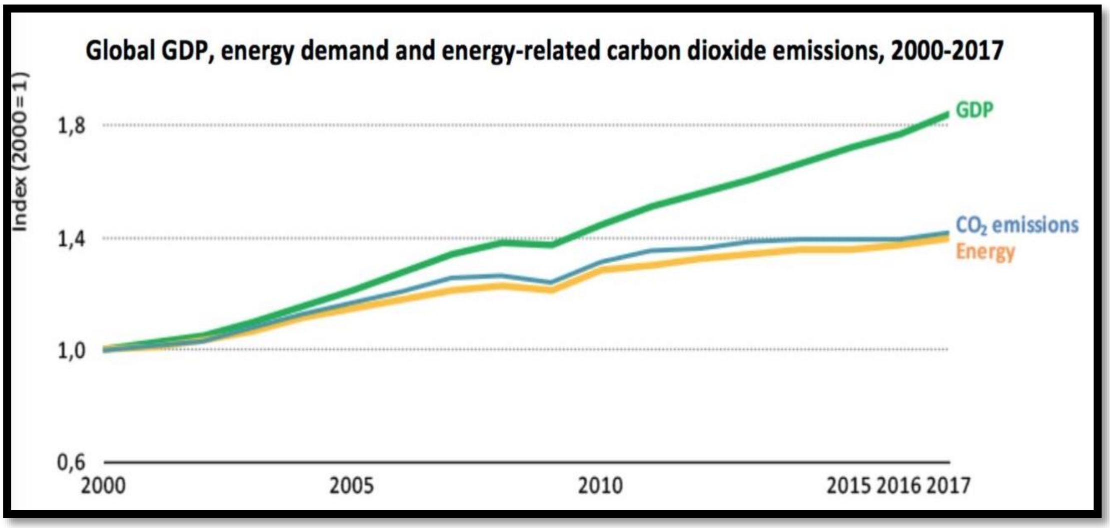


Figure 1.4: Global energy-related CO_2 emissions, 2000-2017 in Global energy and CO_2 status report – 2017 by International Energy Agency, 2018.

International Energy Outlook (IEO) reports that the annual CO_2 emission by developing countries rose by 30% in 2010 compared to developed stable economies. This difference will further increase to 77% by 2030, making the efforts of reducing the carbon emission impossible (Popluechai, 2010). Fossil fuel combustion in industries, for transport, and in power plants accounts for nearly 80% of world-wide anthropogenic CO_2 emission. From the above statistical report, it is evident that growth of global energy-related CO_2 emission is a clear warning for a serious impending ecological crisis.

1.2.2. Increasing temperature of the globe

The excruciating heat experienced even outside the summer days in many parts of the world is a cause of worry. Fossil fuel combustion from various sectors has contributed to global warming. Sun and Hansen (2003) using the data of surface air temperature from 1951 to 2000 have concluded that the temperature of the globe will show an average increase of 0.3°C to 0.4°C in 2050. According to Friedlingstein (2008), the temperature is expected to rise by 6.4°C by the end of the 21st century. The increased level of greenhouse gas emission is the major reason for the increase of temperature in the past fifty years (Barnett et al., 2005). Besides efforts being taken to cut down carbon emission, many other initiatives remain still insufficient to keep the global temperature stabilized well within optimum for life existence. Solutions like conditioning oneself well within the generated cooled air environment only worsen the situation because the heat generated from air conditioners further increases the atmospheric temperature. Hence, sincere efforts must be made to decrease use of fossil fuels so as to decrease CO_2 emission.

1.2.3. Rise of fuel prices

Economic development of a nation depends mainly on transportation of goods across the country. Both export and import relies heavily on the use of fossil fuels. Rapid growth in population has doubled the fossil fuel consumption. Human need for comfort and luxury will cause a huge increase in the number of automobiles in the years to come. Extensive extraction of petroleum products to meet the above need depletes the crude oil in the wells. This ultimately implies a rise in the market value of petro-diesel and the price eventually becomes exorbitant.

The rise in fossil fuel prices is inevitable. The cost of crude petroleum, which is high because of depletion of sources, is further escalated due to political reasons, taxation system and import charges. As transport and other industries heavily rely upon the supply of petroleum products, fuel price rises due to the excessive growth in the automobile and industrial sectors. Such rapid growth brings an imbalance between demand and supply, which obviously makes the price extra ordinarily high. The economic impact of the rise of fossil fuel is felt on the price of other commodities. There has been a six-fold increase in oil prices compared to the last century. During the period of 2000 to 2008, there was a four-fold increase, making the price per barrel jump from $30 to $130. This rise is projected to go up to $200 per barrel by 2030 (Popluechai, 2010).

For a country like ours there is a massive need to import crude oil. The import percentage of crude petroleum products has already increased in China and India. The rise in fuel prices has hence affected India severely (Figure 1.5). In 2018, the prices of petrol and diesel hit an all-time high of ₹ 85 above. Looking at the above scenario, there is an urgent call for energy-efficient management and energy diversification by exploring an appropriate alternative.

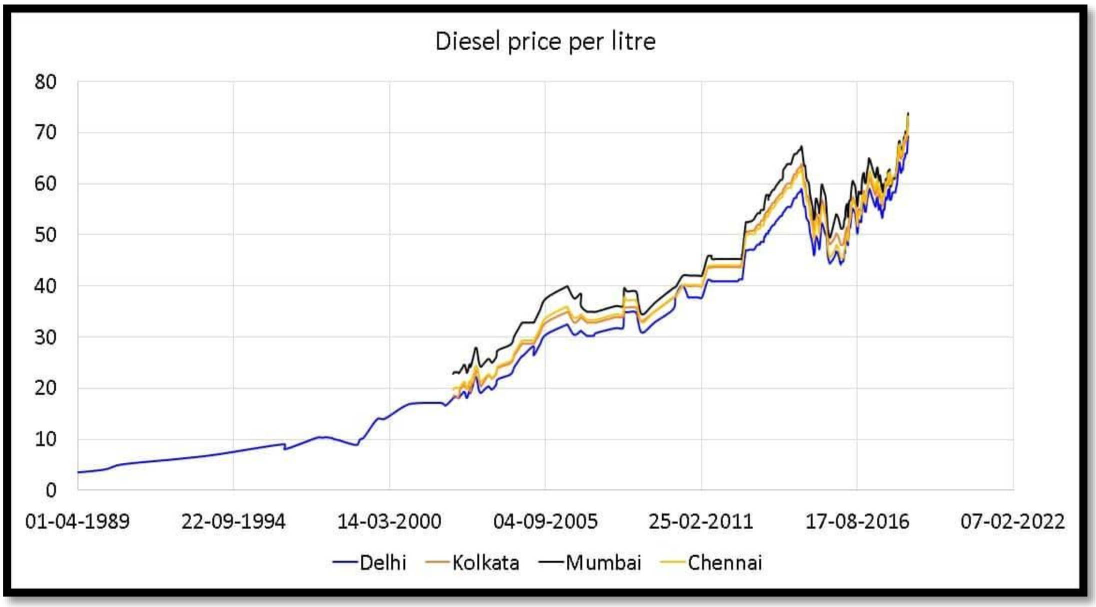


Figure 1.5: Diesel price per litre in metropolitan cities of India. (www.freefincal.com)

1.3. Need for an alternative

Exploiting natural resources to extract petroleum causes damage to and destruction of the earth and its ecosystem. Further, use of fossil fuel use results in emission of huge amounts of hazardous greenhouse gases. To preserve the ecosystem and save life on earth, there is an urgent need to drastically cut down the carbon emission resulting from the combustion of fossil fuels. Various international summits, political and environmental organizations have expressed their concern over the ecological crisis and the impending environmental disaster. Initiatives and investments undertaken by these groups for preserving the ecosystem and its diversity, and reducing carbon emission and global temperature, are steps towards transformation for the enhancement of life both at the macro and micro level. New sources of energy that is renewable, non-toxic and biodegradable are the need of the hour. Projects to develop new technologies for the production of fuel with less carbon emission, and mining of natural gas reserves, will yield renewable energy. Developing nations require a huge amount of energy for their development. Developed nations must invest funds in technologies for the successful production of renewable energy so that preservation of the ecosystem goes hand in hand with development. In this context, ‘Biodiesel’ has drawn a considerable attention as a renewable, biodegradable and non-toxic fuel with less carbon emission (Stavarache et al., 2007).

1.4. Available sources of renewable energy

Most of the renewable energy comes from the Sun. Besides solar energy, wind energy, thermal energy, tidal energy, biomass energy and geothermal energy are viable alternatives.

Solar energy

Solar energy is the largest available source of power that is clean, reliable and safe. The total amount of solar energy available per day is 200 times greater than that used by humans at present (Ashok, 1998).

Biomass energy

This is a renewable energy obtained from biomass and its degradation. Waste material from plants and animals contain solar energy stored in the form of organic matter. This stored energy is released when the chemical bonds in the organic matter break down either due to digestion or decomposition. For example, starch is converted to sugar, which is fermented to extract ethanol, from which biofuel is produced. In the anaerobic digestion of biomass, facultative bacteria digest the biomass to produce methane and carbon dioxide. Methane is another efficient source of renewable energy (Ashok, 1998).

Biofuel from algae

Microalgae are an attractive source of biodiesel (Rawat et al., 2013). Microorganisms like bacteria, fungi, yeast and algae produce oil, and this oil is the best feedstock for biodiesel due to its rich lipid content. Oil accumulated by microalgae contains almost 80% of triglycerides rich in C16 and C18 fatty acids (Meng et al., 2009). An efficient technique for cultivation of and looking for suitable algal strains for rapid growth is the biggest challenge to algal biofuel production (Aliya et al., 2009).

Production of biological hydrogen

Hydrogen gas is another effective alternative fuel and has water as the main by-product. Its high energy content makes it unique and viable. Since it demands high input of energy the commercial production or use becomes inefficient (Ghirardi et al., 1997). Bacteria or algae are used as vectors, as a means to split water and produce hydrogen gas. This process is known as biological hydrogen production (Radmer et al., 1977). Its large-scale commercial production has not been successful.

1.5. Biodiesel – the sustainable and future generation fuel

In the coming two or three decades, the energy demand is projected to grow rapidly. Taking into consideration the environmental damage and high price of fossil fuel, renewable energy should become integral to any energy production and management system. Solar energy, biomass energy, algae energy and hydrogen gas are either unsuccessful or inefficient for large-scale production, or become unfeasible. Thus alternate fuel remains expensive for vehicle use. On the other hand, biodiesel of plant origin does not have this disadvantage, and has drawn world-wide attention as a sustainable fuel for future generations.

1.5.1. Biodiesel of plant origin as the replacement of transport fossil fuel

The total energy consumed by any country in the form of fuel, power, heat and electricity is distributed among transport, industrial, commercial and residential sectors. Among these, the transport sector alone uses 22% of the total energy globally. This eventually accounts for 27% of the global carbon emission (de La Rue du Can and Price, 2008). Therefore, the transport sector becomes the ultimate target for utilization and implementation of biodiesel. By 2030, the energy demand from the transport sector will increase by 2.7% (Popluechai, 2010). Countries like India, China and Brazil where vehicle fitness and carbon emission regulations are not sternly governed would profit by the use of biofuel that is with minimum cost and carbonless. Developed nations may move towards biodiesel as an option for cutting down the carbon emission (de La Rue du Can and Price, 2008).

1.5.2. Biodiesel of plant origin as key to a cleaner ecosystem

Among varied sources of biodiesel, the one from plants becomes the centre of attraction due to the ready and abundant availability of source as feedstock, economic viability in production, feasibility, and no requirement of prior modification or alteration in the engine. Sustainability and oxidative stability of the biodiesel from plant origin are added features.

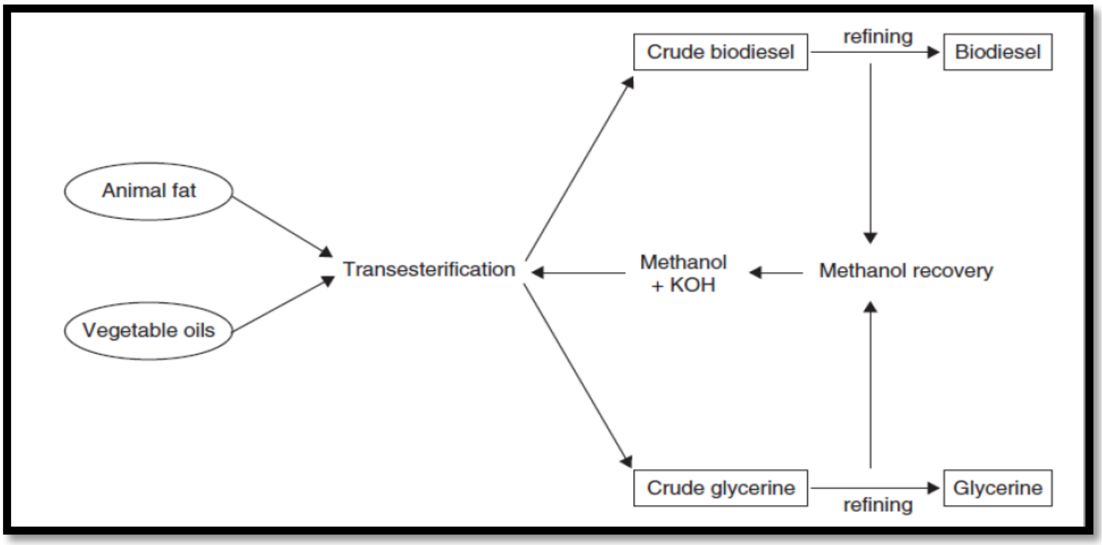


Figure 1.6: Schematic diagram of basic steps in biodiesel preparation (Popluechai, 2010)

The fatty acids from oil rich seeds are esterified to form fatty acid methyl esters. These fatty acid methyl esters (FAMEs) are known as biodiesel. FAMEs are made from either animal fat or vegetable oils through the process of transesterification (Figure 1.6). Glycerol is the by-product which is formed and used in industry for a variety of purposes like soap formation, cosmetics and pharmaceuticals etc. (Figure 1.6).

