ANTIOXIDANT EFFICACY OF MANGO AND JACKFRUIT SEED EXTRACTS ON OXIDATIVE STABILITY OF PALM OIL ON DEEP-FRYING

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Approval Letter

This *dissertation* entitled *Antioxidant Efficacy of Mango and Jackfruit Seed Extracts on Oxidative Stability of Palm Oil on Deep-Frying* presented by Amul Ghimire has been accepted as the partial fulfillment of the requirements for the B. Tech. in Food Technology.

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Abstract

The effects of antioxidants on the changes in quality characteristics of refined, bleached, and deodorized (RBD) palm oil during deep-fat frying (at 180°C) of potato chips for seven consecutive days in five systems were compared in this study. The systems were RBD palm oil without antioxidant (control), with 200 ppm butylated hydroxytoluene (BHT), 200 ppm butylated hydroxyanisole (BHA), 200 ppm mango seed extract, and 200 ppm jackfruit seed extract. Fried oil samples were analyzed for peroxide value (PV), iodine value (IV) and free fatty acid (FFA) content. The extract were prepared by reflux using ethanol solvent in the ratio of sample/ethanol of 1:3 (w/v) and then by vacuum evaporation at 40°C. The yield, flavonoid content, phenol content and DPPH radical scavenging activities of both of the extracts were determined. The average yields were found to be 11.28% and 10.37 % for MSK extract and JSK extract respectively. The total phenol content for Mango Seed Kernel (MSK) extract (117 mg Gallic acid equivalent/g) was found to be significantly higher ($p \leq 0.05$) than that for Jackfruit Seed Kernel (JSK) extract (27.7 mg Gallic acid equivalent/g of the dried extract). Similarly, the total flavonoid content for MSK extract (0.372 mg Rutin equivalent/mg dry extract) was found to be significantly higher ($p \leq 0.05$) than that for JSK extract (0.195 mg Rutin equivalent/mg dry extract). The lowest IC_{50} value of the MSK extract (0.112mg/mL) in comparison to BHA(0.2108), BHT(0.1558) and JSK(0.2884) indicated that it exhibited the highest DPPH (2, 2-Diphenyl-1-Picryl-Hydrazyl) free radical scavenging effect. With respect to PV, the oxidative stability was decreased in the order MSK extract ≈ BHT>JSK extract ≈ BHA>control. Judging the FFA content of the oil at the end of frying operation, the increment of FFA content in oil system was in the order MSK extract<BHT<JSK extract ≈ BHA and the change in IV showed that the effectiveness in protecting oxidation of unsaturated fatty acid was in the order: MSK extract>BHA>JSK extract>BHT>control. Therefore, MSK extract was best among the other antioxidant used such as BHA, BHT and JSK in terms of health aspect and protection against the oxidation process of the oil. Thus, mango and jackfruit seed could be effectively utilized for the preparation of extracts which could be used instead of the synthetic antioxidants such as BHA and BHT for increasing the oxidative stability of palm oil on deep fat frying of potato chips.

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List of Abbreviations

Abbreviation	Full form
AOAC	Association of Official Analytical Chemists
RBD	Refined Bleached and Deodorized
BHT	Butylated Hydroxy Toluene
BHA	Butylated Hydroxy Anisole
TBHQ	Tertiary-Butyl Hydro Quinone
PG	Propyl Gallate
PV	Peroxide Value
IV	Iodine Value
FFA	Free Fatty Acids
MSK	Mango Seed Kernel
JSK	Jackfruit Seed Kernel
FDA	Food and Drug Administration
HPLC	High Pressure Liquid Chromatography
IP	Induction Period
LOX	Lipoxygenases
SOD	Superoxide dismutase
CAT	Catalase
GT	Glutathion

ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
GAE	Gallic Acid Equivalent
RE	Rutin Equivalent
LSD	Least Square Difference
DPPH	2, 2-Diphenyl-1-Picryl-Hydrazyl
A_{AR}	Antiradical Activity
ANOVA	Analysis of Variance
EC	Efficient Concentration
HDL	High Density Lipid
LDL	Low Density Lipid

Part I

Introduction

1.1 General introduction

Antioxidants are chemicals that reduce the rate of oxidation reactions. Oxidation reactions are chemical reactions that involve the transfer of electrons from one substance to an oxidizing agent. Antioxidants can slow these reactions either by reacting with intermediates and halting the oxidation reaction directly, or by reacting with the oxidizing agent and preventing the oxidation reaction from occurring (Rahman, 2007).

Antioxidants play a major part in ensuring that our foodstuffs keep their taste and color and remain edible over a longer period. Their use is particularly important for avoiding oxidation of fats and fat-containing products. When antioxidants are thoroughly mixed with fat or oil, the onset of the final stages of autoxidation when rancidity -development of unpleasant off-flavors and odors becomes evident, is delayed. Another important reason is that certain vitamins and various amino acids can easily be destroyed by exposure to air, and antioxidants serve to protect them. They also help to slow down the discoloration of fruit and vegetables (FDA, 1992). TBHQ, PG, BHA and BHT are the most commonly used antioxidants in food industries. Oxygen reacts preferentially with these antioxidants rather than oxidizing fats or oils, thereby protecting them from spoilage. In addition to being oxidizable, BHA and BHT are fat-soluble. Both molecules are incompatible with ferric salts. In addition to preserving foods, BHA and BHT are also used to preserve fats and oils in cosmetics and pharmaceuticals (FDA, 1992).