1.5.3. Advantages of biodiesel

- Most tested as renewable and sustainable.
- Performance is better than petro-diesel.
- Environment friendly by emitting less carbon, and less hazardous to human health.
- Has been successfully blended with mineral diesel up to 20% and used as fuel. This blend has the same calorific value as mineral diesel.
- Compatible with mineral diesel.
- Biodiesel meets all the quality requirements of storage stability, blending and sampling.
- Reduced emission of sulphur, hydrocarbon and other aromatic compounds.
- Combustion leads to less heat emission.
- Non-toxic, renewable and biodegradable.

Biodiesel is a boon to developing nations. It will inherently enhance growth in all sectors. Agricultural and transport sectors are the direct beneficiaries of the use of biodiesel. Since a continuous supply of feedstock is required for the production of biodiesel, investments by agricultural units will yield a profit if biodiesel becomes a mainstream reality. Profit to the agricultural units means profit to the farmers in rural areas. If the source of feedstock is non-edible, then unused land can be utilized more for producing the feedstock. In any nation if farmers and agricultural units make direct profit, development of other sectors like science and technology, pharmacy and industry will happen eventually.

According to a report in The Times of India, August 30, 2018, the former President and renowned scientist Dr. A.P.J. Abdul Kalam had asked Patna University in 2005 to chalk out comprehensive biofuel plant cultivation in the rural areas of Bihar. He had foreseen the need of biofuel to meet the dual purpose of protecting the ecosystem and meeting the demand of fossil fuel. He also stated that effective cultivation of biodiesel plants like *Jatropha curcas* L. in waste and wetlands in a country like India would yield great revenue and provide employment to over 12 million people. The novelty of *Jatropha curcas* L. was actualized when biofuel developed by the Dehradun-based Indian Institute of Petroleum was used in the maiden flight between Dehradun and Delhi on August 27, 2018.

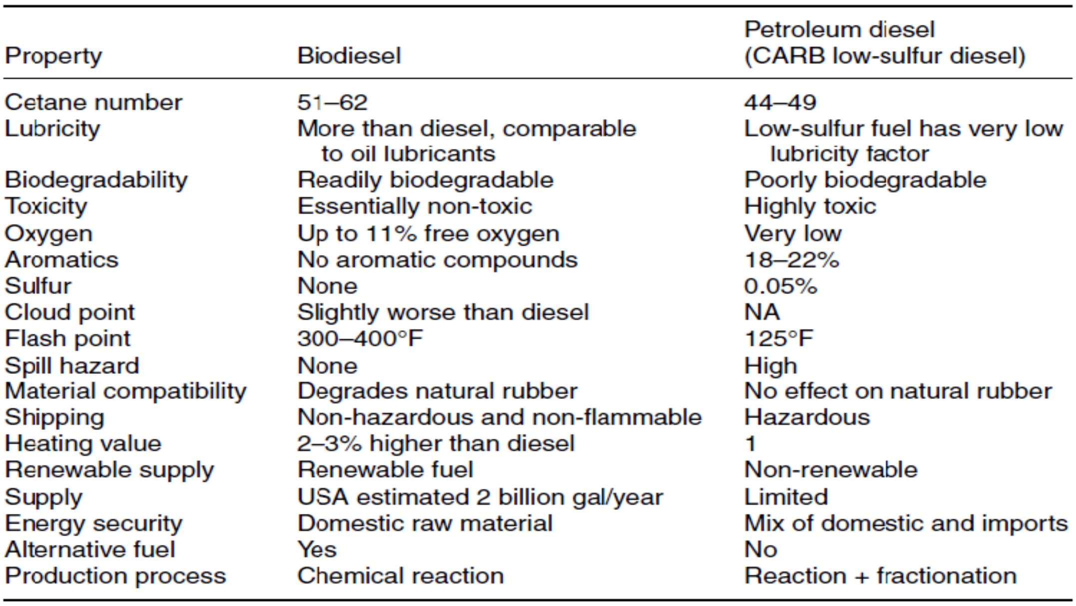
1.5.4. The chemistry of biodiesel

Biodiesel is nothing but fatty acid methyl esters (FAMEs). The extracted oil mainly triglycerides is subjected to pre-treatment to remove contaminants and moisture. The pre-treated oil is then mixed with methanol and a catalyst (sodium hydroxide or potassium hydroxide) to convert all the triglycerides into FAMEs and glycerol. Glycerol is removed and FAMEs is used as biodiesel. During 1800's the technique of transesterification was used to convert triglycerides into glycerine for soap production with FAMEs as a by-product (Popluechai, 2010).

1.5.5. Properties of biodiesel

The properties of biodiesel show that it is not only eco-friendly but also a more efficient fuel than petro-diesel (Table 1.1). Pure biodiesel or 100% biodiesel (B100) gives zero emission of sulphur, sulphate and sulphur dioxide, which are the chief pollutants causing acid rain. B100 also reduces the carcinogen concentration and smog-forming capacity of the air since it results in 67% less unburned hydrocarbons. Compared to petro-diesel there is 48% less CO and 47% less particulate matter (PM) in biodiesel (Popluechai, 2010). The use of B100 increases nitrous oxide emission by 10%, but the potential smog-forming capacity is low. Because there is no SO_2 emission from biodiesel, exhaust gas recirculation (EGR) technology must be introduced in diesel engines to minimise the NO_2 (Agarwal et al., 2006). Biodiesel has a higher cetane value and gives higher ignition quality to the engine. Higher lubricity adds further advantage to the biodiesel (Agarwal et al., 2001). The use of biodiesel in colder regions causes corrosive effects on rubber but in a continent like Asia which is tropical in nature, the corrosive effect becomes irrelevant. Countries like India and Indonesia, where climate change is so drastic and evident, must make biodiesel a preferred option.

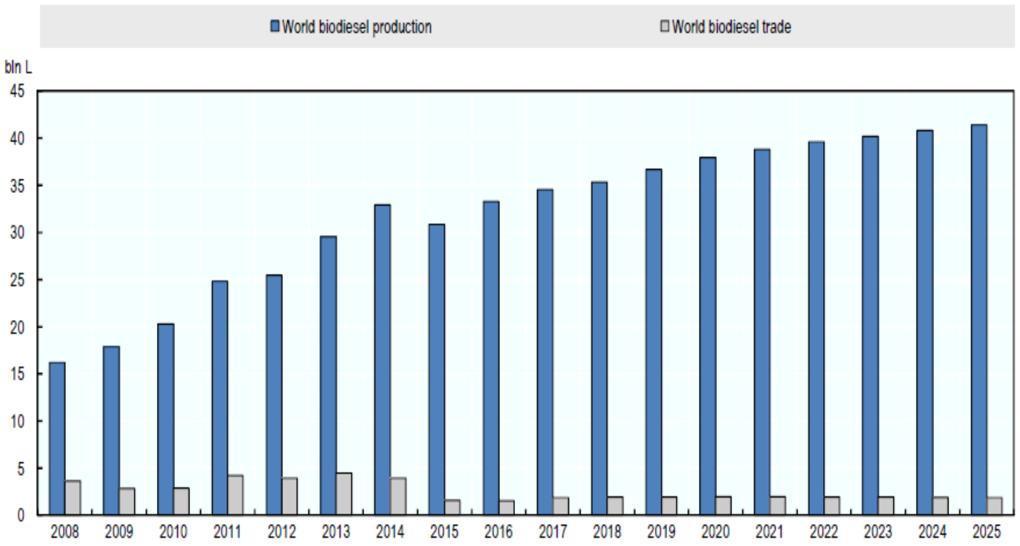
Table 1.1. Comparison of properties between biodiesel and petro-diesel (Agarwal et al., 2001)



Property	Biodiesel	Petroleum diesel (CARB low-sulfur diesel)
Cetane number	51–62	44–49
Lubricity	More than diesel, comparable to oil lubricants	Low-sulfur fuel has very low lubricity factor
Biodegradability	Readily biodegradable	Poorly biodegradable
Toxicity	Essentially non-toxic	Highly toxic
Oxygen	Up to 11% free oxygen	Very low
Aromatics	No aromatic compounds	18–22%
Sulfur	None	0.05%
Cloud point	Slightly worse than diesel	NA
Flash point	300–400°F	125°F
Spill hazard	None	High
Material compatibility	Degrades natural rubber	No effect on natural rubber
Shipping	Non-hazardous and non-flammable	Hazardous
Heating value	2–3% higher than diesel	1
Renewable supply	Renewable fuel	Non-renewable
Supply	USA estimated 2 billion gal/year	Limited
Energy security	Domestic raw material	Mix of domestic and imports
Alternative fuel	Yes	No
Production process	Chemical reaction	Reaction + fractionation

1.5.6. World-wide production of biodiesel

World biodiesel production is steadily increasing. By 2025, world-wide biodiesel production is expected to reach up to 41.4 Bln L (Figure 1.7). The major contributor will be the European Union. The United States, Brazil, Argentina and Indonesia would be other major producers of biodiesel. Brazil would continue to remain in the third position. Argentina would make a drastic cut in the import of fossil fuel and accelerate their biodiesel production to 50% in the coming years. Indonesia’s current installed production capacity of 7 Bln L is sufficient to support the estimated level of 3.7 Bln L by 2025. Malaysia and Thailand would make progress of 0.6 Bln L and 1.2 Bln L by 2025. With government support and other investments from private sectors, India is expected to increase its current biodiesel production to 1.3 Bln L by 2025 (OECD/FAO, 2016).



Source: OECD/FAO (2016), "OECD-FAO Agricultural Outlook", *OECD Agriculture statistics* (database), http://dx.doi.org/10.1787/agr-outl-data-en.

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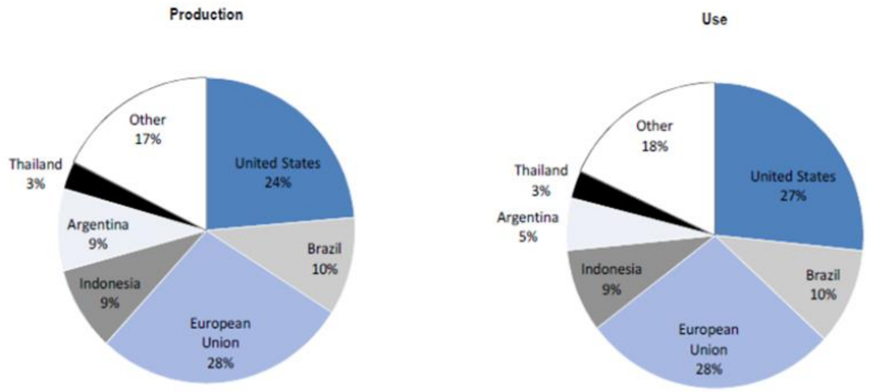

Source: OECD/FAO (2016), "OECD-FAO Agricultural Outlook", *OECD Agriculture statistics* (database), http://dx.doi.org/10.1787/agr-outl-data-en.

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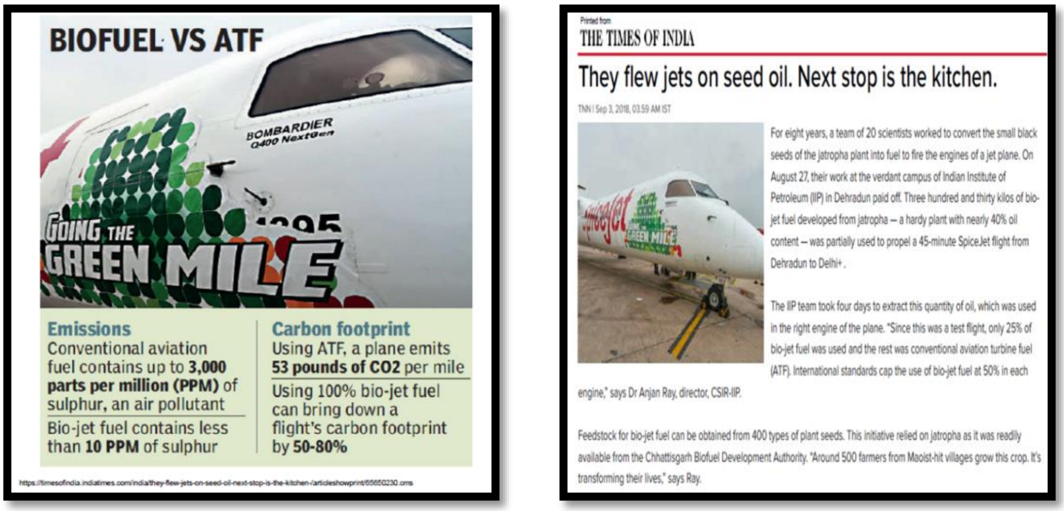
Figure 1.7: World-wide biodiesel production and use.

Blending biodiesel with petro-diesel is a popular way of using biodiesel rather than using B100. A 20% blend of biodiesel (B20) with petro-diesel is most preferred for better utility of biodiesel (Popluechai, 2010). It is encouraging to note that India has successfully propelled a 45 minutes Spice Jet flight from Dehradun to Delhi using a 25% blend of biodiesel with conventional aviation fuel (Figure 1.8).



BIOFUEL VS ATF

Emissions
Conventional aviation fuel contains up to **3,000 parts per million (PPM)** of sulphur, an air pollutant

Bio-jet fuel contains less than **10 PPM** of sulphur

Carbon footprint
Using ATF, a plane emits **53 pounds of CO2** per mile

Using 100% bio-jet fuel can bring down a flight's carbon footprint by **50-80%**

https://timesofindia.indiatimes.com/india/they-flew-jets-on-seed-oil-next-stop-is-the-kitchen-/articleshowprint/65650230.cms

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THE TIMES OF INDIA

They flew jets on seed oil. Next stop is the kitchen.

TNN | Sep 3, 2018, 03.59 AM IST

For eight years, a team of 20 scientists worked to convert the small black seeds of the jatropha plant into fuel to fire the engines of a jet plane. On August 27, their work at the verdant campus of Indian Institute of Petroleum (IIP) in Dehradun paid off. Three hundred and thirty kilos of bio-jet fuel developed from jatropha — a hardy plant with nearly 40% oil content — was partially used to propel a 45-minute SpiceJet flight from Dehradun to Delhi+ .

The IIP team took four days to extract this quantity of oil, which was used in the right engine of the plane. "Since this was a test flight, only 25% of bio-jet fuel was used and the rest was conventional aviation turbine fuel (ATF). International standards cap the use of bio-jet fuel at 50% in each engine," says Dr Anjan Ray, director, CSIR-IIP.

Feedstock for bio-jet fuel can be obtained from 400 types of plant seeds. This initiative relied on jatropha as it was readily available from the Chhattisgarh Biofuel Development Authority. "Around 500 farmers from Maoist-hit villages grow this crop. It's transforming their lives," says Ray.

Figure 1.8: Times of India reporting the use of 25% biodiesel mix with conventional aviation turbine fuel.

1.5.7. Non-edible oil - the preferred source of feedstock for biodiesel

Both the United States of America and the European Union have made remarkable progress in the production of biodiesel. They have retained their top positions world-wide in biodiesel production due to their successful conversion of surplus edible oil to biodiesel. Following these nations, Germany and France also utilize edible oil for conversion to biodiesel. Edible oil is extracted mainly from rapeseed, sunflower seeds and soybean in the United States and Europe. Malaysia and Indonesia cultivate large quantities of coconut along the coastal area, and utilize coconut oil for biodiesel production (Popluechai, 2010). Since India is a developing nation, directing edible oil for a non-nutritional purpose like biodiesel would not only be unethical but would keep the nation underdeveloped. Even though edible oil has more oxidative stability than non-edible oil and is preferred as feedstock for biodiesel, a country like India widens its search for biodiesel feedstock to non-edible oil purely to retain the value of edible oil for nutritional purposes. Non-edible oilseed crops such as *Jatropha curcas* L. and *Pongamia pinnata* are the preferred options. Azam et al., (2005) undertook a comparative study of 75 non-traditional oil seeds. They found that all the species chosen had 30% W/W seed oil, and one-third of these experimental plants were found to contain seed oil with suitable FAME compositions after transesterification and ideal iodine value and cetane number for use as an alternative fuel. The seed oil from these species have the potential to meet the standards of the European Standards

Organization, USA and Germany (ESO; Azam et al., 2005). These putative oil seed plants can further be narrowed down to suit a tropical nation like India. Seed oil content, the amount of productivity of seeds per hectare, the habitat condition required for plant and economic viability, growth habit (tree or shrub), and irrigation level are the other factors for choosing a putative plant for biodiesel. Considering all these criteria, *Jatropha curcas* L. emerges as the best putative plant fulfilling the criteria of having the highest seed oil content, high cetane number, iodine value, and other needed properties for biodiesel. Since it can grow in arid and semi-arid conditions, it can be cultivated successfully in India and other Asian countries. Since the seed contains phorbol esters, the plant remains untouched by cattle and humans.

1.5.8. Putative plants for biodiesel

The following plants have been identified as putative biodiesel sources in India.

- ***Jatropha curcas* (ratanjyot or physic nut)**
- *Azadirachta indica* (neem)
- *Calophyllum inophyllum* (nagchampa)
- *Calotropis gigantia* (ark)
- *Euphorbia tirucalli* (sher)
- *Hevea brasiliensis* (rubber)
- *Pongamia pinnata* (karanj)

These plants are being cultivated in various places both for medicinal and economic purposes. Their suitability for biodiesel has been well studied. They possess unique features of high content of seed oil, oxidation stability etc. Among all these plants, *Jatropha curcas* L. is most preferred plant.

Why *Jatropha curcas* L. is most preferred plant for biodiesel?

- ➢ Easily cultivatable and has low water requirement.
- ➢ Better suited for a country like India which is a tropical region.
- ➢ Not browsed by cattle.

- Barren lands and infertile lands can be utilized for cultivation. Often *Jatropha curcas* L. is used as a hedge plant to protect the cultivated crops.
- Seed of *Jatropha* contains nearly 30% to 40% oil.
- Arid and semi-arid lands can be used for cultivation. Even saline wastelands can be used. Its adaptability for climatic conditions and soils is remarkable.
- It exhibits pest, disease and drought resistance, which leads to optimum crop yield.
- The physio-chemical properties of *Jatropha curcas* L. biodiesel and petro-diesel are similar.
- It has a short gestation period of 2 years.
- It has a long productive life of 50 years.

1.6. REVIEW ON *JATROPHA CURCAS* L.

1.6.1. Scientific classification:

Kingdom: **Plantae**

Division: **Magnoliophyta**

Class: **Magnoliopsida**

Order: **Malpighiales**

Family: **Euphorbiaceae**

Subfamily: **Crotonoideae**

Tribe: **Jatropheae**

Genus: ***Jatropha***

Species: ***curcas***

1.6.2 Taxonomy of *Jatropha curcas* L.

The word “Jatropha” is derived from the Greek words ‘jatros’ and ‘trophe,’ which shows its association with medicinal uses. From the tribe Jatropheae of Crotonoideae in the Euphorbiaceae family, the genus *Jatropha* descends. The Euphorbiaceae family has trees, herbs or shrubs, woody climbers are very rare. It comprises of 180 species. Central and South America are considered to be the origin of *Jatropha*. It mostly grows in tropical and semi-tropical arid countries (Burkill, 1994; Heller, 1996; Openshaw, 2000; Fairless, 2007).

Jatropha curcas is also known as physic nut. It is a drought-resistant species and a dicotyledonous semi-evergreen shrub. It contains 22 chromosomes with 2n diploid species (Soontornchainaksaeng and Jenjittikul, 2003; Jha et al., 2007; Carvalho et al., 2008). *Jatropha* is otherwise also known as poison nut, Barbados nut, purging nut or bubble bush. It grows well in tropical and sub-tropical environments. In India, it grows in almost all biogeographical zones from coastal areas to the outer ranges of the Himalayas (Anonymous, 1959).

The physiological features of *Jatropha* include a short taproot, fine tertiary roots and robust laterals. Even though the plant is branched, the stem is woody, cylindrical, erect and solid. Branches are semi-woody, green and stout. The leaves are usually 5-lobed shallowly with a petiole (3-20 cm long), and the blade has a broadly ovate outline. The petioles are usually 12-19 cm long. Venation is of divergent type, reticulate and multicostate. The inflorescences are often paired, and are either axillary umbel-like cymes or terminal. Solitary female flowers terminate at each major axis with male flowers largely on lateral branches. An average of 20 – 25 male flowers to each female are produced on the same inflorescence (De Oliveira et al., 2009). *Jatropha curcas* flowers usually produce nectar and flowers are scented. The nectaries are present deep in the corolla, and only insects with long proboscis can access the nectar. Greenish yellow-coloured flowers with a sweet and heavy perfume are a unique feature of *Jatropha.* They are usually pollinated by moths.

Fruits measure 2.5 – 3 cm and are broadly ellipsoid capsules. They are smooth-skinned and fleshy and green initially, but turn yellow and eventually become black and dry. They are 3-seeded fruits (Figure 1.9). Seeds are 1-2 cm long and are ellipsoid, coarsely pitted and mottled black. Seedlings generate taproot and 4 peripheral roots (Anonymous, 1959; Singh, 1970; Heller, 1996; Raju and Ezradanam, 2002; Bhattacharya et al., 2005; Achten et al., 2008; Brittaine and Lutaladio, 2010).

Figure 1.9: Cross-section of *J. curcas* seed and matured seeds (King et al., 2009)

1.6.3. Agronomy of *Jatropha curcas* L.

Jatropha curcas L. plants are mostly grown as a hedge or as fence. Cattle and animals do not browse them. They are easily propagated by cuttings (Heller, 1996; Kumar and Sharma, 2008). Since *J. curcas* L. is well adapted to arid and semi-arid conditions, it is often used for preventing soil erosion (Martinez-Herrera et al., 2006). Rainfall regimes from 200 to 1500 mm per annum suits the cultivation of *Jatropha* (Achten et al., 2008). *Jatropha curcas* L. can grow in low fertility and low moisture conditions. It can also tolerate high temperatures (Augustus et al., 2002). It can survive in even poor stony soils (Aderibigbe et al., 1997). Leaf-shedding activity is utilized by the plant as an adaptation mechanism to survive in harsh environments. Shed leaves usually decompose and provide nutrients for the plant, and this even reduces water loss during dry season. Thus, *Jatropha* is known for its adaptability to various types of soil, including soils that are sandy, stony and saline, which are poor in nutrition (Juan et al., 2011).

From 2013, commercial production of *Jatropha* got a boost. Project developers have turned their attention towards greater yield and profit through the cultivation of *Jatropha* in recent years (Singh et al., 2006). Due to the presence of toxic protein called curcin, which is similar to ricin in castor, cattle and animals do not browse either the plant or seeds. Since it has the potential to become a good organic manure

(Staubmann et al., 1999; Gubitz et al., 1999), the compost of *Jatropha* seeds can replace many of the chemical fertilizers. It has nitrogen content ranging from 3.2 to 3.8% similar to that of cow dung manure and neem oil cake (Kumar and Sharma, 2008).

1.6.4. Chemical content of *Jatropha*

Jatropha seeds contain 47.25% fat, 24.60% crude protein and 5.54% moisture (Akintayo, 2004). The seeds are known for their high specific weight and they can be transported without deterioration at low cost. Mature seeds of *Jatropha curc*as L. also contain other chemical compounds, such as glucose, galactose, fructose, raffinose, saccharose, stachyos and protein (Achten et al., 2008). Li et al., (2010) identified a compound with strong anti-oxidative activity from the seeds of J. curcas L. named jatrophasin A (3, 4, 4', 5'-tetrahydroxyl-3'-methoxyl-bisepoxylignan).

Alkaloids such as jatrophine, jatropham, curcacycline A, curcain, and other secondary metabolites like tannins, glycosides, flavonoids and sapogenins with anti-cancerous properties are present in the leaf, bark and latex of *Jatropha* (Van den Berg et al., 1995; Thomas et al., 2008; Debnath and Bisen, 2008). The seeds of *J. curcas* also contain toxic compounds such as phorbol esters, diterpenoids and a protein called curcin (King et al., 2009).

Pant et al., (2006) showed that depending on the altitude of cultivation, types of species grown and climatic condition, the yield of *Jatropha curcas* L. seeds varies. The study showed that elevation ranges of 400–600m, 600–800m and 800–1000m give an average oil yield of 43.19%, 42.12% and 30.66% of their seed weight, respectively. Seeds contain about 30 - 40% of viscous oil on an average. Depending upon the area of cultivation and care given with sufficient water and nutrients, the oil content of the seed can cross over 50% (Jingura et al., 2011).

1.6.5. Chemical content of *Jatropha* seed oil

Jatropha curcas L. seed oil is chiefly made up of triacylglycerol containing Oleic acid, stearic acid, palmitic acid, linoleic acid, meristic acid, lauric acid**,** arachidonic acid, arachidic acid and behenic acid (Adebowale and Adedire, 2006; De Oliveira et al., 2009). Akbar et al., (2009) studied the characteristics and composition of *Jatropha curcas* oil seed from Malaysia, and showed that it is the best biodiesel feedstock. Oleic acid (44.7%) and linoleic acid (32.8%) are the dominant unsaturated fatty acids, while palmitic acid and stearic acid are the saturated fatty acids found in

Jatropha oil. Since the oil extracts have physico-chemical properties closely resembling conventional diesel, *Jatropha curcas* L. oil has been accepted as the best feedstock for biodiesel.

1.6.6. Uses of *Jatropha curcas* L. oil

In the areas of agriculture, biofuels and healthcare management, *Jatropha curcas* L. has emerged as an interesting multipurpose plant. Different parts of *Jatropha curcas* L. are used for various purposes. The fruit and the wood of *Jatropha* can be used for numerous purposes including fuel. Besides the chief purpose of feedstock for biodiesel, the seed oil of *Jatropha* can be used for manufacturing soaps and candles and in the cosmetics industry as a paraffin substitute or extender. Glycerol, which is a by-product in the trans-esterification of seed oil, has many useful industrial applications as a raw material for the synthesis of 1, 3 propane-diol and other polymeric materials (Vinayak and Kanwarjit, 1991; Berchmans and Hirata, 2008). *J. curcas* oil and other parts of *Jatropha* plants are used to produce glycerol, cosmetics, soap, dye, varnish, bio-pesticide, fertilizer and molluscicide (Heller, 1996; Mangkoedihardjo and Surahmaida, 2008; Bar et al., 2009; Jamil et al., 2009; Sharma and Pandey, 2009; Agamuthu et al., 2010; Brittaine and Lutaladio, 2010; Pandey et al., 2012; Warra, 2012).

1.6.7. Medicinal property and uses of *Jatropha curcas* L.

Jatropha curcas L. is known for its medicinal uses. Medicinal compounds for external application and internal intake are made from *Jatropha* (Agbogidi and Ekeke, 2011). Glycosides, phytosterol, flavonoids, tannins, and steroidal sapogenins are present in various parts of the plant such as leaves, latex, fruits and bark. These compounds have a wide range of medicinal properties (Duke, 1994; Edeoga et al., 2005; Agbogidi and Eruotor, 2012). Apigenin, vitexin, isovitexinin and other factors present in *J. curcas* leaves are useful for treating malaria, and rheumatic and muscular pains (Thomas et al., 2008; Agbogidi and Ekeke, 2011). Jatropham and jatrophone are anti-tumour components identified from other species of *Jatropha* (Oskoueian et al., 2011). Flavonoids obtained from *Jatropha* and are often used for allelopathy and for inhibition of germination and growth. Stryptic, vermifuge and vulnerary are the other ethno-medicinal uses of *J. curcas*.

Various parts of *Jatropha* are used as a folk remedy for heartburn, ulcer, stomach ache, fever, cough, diarrhoea, jaundice, paralysis, erysipelas, rheumatism, alopecia, ascites, carbuncles, burns, convulsions, rashes, yellow fever and tetanus. Traditionally this plant is used for treating dysentery and diarrhoea. *Jatropha* is effectively used for its antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

The alkaloid “jatrophine” present in the latex of *Jatropha* is used for rheumatism, skin diseases and for sores on domestic livestock (Das Gupta et al., 2010). Nath and Dutta (1992) demonstrated that *Jatropha* also has wound-healing properties derived from curcain, a proteolytic enzyme isolated from latex. The paste of *Jatropha* leaves is used to treat scabies and as rubefacient for paralysis and rheumatism (Duke et al., 2002; Aliyu, 2006). The decoction of *Jatropha* leaves is an effective remedy for cough and an antiseptic after birth. The sap flowing from the stem arrests the flow of blood from wounds and heals wounds.

Muanza et al., (1995) revealed that a methanol extract of physic nut leaves is effective against the cytopathic effects of human immunodeficiency virus, protecting cultured human lymphoblastoid cells. In guinea pigs, cardiovascular diseases can be cured by extract of the leaves, and the leaves could be a source of Beta-blocker agents (Fojas et al., 1986). Control of *Sclerotium* sps, an *Azolla* fungal pathogen, is achieved through the aqueous extracts of physic nut leaves (Garcia and Lawas, 1990). In laboratory experiments, ground physic nut showed molluscicidal activity against the host of liver fluke (Agaceta et al., 1981). Urinary infection has been successfully treated by using the young leaves. The tender twig can be used as a tooth brush (Gill, 1992).

Various extracts of *Jatropha* showed activities like haemagglutination (lectins), anti-inflammatory effects and curing skin irritation, and also proved to have molluscicidal and insecticidal effects. The seed is poisonous because of the presence of toxic phorbol esters, and toxalbumin called curcin and cyanic acid. The seeds are highly toxic to both animals and humans (Adolf et al., 1984; Nath and Dutta, 1991; Levin et al., 2000; Rai and Lakhanpal, 2008). Medical literature has reports of toxicoses: if a child ingests four seeds it can be highly toxic, with symptoms similar to that of organophosphate insecticide intoxication, having no antidote (Nath and Dutta, 1991; Gubitz et al., 1999; Joubert et al., 1984; Koltin et al., 2006).

Skin ailments and rheumatism are cured using the oil as a purgative (Heller, 1996; Marroquin et al., 1997). *Jatropha* seed kernel oil contains 36% linoleic acid (C18:2), which is effective for skin care. In addition, rural communities use *Jatropha* oil in torches and lanterns. Since the oil burns with a smoke-free flame, it causes no environmental pollution (Tigere et al., 2006). Oil-rich seeds and seed oil are used to expel internal parasites even though the oil causes strong irritation of the gastro-intestinal tract and even poisoning. Seeds are used as purgative agents, but the bark and leaves also have the purgative effect (Henning, 2007). The seed oil (Jamalgota) is applied to treat skin diseases like eczema and to reduce rheumatic pain (Prasad et al., 2012).

Jatropha oil has well-recognized anti-HIV, anti-cancer and antimicrobial activity. There are a very few previous reports confirming the specific anti-HIV activity. One such study showed that HIV was strongly inhibited via induced cytopathic effects with low cytotoxicity by the use of water extracts of the branches of *Jatropha curcas* L. It also possesses specific in vitro anti-reverse transcriptase enzyme activity (Igbinosa et al., 2009).

1.6.8. *Jatropha* seed oil as biodiesel

Jatropha curcas L. has received world-wide attention in recent years for biodiesel conversion. It is highly desirable that a non-edible oil like *Jatropha* be used as feedstock so that edible oils can be used for nutrition. The presence of toxic compounds makes it unsuitable for human consumption. Biodiesel is renewable and clean fuel composed of monoalkyl esters of fatty acids derived from vegetable oils or animal fats, produced when vegetable oil is subjected to transesterification with methanol or ethanol (Knothe et al., 2006). The use of biodiesel has grown enormously during the last few years. *Jatropha* used as feedstock accounts for a large percentage of the direct biodiesel production, impacts capital income and involves less expense (Bozbas, 2005).

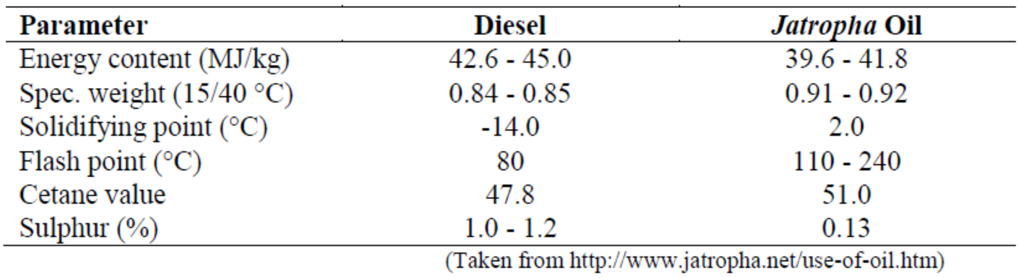
Biodiesel from *Jatropha curcas* L. is mostly preferred because of the superior properties of *Jatropha* seed oil compared to other vegetable oils. It has good oxidation stability and low acidity compared to soybean oil. It has low viscosity, relative to castor oil, and possesses better cooling properties than palm oil. It also has a fatty acid composition with a high percentage of unsaturated fatty acids (Gubitz et al., 1999).

Biodiesel produced from *Jatropha* can be used directly without modifying the engines (Gubitz et al., 1999). Fuel produced from the feedstock of *Jatropha* oil meets the standard requirements put forward by American and European countries (Vinayak and Kanwarjit, 1991; Azam et al., 2005; Tiwari et al., 2007).

Bhagat and Kulkarni (2009) reported the variations in the oil content, fatty acid profile and physico-chemical analysis of various species of *Jatropha*. In addition, free fatty acid level, the density of oil and viscosity of the biodiesel produced from *Jatropha* oil are stable during storage period (Sandquist and Matas, 2012). Moreover, biodiesel from *Jatropha* seeds has a higher flash point ensuring safer storage and handling (Table 1.2). Berchmans and Hirata (2008) have even succeeded in obtaining biodiesel from *Jatropha* oil with low free fatty acid content (1%) by a two-step esterification process.

Popluechai et al., (2008) showed that the major fatty acids present in *Jatropha* oil were oleic (36% – 44%) and linoleic (29% – 35%) as unsaturated fatty acids and palmitic (12 – 14%) and stearic (8 – 10%) as saturated fatty acids. The elevated level of unsaturated fatty acids helps to make the respective fatty acid methyl esters, which make the biodiesel more stable. Reksowardojo et al., (2006) undertook a study in which they compared petro-diesel with biodiesel generated from different vegetable oils including *Jatropha curcas* L. They revealed that biodiesels more specifically from *Jatropha* origin have more efficiency in direct injection (DI) engines.

Table 1.2. Comparison between the properties of diesel and *Jatropha* oil.



Parameter	**Diesel**	***Jatropha* Oil**
Energy content (MJ/kg)	42.6 - 45.0	39.6 - 41.8
Spec. weight (15/40 °C)	0.84 - 0.85	0.91 - 0.92
Solidifying point (°C)	-14.0	2.0
Flash point (°C)	80	110 - 240
Cetane value	47.8	51.0
Sulphur (%)	1.0 - 1.2	0.13

(Taken from http://www.jatropha.net/use-of-oil.htm)

Shuit et al., (2010) explored the feasibility of *in situ* extraction and transesterification of *J. curcas* L. seeds to biodiesel. The results revealed that the amount of oil extracted and yield of FAME depend upon the size of the seed and reaction period. Using *n*-hexane as co-solvent and seeds with a size of 0.355 mm, reaction period of 24 h, reaction temperature of 60 °C, methanol to seed ratio of 7.5 ml/g and 15 wt% of H_2SO_4, the efficiency of oil extraction and FAME yield reached up to 91.2% and 99.8%, respectively. Thus, a single-step reactive process can give the best yield of biodiesel and reduce the cost and processing steps.

Applying direct base-catalysed trans-esterification process using sodium hydroxide and methanol as catalyst, a yield of 85.3% of methyl ester was obtained within two hours of reaction. 0.6g of sodium hydroxide in 20ml of methanol was reacted with 100ml of *Jatropha* seed oil to gain maximum yield of biodiesel. The biodiesel obtained with this proportion had a specific gravity of 0.878 and viscosity of 7.891 centistokes (at 20^0C), and 85.3% ester content (Ntaganda et al., 2014).

1.7. SEED STORAGE

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 reported an increase in seed yield from *Jatropha curcas* L. by exogenous application of phytohormones. However, it was 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 take place (Yamane et al., 2007; Dunn Robert, 2008). Factors like hydrolysis and oxidation are most deleterious process that make 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.

Seeds are considered viable until they retain their genetic and physiological quality. Seed longevity depends on the way in which the seed interacts with the storage environmental conditions (Arc et al., 2011). The purpose of seed storage varies from preservation and sustenance of the seed genetic resources at seed banks to distribution of quality seeds for species that are used in crops and production. Prolonged seed storage generally leads to seed aging with the manifestation of irreversible metabolic and cellular changes such as oxidation of proteins, nucleic acids, and lipids. Seed aging also involves enzyme inactivation, membrane perturbation, and impairment of RNA and its biosynthesis (Kibinza et al., 2006; El-Maarouf-Bouteau et al., 2011; Hu et al., 2012). Even if an optimal environment is provided, seed deterioration is inevitable and this is a major hurdle for agriculture and germplasm conservation (Li et al., 2017). Storage of oilseeds is a greater challenge than that of cereal grains as oilseeds are more susceptible to seed deterioration.

Hence, systematic studies of all changes that happen during storage are essential. Study on storage of oil seeds will help understand seed behavior during storage and kinetics of seed deterioration. Knowledge of the behavior of seeds during storage is essential in order to increase their longevity and to prevent a significant loss in their genetic and physiological features.

1.7.1. Types of seeds

Seeds are divided into two groups based on their storage characteristics: orthodox and recalcitrant. Orthodox seeds are those that can be dried to hold the moisture contents of 10% or less. Recalcitrant seeds, on the other hand, are those that cannot be dried below relatively high moisture levels (25 – 45%) (Roberts, 1973). If they are desiccated below 10% they lose their viability and get deteriorated.

1.7.2. Seed morphology

Some of the orthodox seeds (e.g., species of the Leguminosae) morphologically have a hard seed coat that helps to maintain a low level of metabolism in dry state. In *Carya* Nutt, *Cornus* L., and *Nyssa* L. the hard seed coat is to protect the embryo from mechanical damage. Most of the seeds with hard seed coat are meant for long-term storage. On the other hand, seeds with thin seed coat are meant for short period of storage because of bruising of internal tissues or rapid uptake of moisture and oxygen (Franklin, 2008). Seeds that contain mucilage around them are likely to be prone for early seed deterioration since the mucilage helps in transfer of moisture from surroundings into the seed (Cheah and Osborne, 1978). The oleaginous seeds have tendency to deteriorate rapidly due to lipid peroxidation that occurs during the storage.

1.7.3. Chemical composition of seed

Seed deterioration during storage depends upon also the chemical composition that a seed contains. Seeds that contain lipids in endosperm will easily get deteriorated compared to starch containing seeds. Even under ambient storage conditions oil seeds like sunflower, groundnut, and sesame lose their viability due to rancidity of the oil present within the seed. Storage potential of the oil seeds can vary anything from 9 to 23 months under suitable storage conditions (Begum et al., 2013).

1.7.4. Storage environment

The purpose of seed storage is the preservation of seeds with their genetic and physiological quality. A suitable environment has to be provided that would ensure seeds being less susceptible to deterioration. Moisture levels in the environment is detrimental to seed quality. Moisture supports growth of microorganisms. Entry of microorganism and formation of microbial colonies on the surface of seeds will eventually lead to penetration of these microorganisms into the seeds and cause seed deterioration. Therefore, any effort towards maintaining low moisture content will disable the microbes to survive thus safeguarding the viability of seeds.

Seed quality on storage is also affected by ambient temperature (Shelar et al., 2008; Balesevic- Tubic et al., 2005; Khatun et al., 2009; Biabani et al., 2011). Lower temperature delays the process of seed deterioration thereby sustaining the seed viability during storage (Balesevic-Tubic et al., 2005; Mohammadi et al., 2012).

1.7.5. Role of reactive oxygen species (ROS) in seed biology

Reactive oxygen species (ROS) are considered to be toxic and deleterious molecules and play a key role in causing damage to seeds during desiccation especially in recalcitrant seeds. However, there is also increasing evidences that ROS such as O_2^-, H_2O_2, and OH can play a dual role of both being toxic molecules causing damage to lipids, proteins, and nucleic acids and also as signaling molecules for programmed cell death, hormone signaling, growth and development, and responses to biotic and abiotic stresses in seeds (Mittler et al., 2004) (Figure 1.10). The changes in ROS content in different stages of sunflower seeds (*Helianthus annuus*) is reported by Bailly et al., (2008) (Figure 1.10). The content of H_2O_2 was measured and found to be altered differently throughout the various stages of seed formation, dormancy, imbibition and seed dry storage. During the initial phase of seed development until the phase of mass maturity, the H_2O_2 content was high due to the presence of high metabolic rate brought about by high moisture content. But during the process of desiccation or maturity drying the high content of H_2O_2 subsequently reduced. When seeds undergo imbibition there is a noticeable increase in H_2O_2 in seeds. This increase can be attributed once aging to the moisture content because of which there is resumption of metabolic activities.

During this process, there is a formation of superoxide anion and hydrogen peroxide, formed from the utilization of 2 – 3% of oxygen (Oracz et al., 2007). Even though the level of H_2O_2 increased yet the seed remained ungerminated because the dormancy was still maintained by seed.

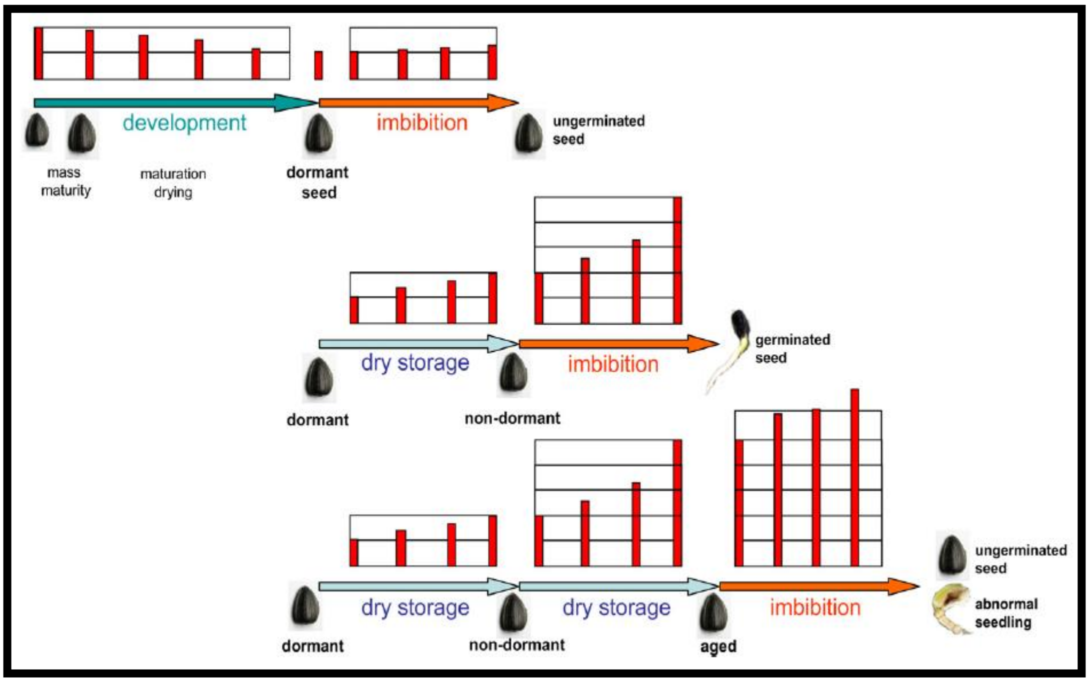


Figure 1.10: Schematic representation of changes in H_2O_2 content in sunflower seeds during their development, after-ripening, germination and aging (Bailly et al., 2008).

On the other hand, when dormant seed undergo dry storage, H_2O_2 significantly increases and seed becomes non-dormant and this eventually results in germination upon imbibition. This indicates that the level of H_2O_2 is maintained differently in these two types of seeds. The increase of H_2O_2 during dry storage helps the seed break dormancy and becomes non-dormant. H_2O_2 increase during imbibition of non-dormant seeds functions as signaling molecule causing germination. At the end of the first phase of dry storage, the dormancy of the seed gets broken and seed becomes non-dormant. If this non-dormant seed undergoes further dry storage instead of imbibition then the level of H_2O_2 produced reaches above the thresh hold point to function as signaling molecule. This highly elevated H_2O_2 produced during second phase of dry storage becomes detrimental and deleterious to cause seed deterioration. In this case, H_2O_2 play a detrimental role (Bailly et al., 2008).

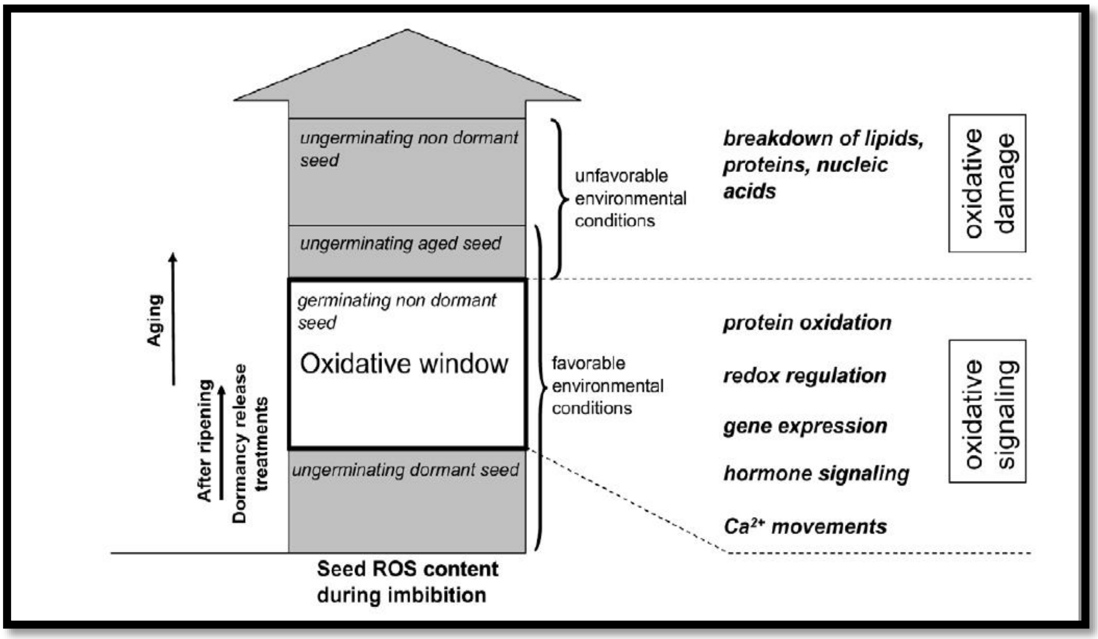


Figure 1.11: The oxidative window. A model to show the dual role of ROS (Bailly et al., 2008).

From the beginning of seed formation to the end of seed germination, the sources of ROS fluctuate substantially. This variability of sources is related to cytoplasmic viscosity and molecular mobility. These two factors govern the rate of metabolic reactions (Walters, 1998). In dry seeds where the metabolic processes are on hold, the process of lipid peroxidation becomes a mechanism for ROS production through non-enzymatic chain reactions (Priestley, 1986). In certain cases ROS gets formed through Amadori and Maillard reactions especially for non-oleaginous seeds (Sun and Leopold, 1995).

Due to lone pair of electrons, ROS are highly reactive with the macromolecules such as nucleic acids, lipids, proteins, and sugar. The shift from signaling role to deleterious role is associated with the accumulation of ROS above the thresh-hold level leading to cellular alteration and damage. Seed desiccation and seed aging are two major physiological processes when ROS levels become proliferative and deleterious for seeds (Bailly et al., 2008) (Figure 1.11). Orthodox seeds are capable of withstanding desiccation as they produce less ROS than the recalcitrant seeds (Vertucci and Farrant, 1995; Kermode and Finch Savage, 2002).

Various protective and defense mechanisms including antioxidant pools become inactive and incapable of scavenging free radicals under dehydrated state of seeds. During the long-term storage of oil seeds, these free radicals inactivate antioxidant enzymes leading to severe cellular damage. Bailly et al., (1998) have shown that long-term storage of sunflower seeds lead to loss of viability and loss of catalase activity. They have shown a surge in free radicals during storage which causes severe damage to the antioxidant enzymes that scavenge the free radicals. This leads to loss of viability. The loss of seed viability cannot always be attributed to lipid peroxidation during storage. For eg. sunflower seeds with moisture content below the thresh-hold value of ca. 0.21 H_2O g DW^{-1} and stored at 35˚ C had an accumulation of hydrogen peroxide without causing lipid peroxidation. This indicates that the oxidative damage occurs in non-lipidic cellular fractions like nucleic acid and protein. On the other hand, when the stored seeds contains moisture content above the thresh-hold level there was loss of seed viability associated with lipid peroxidation (Bailly et al., 2008).

Ratajczak et al., (2015) determined the level of ROS (H_2O_2, $O2^{-\bullet}$ and $OH^{\bullet}$.) in both cotyledons and embryonic axes of common beech seeds during long-term storage. They reported increase in all species of ROS with increase in storage period. The concentration of ROS was found to be less in cotyledons compared to embryonic axis. The level of H_2O_2 was found to be lowest in 2-year-old stored seeds. But the value reached its peak after 13 years of storage.

1.8. Seed deterioration during storage

Seed deterioration is attributed to series of biochemical reactions. The catabolic process of seed deterioration is irreversible. There is formation of hydrogen peroxides, oxygenated fatty acids, and other free radicals when the lipid rich seeds are stored for a long period of time. By nature, free radicals are unstable and thus react with molecules like lipids, proteins, and nucleic acids. The free fatty acids formed during the storage of lipid seeds are responsible for rancidity and seed deterioration. Reduction of seed oil content, increased free fatty acid content, decreased unsaturated fatty acids content and damage to oil bodies are specific deleterious effects seen in oil seeds during storage.

Seed deterioration is reflected at molecular level, cellular level, and structural level. Impairment of RNA and protein synthesis, damage to DNA, chromosome aberrations, cell death and necrosis, loss of membrane integrity, altered enzymes activity, and decreased food reserves are the list of events associated with seed deterioration during seed storage (Kibinza et al., 2006). Physiologically, loss of seed viability and vigor, low germinations, abnormal seedlings, poor field emergence, and seed death are the other manifestations of seed deterioration.

1.8.1. Lipid peroxidation

The main cause of oil seed deterioration is lipid peroxidation. This results in deteriorative biochemical changes in seeds. Lipid peroxidation leads to increase of free fatty acids content thus changing the cellular pH to an unfavorable intracellular toxic condition (Balesevic-Tubic et al., 2005).

The end product of lipid peroxidation is hydroperoxide (ROOH). This further gets broken down into aldehydes and ketones, including the formation of malondialdehyde (MDA) (Figure 1.12). Extend of lipid peroxidation is estimated by measuring the amount of MDA being formed. Formation of free radicals takes place either by the action of enzymes like lipoxygenase present in many seeds or by non-enzymatic way of auto-oxidation of lipids. Free radicals are the causative agents that bring about the lipid peroxidation. Free radicals production is initiated by oxygen, which primarily targets the lipids and other molecules in the cell. This results in many undesirable events occurring within the cell.

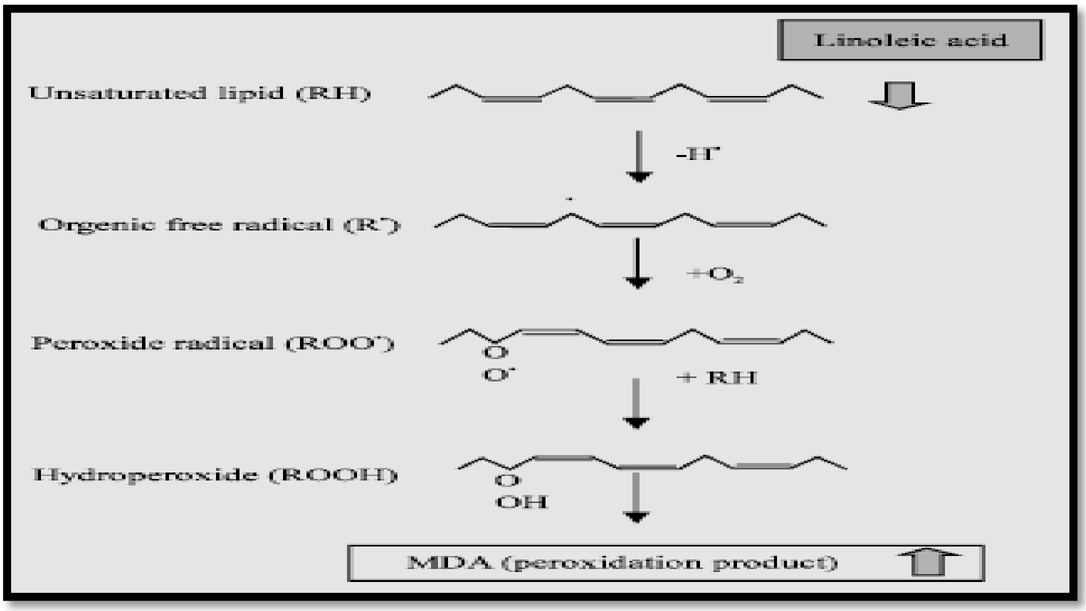


Figure 1.12: Peroxidation reaction of polyunsaturated fatty acids resulting in MDA production.

Sunflower seeds subjected to storage resulted in loss of viability caused by increased level of MDA amounted from lipid peroxidation. This increased level of MDA further declined the activity of antioxidant enzymes indicating the attack of the free radicals on the antioxidant enzymes (Kibinza et al., 2006). Membranes are the early and easy target of lipid peroxidation. The free radicals formed through lipid peroxidation target the lipid bilayers of the membrane resulting in membrane damage and electrolyte leakage due to the loss of membrane integrity (Ghassemi-Golezani et al., 2010). Lipid peroxidation a complex process and takes place in two different stages. First stage occurs in the initial phase of seed storage, where there is spontaneous oxidation of the unsaturated fatty acids and saturated fatty acids remain unaffected. The second stage occurs at the later part of storage where both unsaturated and saturated fatty acids are oxidized resulting in loss of seed viability and germination (Motlagh and Shaban, 2014). There is a significant increase in MDA content in soy seeds kept for 12 months of natural aging (NA) (Balešević-Tubić et al., 2011). Elevated levels of MDA were found in studies done on cotton (Goel et al., 2003), sunflower (Balešević-Tubić et al., 2005), and sweet pepper seeds (Kaewnaree et al., 2011). Xin et al., (2014) showed that there is 20% and 72% increase in MDA content compared to control when soybean seeds are subjected to artificial aging for 18 and 41 days. This increased level of MDA and leachate conductivity is negatively correlated to seed viability. The results suggest that artificial aging causes intense lipid peroxidation and oxidative damage and can be used to mimic changes seen during seed storage.

1.8.2. Membrane disruption

One of the early targets of free radicals is the cell membrane. Lipid bilayer of the membrane serves as the platform for lipid peroxidation causing severe damage to membrane. When there is a loss of membrane integrity then there is leakage of the electrolytes and other essential components from the cell. As a result, cells are not able to retain their normal physical condition and function. Free radicals and the formation of free fatty acids are chief causative agents of membrane disruption. Lipid peroxidation can result in not only destruction of the outer bilayer of lipid of the cell but later can target the membrane of intra cellular organelles as well (Ghassemi-Golezani et al., 2010). The membrane damage is brought about by excess accumulation of ROS (Varghese and Naithani, 2008; Roach et al., 2010; Ratajczak et al., 2015).

Increased leaching of seed constituents is brought about by harsh storage conditions as a result of alteration in membrane permeability. Eventually the increased leaching leads to loss of viability of the seed. Decline in seed vigor, seed germination, field emergence are associated with high level of electrolyte leakage. Diminishing of normal cell functions and energy productions are due to alteration of membrane systems such as tonoplast, endoplasmic reticulum, and plasmalemma. Loss of permeability and membrane disruption are early events of seed deterioration. Increasing electrolyte leakage was reported in aged seeds of sunflower (Kibinza et al., 2006), soybean (Xin et al., 2014), sycamore maple and norway maple (Pukacka and Ratajczak, 2007), yellow-lupin (Garnczarska et al., 2009), and neem (Varghese and Naithani, 2008).

1.8.3. Alteration of proteins during aging of seeds

The free radicals besides mediating the lipid peroxidation are capable of targeting the thiol groups present proteins resulting in oxidation. This causes the inactivation of the enzymes involved in maintaining cellular oxidative homeostasis which results in formation of more reactive and deleterious molecules (Halliwell and Gutteridge, 1984). The overall negative effect of lipid peroxidation decreases the membrane fluidity, which in turn facilitates the exchange of phospholipids between the two halves of the bilayer of the membrane causing severe damage to membrane proteins, increasing the leakiness of the membrane, inactivates the receptors, ion channels, and enzymes (Halliwell, 2006). In aging seeds, the generation of ROS is mostly by non-enzymatic reactions. Even at very low moisture content non-enzymatic reaction like Amadori and Maillard takes place (Priestly, 1986; Sun and Leopold, 1995). It is a process of glycosylation where reducing sugars are attached to amine group of the amino acids and the proteins becomes glycosylated. This glycosylated protein formed during storage plays a deleterious role in impairment of transcription leading to faulty synthesis of protein and DNA damage during germination (McDonald, 1999). Wettlaufer and Leopold (1991) investigated the correlation between Maillard reactions and loss of seed vigor and showed loss of germination in soybean seeds due to accumulation of Maillard products. Sun and Leopold (1995) established a loss of germination in seeds with high content of Maillard products formed during long-term storage and accelerated aging of seeds.

1.8.4. Antioxidant enzymes in aging seeds

Antioxidants play a significant role in scavenging the free radicals produced during aging of the seeds (Figure 1.13). When there is a perfect balance maintained between the pool of antioxidants and free radicals produced during storage and accelerated aging, then the homeostasis of the seeds is sustained and viability and seed vigor are retained.

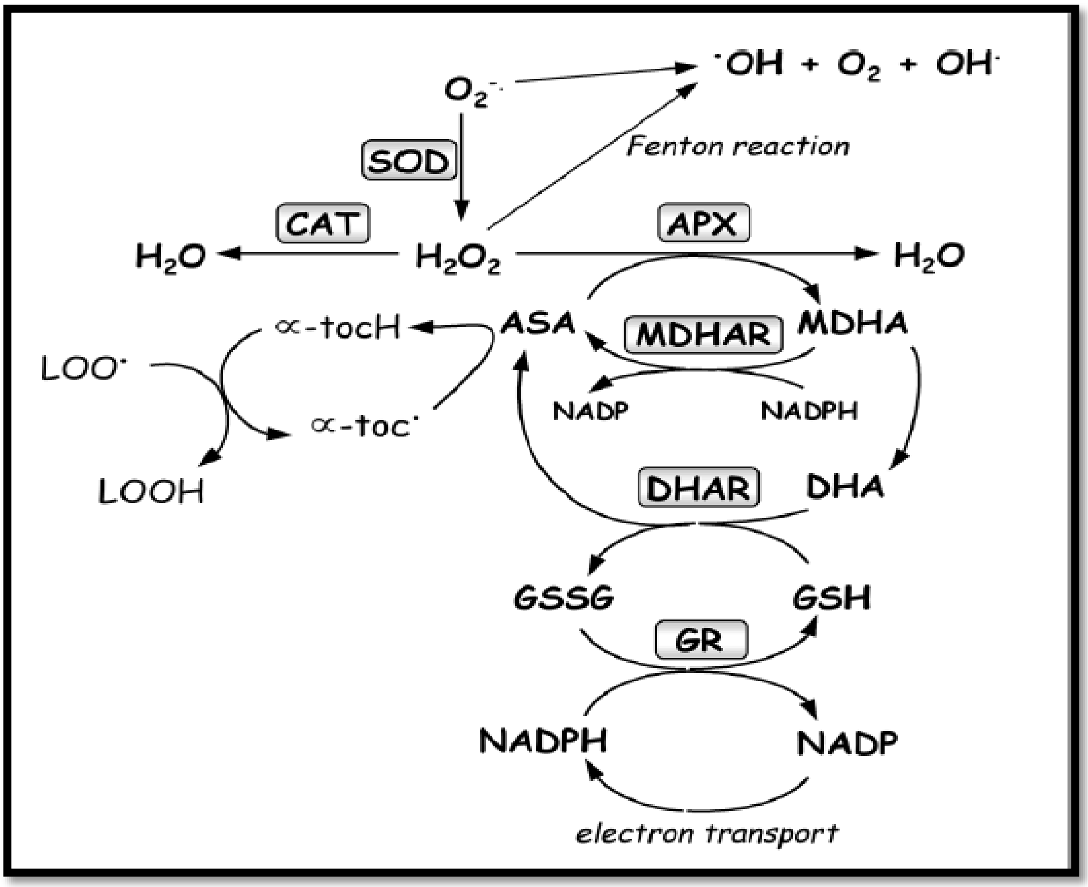


Figure 1.13: Detoxifying system of antioxidant enzymes and non-enzymatic antioxidants - superoxide dismutase (SOD); ascorbate peroxidase (APX); monodehydroascorbate reductase (MDHAR); dehydroascorbate reductase (DHAR); glutathione reductase (GR); ascorbate (ASA); monodehydroascorbate (MDHA); dehydroascorbate (DHA); oxidized glutathione (GSSG); reduced glutathione (GSH); α-tocopherol; α-tocopheryl (Bailly, 2004).

Combination of enzymatic and non-enzymatic antioxidants play a protecting role of detoxifying the free radicals (Figure 1.13). In dry seeds or stored seeds the auto-oxidation begins with generation of superoxide free radical (O_2^-). This most harmful free radical is converted to less harmful free radical of hydrogen peroxide through the action of SOD which can be mitochondrial, cytosolic, or chloroplastic. On one hand, this hydrogen peroxide further gets converted to water through the action of CAT which is present in glyoxysomes and peroxisomes and on the other hand, as ascorbate is reduced to monodehydroascorbate (MDHAR), hydrogen peroxide is converted to water by ascorbate peroxidase (APX) as well. Through series of reactions of reduction and oxidation, there is a formation of many reducing equivalents which end up transferring electrons to electron transport chain. GR as part of the antioxidant enzymes system also plays significant role in detoxifying the effect of free radicals. APX, MDHAR, DHAR, and GR are present in chloroplasts, cytoplasm, mitochondria, peroxisomes, and apoplast (Mittler, 2002). These enzymes are involved in regenerating powerful non-enzymatic antioxidants like ascorbic acid (vitamin C), reduced glutathione, and tocopherol (vitamin E) (Bailly, 2004). Non-enzymatic antioxidant tocopherol converts a more harmful molecule like lipid radical ($LOO^{\cdot}$) to a less harmful molecule of lipid peroxide.

During storage since there is continuous formation of free radicals, there emerges a time point where there is an imbalance between the concentration of the free radicals and the pool of antioxidant enzymes to check their ill-effects. Prolonged storage and inappropriate storing environment condition such as high temperature and high humidity will aggravate this process of seed deterioration through increased free radical synthesis (Bailly, 2004). Scialabba et al., (2002) showed a decreased activity of POX in stored seeds of radish compared to control. Pallavi et al., (2002) also reported similar kind of decrease in sunflower seeds. In the younger seeds of *Chenopodium rubrum*, CAT and POX activity was reported to be high (Mitrovic et al., 2005).

Balesevic-Tubic et al., (2011) reported that there is a decreased activity of both SOD and POX after 12 months of NA of soybean seeds. When subjected to accelerated aging, there was a drastic decrease in the activity compared to NA. Both SOD activity and germination got decreased linearly in relation to storage condition and duration of storage in soybean seeds.

Demirkaya, (2013) studied the relationships between antioxidant enzymes and physiological variations that occur during aging of pepper seeds. Seeds of three different cultivars of pepper seeds were subjected to aging. It was reported MDA and electrolyte leakage were increased and CAT, POX, and SOD decreased significantly in all the cultivars of seeds. This increase and decrease of the above-mentioned parameters was more vivid once the seed vigor dropped to below 60%. Negative correlation was established between lipid peroxidation and seed viability. A positive correlation was found between the loss of seed viability and decreased activity of SOD, POD, and CAT. This suggests that the activities of antioxidant enzymes are important to maintain seed viability.

Reduced mitochondrial and ascorbate–glutathione levels in artificially aged soybean seeds was studied by Xin et al., (2014). Two sets of seed lots were subjected to accelerated aging for 18 h and 41 h. Mitochondrial antioxidant enzymes such a SOD, ascorbate peroxidase, reduced GR, MDHAR, and DHAR were significantly reduced in aged seeds. The elevated levels of superoxide radical and hydrogen peroxide confirms the deleterious role of free radicals in artificially aged seeds. It was concluded that destruction of mitochondria in artificially aged seeds was associated with ROS accumulation. Ratajczak et al., (2015) reported that there is an increased electrolyte leakage and accumulation of superoxide radicals, hydrogen peroxide, and hydroxyl radicals in beech seeds stored for 13 years. Decreased catalase activity was observed both in embryonic axis and cotyledons of the stored seeds.

Sahu et al., (2017) studied the reactive oxygen species metabolism in neem (*Azadirachta indica*) seeds exposed to NA and controlled deterioration (CD). They reported that increased level of SOD, CAT, and APX were highly positively correlated with seed vigor and seed germination. Increase in the amounts of hydrogen peroxide and OH- radicals were associated with the loss of CAT and APX activity during both types of aging. At the initial period of aging, the SOD activity was increased and was associated with elevated level of hydrogen peroxide.

From the above studies, it is evident that antioxidant enzymes play a very significant role in protecting the seeds from deleterious effects of free radicals under storage. Conditions like increased temperature and moisture or prolonged storage results in an imbalance between reactive oxygen species and antioxidant enzymes, resulting in complete loss of seed vigor, viability and germination, which culminates in complete seed deterioration.

1.8.5. Non-enzymatic antioxidants levels with aging in seeds

Besides the enzymatic antioxidants there are a plethora of non-enzymatic antioxidants that play a vital role in protecting the seeds during the storage. Among these non-enzymatic antioxidants, vitamin E (tocopherol) and vitamin C (ascorbic acid) are the major ones that contribute to seed oil stability and seed longevity.

Tocopherol, a non-enzymatic antioxidant, along with other antioxidants exerts a synergetic action to enhance oxidative stability of the seed oil (Mohamed and Awatif, 1998). Since *Jatropha curcas* L. seed oil constituted of high amount of polyunsaturated fatty acids (PUFA), tocopherol is an essential component of the seed oil to protect these PUFA from oxidation during storage.

Tocopherols are the lipophilic antioxidants present in seed oils (Saloua et al., 2009). There are four different types of tocopherol synthesized in plants. Depending upon the number and position of methyl substituents present on the chromonal ring they are termed as alpha, beta, gamma, and sigma. They are amphipathic in nature, where the hydrophobic tail is associated with the lipid membrane and the polar group remains on the surface of the membrane. In plants, plastid is the source of synthesis and localization of tocopherol (Arango and Heise, 1998). Seed contains the highest among all the other tissues of the plants (Sheppard et al., 1993). The main function of tocopherol is to scavenge the hydroperoxides intermediates thus stopping the chain reaction of autoxidation (Tuberoso et al., 2007). Tocopherols are one of the major antioxidants that have an ability to donate their phenolic hydrogen to lipid peroxides thus terminating the chain reaction of lipid peroxidation (Seppanen et al., 2010). Tocopherols are basically involved in two processes of oxidation. Firstly, tocopherol itself gets oxidized by transfer of one electron to a tocopheryl-radical and secondly tocopherol forms hydroperoxide by reacting with singlet oxygen (Neely et al., 1988; Krieger-Liszkay and Trebst, 2006). Both these reactions can be reversed when tocopheryl-radical and hydroperoxide react with ascorbic acid and the tocopherol is retrieved. Hence, a single molecule of tocopherol participates in many chain breaking event of lipid peroxidation before being subjected itself for degradation due to long-term storage (Sattler et al., 2004). Tocopherol is converted to tocopherylquinone when there is an acidic condition through the splitting of hydroperoxide, which is an irreversible reaction (Krieger-Liszkay and Trebst, 2006). Tocopherols are considered efficient antioxidants since they generate stable antioxidant radicals. They also have the power to compete with the lipid substrate for oxygen (Van Aardt et al., 2004). Tocopherol is essential for seed longevity and absence of tocopherol in seeds increased the oxidation of PUFA and raised the lipid radicals content from three to four fold (Sattler et al., 2004).

In vitro efficiency of tocopherol depends upon the other chemical species functioning as pro-oxidants and synergists in system (Kamal-Eldin and Appelqvist, 1996). Rodrigues et al., (2015) established the protective role of gamma tocopherol in seeds of *Jatropha curcas* L. seeds during aging and not in extracted oil.

Gawrysiak-Witulska et al., (2011) investigated that rapeseeds stored for 18-days showed a 14.4% decrease in tocopherol content when the moisture content reached 15.5% and temperature of 30 °C. When the seeds were stored with 10% moisture content then there was 4% loss of tocopherol content in the same seeds. Gopalakrishnan et al., (1996) showed that rapeseeds stored for 10 days at room temperature had a loss of 50% in tocopherol content. Goffman and Möllers (2000) observed a change in tocopherol content in rapeseeds when they were subjected to storage at different temperature of 5°C, 20°C, and 40°C. Significant decline in tocopherol content was found only at 40° C. Gawrysiak-Witulska et al., (2009) observed when rapeseeds were dried and stored at 10°C there was a 23 % – 30% decline in tocopherol content.

Vaidya and Eun (2013) studied tocopherol stability in oil extracted from both unroasted and roasted walnut seeds stored in darkness. They showed that unroasted walnut oil had a tendency to lose more tocopherol during storage than roasted walnut oil. In case of the unroasted walnut oil, the total tocopherol content was significantly decreased from 314.88 µg/g to 234.58 µg/g after 12 days of storage with a rate of reduction of 2.17% per day. On the other hand, the tocopherol content in roasted walnut oil decreased from 277.77 µg/g to 238.63 µg/g and the rate was 1.18% per day during the same period. Continuous degradation of tocopherol is evident from the negative correlation between tocopherol content and storage period. As the storage period gets increased the stability of the tocopherol gets decreased and its degradation increases. The reason for degradation is connected with their role as antioxidants during the autoxidation of the lipids (Lee et al., 2007).

Vaidya and Eun (2013) through their studies on walnut also showed that alpha-tocopherol was least stable during storage and delta-tocopherol was most stable. Low stability can be attributed to its low redox potential. When there is an increase of methyl groups, it enhances the power of hydrogen donation. Therefore, alpha-tocopherol, which is highly methylated, have stronger tendency to donate hydrogen atom to lipid peroxy radicals than gamma and delta tocopherol (Kamal-Eldin and Appelqvist, 1996). Thus, alpha tocopherol is more vulnerable to deterioration during storage (Isnardy et al., 2003).

De Camargo et al., (2012) studied the changes in tocopherol content during 6 months of storage of the gamma radiation-induced oxidation of peanuts. The results showed that among all the other species of tocopherols, alpha tocopherol is most sensitive to gamma radiation. As the storage period prolonged, alpha tocopherol content got declined more than the other forms of tocopherols.

The above literature indicates an essential role of tocopherol in seed longevity and seed protection during storage of oil seeds. Storage period and total tocopherol content in oil extracted from seed have negative correlation indicating the degradation of tocopherol during prolonged period of storage.

1.8.6. Effect of seed storage on germination

Germination test for long time was the only method to check the seed quality and viability after storage (Maeda et al., 1986). During storage if the seed quality is not maintained then it reflects directly on the percentage of germination of seeds. Pradhan and Badola, (2012) concluded convincingly that the storage condition highly affected *S. chirayita* seed germination percentage and as storage duration increased it declined the percentage of germination irrespective of storage condition. Fantazzini et al., (2018) observed a decrease in germination of naturally stored coffee seeds for 6 months. Aged seeds of sunflower (Kibinza et al., 2006), soybean (Xin et al., 2014), sycamore maple and norway maple (Pukacka and Ratajczak, 2007), yellow-lupin (Garnczarska et al., 2009) and neem (Sahu et al 2017) showed a decline in germination. The evaluation of germination and seed vigour are meant to evaluate the quality of seeds. The purpose of maintaining high quality in seeds during storage may include: (i) to ensure high germination percentage, (ii) production of seedlings that may have better potentials to withstand abiotic and biotic stresses, (iii) rapid and uniform germination, (iv) uniform enhanced crop maturity and increased efficiency of harvest, and (v) production of desired plant population (Moncaleano-Escandon et al., 2013).

Decrease in germination is found in various seeds due to NA and accelerated aging. Seeds like peanut (Sung and Jeng, 1994), cotton (Iqbal et al., 2002), chickpea

(Kapoor et al., 2010), rice (Kapoor et al., 2011), soybean (Xin et al., 2014), pea (Veselova et al., 2015) and rice (wang et al., 2018) showed significant decrease in seed germination, when they were stored for long period of time or subjected to artificial aging.

In all the above studies, declined seed germination is associated with increased MDA level, electrolyte leakage, and decreased antioxidant enzymes activity. Lowering of biochemical activities or termination of such activities during prolonged or artificial aging might be a possible reason for loss of seed germination. During storage, the stored products are utilized to obtain energy for sustenance of seed viability and seed vigour. The enzymes responsible for conversion of reserve food to energy, get either impaired or destroyed or their activities get decreased during storage (Iqbal et al., 2002). The power house of the cell- mitochondria undergoes membrane damage during storage. Damaged mitochondria is inefficient to supply the needed energy for emergence of radicle from seeds (Gidrol et al., 1998). At a molecular level, there is a loss of DNA integrity, DNA damage, and DNA degradation during prolonged or artificial aging. Such changes at the molecular level are associated with decline in germination and in shoot length, root length, and seed vigour index. Impaired transcription leading to incomplete or faulty protein and enzymes synthesis essential for seed germination is attributed to DNA damage and loss during storage (Kapoor et al., 2010).

Decreased seed germination is also associated with high relative humidity and temperature during storage or accelerated aging (Powell and Matthews, 1977; Suma et al., 2013). Seed moisture content certainly gets elevated when the surrounding relative humidity is high during storage. High moisture content beyond thresh-hold point is detrimental to seeds (Abba and Lovato, 1999). A series of unwanted metabolic processes is triggered when there is high relative humidity, temperature, and ambient oxygen. These metabolic processes which are undesirable cause accumulation of reactive oxygen species, increase in respiration and consumption of storage reserves, loss of membrane integrity, and finally seed deterioration. The deteriorated seeds yield less or no germination (McDonald, 1999; Liu et al., 2016).

1.8.7. Seed oil content during seed storage

Bhattacharya and Rah (2002) have shown that there is a decrease in oil content of maize, groundnut, and soybean seeds due to storage with increase of free fatty acids. Simic et al., (2007) reported that average seed oil content was significantly reduced by 0.82% in maize seeds, 8.53% in sunflower seeds, and 2.19% in soybean seeds due to prolonged storage of these seeds. Oil content was negatively correlated with storage longevity (Sisman and Delibas, 2004; Ghasemnezhad et al., 2007)

Along with the decrease in oil content there was rancidity observed in the oil due to storage longevity. Deterioration of lipids due to oxygen is found to be the predominant reason for both decrease and rancidity of oil content during prolonged storage (Ahmadkhan et al., 2000; Morello et al., 2004).

Gupta and Rao (2008) subjected the *Jatropha curcas* L. seeds for 80 days of storage under ambient temperature. After 80 days of storage, there was a significant decrease in oil content. Similar kinds of observation were made when rape seeds were stored for 9 months at 10°C (Savi´c et al., 2009). Such studies have also been done with argon kernel oil and *Ricinodendron heudelotti* kernels (Harhar et al., 2010; Tchiegang et al., 2005).

Abreu et al., (2013) studied the deterioration of sunflower seeds during storage. In this study, changes in seed quality when stored in different package systems under different environmental condition was investigated. It was reported that decrease in oil content was evident after 12 months of storage irrespective of package and environmental condition. Lipid peroxidation and formation of free fatty acids are the two major reasons for reduction in oil content and rapid deterioration of the oil-seed (Balesevic-Tubic et al., 2005). A similar study was undertaken by Lins et al., (2014) and Chew et al., (2017) in sunflower seeds (cv. BRS 122).

Two major reasons that can be attributed to reduction of oil content in seed during storage are hydrolysis and oxidation of lipids (Sathya et al., 2006). Hydrolysis of triacyl glycerol (TAG) may be an early occurrence but oxidation is a multi-step process leading to the formation of multiple undesired products leading to seed deterioration (List et al., 2005). Since oil is nothing but lipids that has many double bonds in the form of unsaturated fatty acids, they are susceptible to oxidation during storage regardless of whether there are esterified in triacylglycerol molecule or there are in free state. During the long-term storage since there is a greater exposure to atmospheric oxygen there is increased amount of oxidation taking place at the seeds. This could be considered one of the reasons for the reduction of the oil content in seeds during storage. On the other hand, to maintain the seed viability the stored lipid products are utilized as energy source. This could be another reason for the reduction of oil during storage. Enzymes such as acyl-CoA oxidase, citrate synthase, malate synthase, lipases, and catalase are enzymes that are mainly involved in oil and fatty acids metabolism of the seeds (Kindle, 1987). These enzymes are highly oxygen-dependent for their optimal activities. This can be considered another reason for the reduction of the oil in seed during prolonged storage. Temperature also has great influence in the reduction of seed oil during storage.

1.8.8. Free fatty acids content during seed storage

Oil properties (Kulkarni and Dalai, 2006), oil refinement, oil quality (Ramos et al., 2009), and transesterification process of raw material determine the properties of biodiesel. Fatty acid composition of the raw material determines the biodiesel properties (Wang et al., 2012). Degree of unsaturated fatty acids (DU) and long chain saturated fatty acids (LCSF) present in the extracted oil contribute to the stability of the biodiesel (Ramos et al., 2009). During the process of transesterification process, the fatty acid compositions of the raw material do not undergo any changes. To ascertain the oxidative stability of oil; parameters like cetane number (CN), iodine value (IV), oxidation stability (OS) and cold filter plugging point (CFPP) are determined. Fatty acid composition in biodiesel determines these critical parameters (Ramos et al., 2009). Ramos et al., (2009) undertook the study of correlations of the edible oil derived from olive, corn, palm, and rape to find out the best-suited raw material for biodiesel conversion. Since biodiesel oxidation stability mainly depends on the amount of double bond present, it is highly important that OS and DU are correlated to find the suitability and quality of biodiesel. More over estimating and evaluating the amount of monosaturated, polysaturated, and unsaturated fatty acids of non-edible oil for feedstock helps to decide the quality of biodiesel (Chuah et al., 2016).

Reduction in the oil content/lipid or changes in fatty acid profile of the seed oil is reported by Sathya et al., (2006). They have reported that hydrolysis and oxidation are responsible for both decrease of oil content and change in fatty acid of the seed oil due to storage of oil seeds. High moisture present during storage causes hydrolysis of the triglycerides leading to the release of fatty acids from triacylglycerol moieties. Many vegetable oils contain significant amount of monounsaturated and polyunsaturated fatty acids which render stability (Knothe, 2007). Hence, due to prolonged storage and high moisture and temperature there occurs the oxidation of fatty acids present either within the triacylglycerol moiety or in a free state. The oxidized fatty acid leads to the formation of various undesired and detrimental components.

Decline in phospholipids and PUFA that lead to loss of seed vigour and seed deterioration are associated with the lipid-related changes of oil seeds during storage longevity (Priestley and Leopold, 1983). Zacheo et al., (1998) subjected almond seeds to accelerated aging by exposing them to 80% moisture and 20% temperature for 40 days. He showed that there is decrease in lipid content along with levels of linoleic acid (C18:2) and linolenic acid (C18:3) after 20 days of accelerated aging.

Ouzouline et al., (2009), using two soft wheat varieties studied the effect of accelerated aging. Eight days of accelerated aging resulted in loss of germination and decrease in unsaturated fatty acids. In both the varieties, oleic acid and linoleic acid content decreased. As compared to neutral lipids the amount of polar lipids got decreased more. In natural lipids there was an increase of free fatty acids on one hand and on the other hand decrease of triacylglycerol. Among polar lipids phosphatidylcholine decreased during accelerated aging. It was concluded that decrease in lipid content is associated with loss of seed vigour and inhibition of germination in wheat seeds under accelerated aging. Tammela et al., (2000) undertook studies on the storage of pine seeds for 29 years and observed a decrease in phospholipids and triacylglycerol contents because of lipid peroxidation. Increase in free fatty acids, MAG and DAG contents were also reported.

Decrease in lipid content was also seen in seeds of pea (Powel and Matthews, 1981), soybean (Stewart and Bewley, 1980), peanut (Pearce and Abdel Samad, 1980), tomato (Francis and Coolbear, 1984), sunflower (Gidrol et al., 1989), and cotton (Freitas et al., 2006) on storage and accelerated aging. Tatić et al., (2012) studied the soybean seed viability and changes in fatty acids affected by seed aging. They investigated that linoleic acid and oleic acid content were decreased due to NA of 12 months and accelerated aging of 5 days.

Fotouo et al., (2016) studied the effect of storage of *Moringa oleifera* Lam. seed oil. It was observed that no significant changes in oil content was found until 12 months of seed storage but thereafter, 24 months of storage resulted in decrease of seed oil. On the other hand, the content of free fatty acids increased significantly even after 12 month of storage and overshoot the recommended value of 2% for biodiesel in 24 months of storage.

Sharma et al., (2013) subjected soybean seeds to NA of 180 days and reported that lipid peroxidation increased with aging which was associated with decreased activities of antioxidant enzymes. They also reported that the lipid peroxidation was positively correlated with the increase of free fatty acids. Increased free fatty acids result in loss of seed viability and seed deterioration.

1.8.9. Molecular level changes during seed storage

1.8.9.1. *DNA damage*

During long-term storage there is a loss of seed viability and seed deterioration. Seeds when subjected to storage undergo a process of desiccation in which the cytoplasm of the seed cells undergo transformation from fluid state to glassy viscosity. This glassy state suits the seed to withstand the ongoing desiccation during long-term storage. Cellular components are stabilized and their mobility is restricted severely in glassy state (Buitink and Leprince, 2008). When there is low ambient temperature and high humidity, the seed longevity or seed viability comes under the threat of deterioration. High temperature and humidity promotes irreversible aggregation of denatured protein due to increased fluidity of cytoplasm. Deleterious molecules “Reactive oxygen species” bring in oxidation of proteins, lipids, DNA, RNA, and cell membranes (Osborne, 1994; Bailly, 2004; Rajjou and Debeaujon, 2008; Rajjou et al., 2008).

During storage loss of seed viability, vigour and seed deterioration are often connected with the accumulation of DNA lesions and DNA strand breakage resulting in loss of DNA integrity and later DNA disintegration (Cheah and Osborne, 1978). The free radical molecules generated during aging oxidize the DNA causing strand breakage. ROS cuases DNA strand break either by direct desaturation of deoxyribose units or through covalent modifications of bases (Bray and West, 2005) (Figure 1.14). The hydroxylation of the C-8 position of guanine to form 7, 8-dihydro-8-oxoguanine

(8-oxoG) is one of the modifications that takes place (Bray and West, 2005; Biedermann et al., 2011) (Figure 1.14). 8-oxoG is mutagenic in nature thus it can easily pair with adenine (A) or cytosine (C), and the mispairing with A residue forms GC to TA trans version at the time of replication. When seeds with DNA damage occurred during storage are subjected to imbibition, it results in DNA repair provided there is no severe DNA damage beyond the point of repair. Base excision repair system (BER) mainly targets to remove the mutagen 8-oxoG and stopping it from further mismatch of base pairing (Bray and West, 2005; Biedermann et al., 2011; Chen et al., 2012). The first target of BER is to cleave N-glycosyl-bound DNA between the base and sugar to excise 8oxoG base to create apurinic/apyrimidinic (AP) sites. This is catalyzed by DNA glycosylases of BER. Through the activity of endonuclease, AP site is hydrolyzed at the phosphodiester bond resulting in the cleavage of DNA backbone. Finally, DNA polymerase and ligases come into action to complete the rest of the repairing system of BER (Sano et al., 2016).

Tolentino et al., (2003) subjected long leaf pine seeds to natural and accelerated aging. Both types of aging caused reduction in DNA content and hence germination. Gel electrophoresis analysis revealed that seeds stored for 270 days at 30°C showed a marked DNA fragmentation. Beyond 270 days of NA at 30°C caused further severe fragmentation. With respect to accelerated aging, there occurred DNA fragmentation in seeds leading eventually to seed deterioration. However, seeds stored at 4°C did not reveal any DNA fragmentation. Oxidative stress and lipid peroxidation are the two major causes of seed DNA fragmentation, loss of germination, and DNA content. Accelerated aging imparts more intense deleterious effects on seeds than NA since it maintains high temperature and moisture.

El-Maarouf-Bouteau et al., (2011) investigated the effect of sunflower seed aging on DNA to determine the correlation between loss of seed viability with programmed cell death (PCD). After 7 days of aging at 35°C at various moisture content, it was reported that higher the moisture content, lower the seed viability. Epigenetic phenomena play an important role in plants. Sahu et al., (2013) showed that when carbon at fifth position of cytosine gets methylated it acts as a conserved epigenetic marker and functions as silencer in gene activity. Transcription of gene is blocked when cytosine of gene is methylated at promoter region (Li et al., 2014). This epigenetic modification is quiet abundant in most of the plant species indicated by presence of 5-methylcytosine (m5C) (Liu et al., 2013). The specific feature of this epigenetic phenomenon is that the pattern of genome wide methylation is static but specific modifications such as 5-methylcytosine (m5C) and histones modification occur only by specific manner as per the situation (Brautigam et al., 2013). Plants maintain genomic plasticity towards rapid adaptation to new external conditions of both biotic and abiotic stress without allowing any alterations in the sequence of DNA. This plasticity is achieved through DNA methylation (Causevic et al., 2005; Sahu et al., 2013). Several reports have shown the importance of this DNA methylation for seed development and viability (Adams et al., 2000; Xiao et al., 2006a, b; Gehring et al., 2009). Michalak et al., (2015) studied 5-methylcytosine alterations in DNA during aging of Quercusrobur seeds. There was a decrease in the total amount of 5-methylcytosine in seeds after 18 months of storage. This was significantly correlated to decrease in seed viability, germination, and seedling emergence.

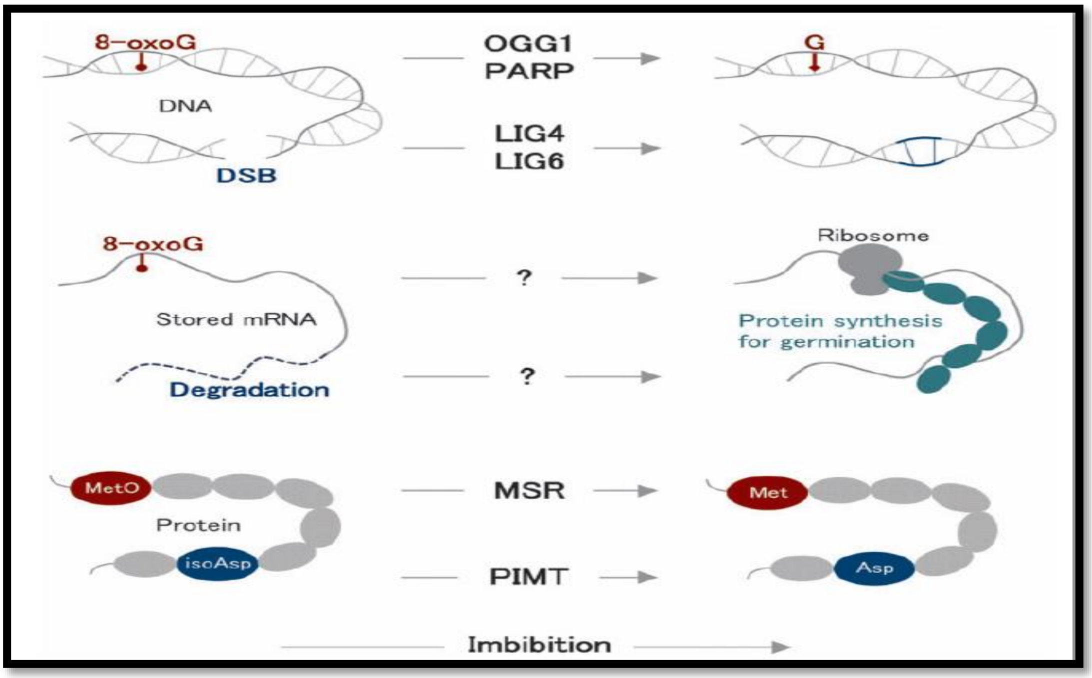


Figure 1.14: DNA, mRNA, and protein repair system in germination and seed longevity. Damaged DNA, RNA, and protein molecules accumulate in dry seeds and are repaired upon imbibition. 8-oxG, 7, 8-dihydro-8-oxoguanine; DSB, DNA double-strand break; Met, methionine; MetO, methionine sulfoxide; Asp, L-aspartyl; isoAsp, L-isoaspartyl (Sano et al., 2016).

1.8.9.2. *mRNA damage*

Stored dry seeds contain either stored mRNA or long-lived mRNA (Sano et al., 2015). More than 10,000 mRNA species were found in the dry seeds of *Arabidopsis* (Nakabayashi et al., 2005). These mRNAs are essential for seed germination (Sano et al., 2015). The stored long-lived mRNAs are important for rapid seed recovery related metabolic activities upon imbibition (Brooker et al., 1978, Martin and Northcote, 1981, Galland et al., 2014, Sano et al., 2015). Kranner et al., (2011) have shown reduction in mRNAs and loss of mRNA integrity in deteriorating seeds of *Pisum sativum* to correlate with loss of germination. Due to the single strand structure of RNA, it is more vulnerable than DNA to reactive oxygen species attack. Seed longevity depends on the stability of mRNA as it controls translation and any loss of translational activity in imbibed seeds would result in non-viable seeds (Rajjou et al., 2008). Loss of integrity of RNA impacts the seed longevity to bring in early seed deterioration (Figure 1.14). Molecular mechanism for repair of damaged mRNA in aging seeds is yet to be elucidated (Sano et al., 2016).

1.8.9.3. *Protein damage*

If stored seeds do not undergo imbibition within the stipulated time and continue to be in storage then proteins are either spontaneously oxidized by free radicals or undergo covalent modification which results in loss of functions (Rajjou et al., 2008).

According to Stadtman (2006), ROS in stored seeds targets the methionine of the protein moiety and oxidizes it to convert into methionine sulfoxide. This is one of the major forms of damage caused to protein. This oxidized methionine sulfoxide reversibly gets converted to methionine by methionine sulfoxide reductase (MSR) and repairs the damage (Weissbach et al., 2005). Weissbach et al., (2005) showed that seed longevity is connected with the increased activity of methionine sulfoxide reductase in two *M. truncatula* genotypes. MSR is essential for the seed longevity.

In older proteins L-aspartyl or asparaginyl residue gets covalently modified to abnormal isoaspartyl (isoAsp) residue resulting in loss of function (Lowenson and Clarke 1992). L-Isoaspartyl O-methyltransferase (PIMT) successfully repair such damaged protein and helps protein to gain function. In almost 45 plant species, PIMT activity has been found and activity of PIMT is highly localized in seed (Mudgett et al., 1997).

1.8.10. Oil body changes during seed storage

Seed lipids are well gathered in the form of oil bodies (OB). Oil bodies are distinctive, large, and prominent structures within the vacuoles of endosperm of the seed. Triacylglycerol (TAG) is present as a core constituent of the oil bodies. This TAG is surrounded by phospholipids which in turn is stabilized by amphipathic embedded proteins. Among these proteins, oleosins dominate in number. The size and structure of oil bodies are determined by these types of proteins, which give stability to oil bodies. They are amphipathic proteins and have molecular weight of 15 – 26 kDa. Depending on the size, they are classified into high molecular weight oleosins (H-form) and low molecular weights oleosins (L-form). Oleosins normally contain two domains: (i) Hydrophilic and (ii) Hydrophobic. The hydrophilic region contains N and C domain, which differ with respect to the amount of amino acids they contain. Hydrophobic domain which is central and conserved contains 75 uncharged aliphatic amino acids that includes three proline residues and a motif of one serine residue. These two types of residue interact with each other in such way that they form proline knot, which is characteristic of oleosin. Caleosins and steroleosins are the other two types of oleosin-like oil body proteins (Popluechai, 2010).

There are three types of oleosin found in the seeds of *Jatropha curcas* L. They are oleosin 1 (*JcOle1*), oleosin 2 (*JcOle2*), and oleosin 3 (*JcOle3*). All three were isolated and characterized at both gene and transcript level where as L form of Oleosin 3 was characterized at protein level as well (Popluechai, 2010). Surface properties of oil bodies and their integrity are maintained by these three oleosins in *Jatropha curcas* L. seeds. When the oil bodies were subjected to trypsin digestion it damaged the surface oleosins of the oil bodies thus reducing the electronegative charge and steric hindrance contributed by oleosins. Coalescence of oil bodies occurred due to reduction of electronegative charge and steric hindrance. Such kind of stability afforded by oleosin to oil bodies within seeds was also found in seeds of other oil plants (Popluechai, 2010). Even though isolation and characterization of oleosins were done in many seeds of oil plants, yet the effect of aging on oleosin in oil seeds, particularly in *Jatropha curcas* L. seed has not been done so far.

Aim of this study

Systematic and detailed evaluation of the biochemical, molecular and physiological changes occurring during storage/aging in post-harvested seeds of *Jatropha curcas* L. has not been reported so far. Such a study will help in mapping the biochemical changes and seed deterioration occurring during storage. In order to study the effect of storage/aging on orthodox seeds like *Jatropha curcas* L., the seeds need to be stored for a longer period of time. To overcome this difficulty, accelerated aging protocols are used to enable accurate measurement of the changes occurring during seed storage but within a short time frame. Both accelerated aging and salt-accelerated aging are widely accepted as models to study changes occurring in seeds during storage. In these techniques, seeds are exposed to higher temperature and humidity in order to accelerate their aging artificially (Powell and Mathews, 1984 and Mendonca et al., 2008).

The economic potential and selection of *Jatropha curcas* L. seeds as best feedstock for biodiesel depend on the quality of the oil produced from the seeds. This study will help us know the measures to be taken to ensure seed quality and longevity. Thus, the present study aims to investigate the sensitivity of *Jatropha curcas* L. seeds to storage under natural as well as accelerated aging and to evaluate the biochemical and physiological changes occurring in these seeds during storage and accelerated aging.