Although there is vacuum packaging or packing under inert gas to include oxygen and refrigeration/freezing, which generally reduce the rate of autoxidation unfortunately they are not always applicable. Furthermore it is a fact that little oxygen is needed to initiate and maintain oxidative process and it is quite impossible or expensive to remove the least traces of air from a food product. Also antioxidants are only one means of fending off oxidation (Allen and Hamilton, 1983). Antioxidants are generally effective, easily applied and inexpensive. Other justification for need of an antioxidant use are- an antioxidant can extend the shelf life of a food, reducing wastage and complaints it can reduce nutritional

losses (oil soluble vitamin) and more important it can widen the range of fats which can be used (Allen and Hamilton, 1983).

Modern lipid science discovered that antioxidants are widely distributed in nature (Bracco *et al.*, 1981). Natural antioxidants seem to be more adequate for protection against oxidation and have many inherent qualities unsuppressed by the synthetic antioxidants (Loliger, 1983). It imparts no adverse effect in its long run of use; natural antioxidants do not have a quantitative tolerance limit in Federal Regulations. Natural antioxidant have to be used in a number of cases even when there is no choice for anything else, because of company policy or food legislations and public pressure group. There are some scientific evidences which align sufficiently in support for using natural antioxidants (Bauernfeind and Cort, 1974).

Plants have excellent antioxidant properties and these effects are mainly attributed to their phenolic constituents. The phenolic constituents of mango seed kernel are reported to be mainly gallic and ellagic acids, as well as gallates. Gallotannins and condensed tannin-related polyphenols are also reported to be present. A phytochemical investigation of mango stem bark extract has led to the isolation of gallic acid, 3,4-dihydroxy benzoic acid, gallic acid methyl ester, gallic acid propyl ester, mangiferin, (+)-catechin, (-)-epicatechin, and benzoic acid and benzoic acid propyl ester. Antioxidative activity of tamarind seed coat is due to the presence of 2-hydroxy-3,'4'-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenyl acetate and epicatechin. Avocado seeds are claimed to be rich in a complex mixture of polyphenolic compounds, ranging from (+)-catechin and (-)-epicatechin to highly polymeric substances; a proanthocyanidin has also been identified (Soong and Barlow, 2004).

1.2 Statements of the problems and the justifications

The major problem associated with fats and oils is related to its instability in different conditions. Since they are widely used as the chief ingredients in industrial processes such as deep frying and culinary practices, more concern must be given towards the oxidative stability. Though different types of synthetic antioxidants are being used, such as BHA, BHT, PG and TBHQ, more concern should be being given towards the natural source of antioxidants which would be free of any hazards and side effects while using in food products. Extracts of seed kernel of different fruits could be used industrially for the

preservation of food so that the cost of food production might be reduced in addition to the minimum side effects of natural antioxidants. Thus, this work is focused to utilize the by-products of fruits, explore the antioxidant capacities in seed kernels of various fruits and reduce the use of synthetic antioxidants.

Several natural antioxidants have already been isolated from different kinds of plant materials, such as oil seeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs. Antioxidant compounds have been identified in the seeds of citrus, grape, mango, canola, sunflower, primrose, sesame, flaxseeds and lupin; yet, studies relating to the antioxidant activity of tropical and subtropical fruit seeds have been sparsely report (Soong and Barlow, 2004)

Fruit seeds have not generally received much attention as antioxidant sources and this could be due to their lack of popularity and lack of commercial applications (unlike oil seeds). However, there are considerably higher ratios of by-products arising from fruit-processing plants as fruit juices and derived products have experienced growing worldwide popularity. For example, there is about 3×10^5 ton of dry mango seed kernels available annually in India after consumption or industrial processing of mango fruits. It would be beneficial, in improving the complete utilization of the seeds, if they could be used as a source of natural food additives and ingredients (Soong and Barlow, 2004).

1.3 Objectives

1.3.1 General objectives

The general objective of the study was to compare the antioxidant efficacy of mango and jackfruit seed extracts on oxidative stability of palm oil on deep-frying of potato chips.

1.3.2 Specific objectives

The specific objectives of the study were to:

a. Compare the antioxidant activities of seed of mango and jackfruit with the synthetic antioxidants.
b. Estimate the level of usage of individual seed kernel as antioxidants.
c. Identify the highest antioxidant capacity among the used seed kernels.
d. Study the oxidative stability of palm oil at frying temperature using the natural antioxidants from the seed kernel.

e. Utilize the by-products of the fruits to minimize the cost of added synthetic antioxidants in the frying oil.

1.4 Significance of the study

This work is focused on to the study about the antioxidant characteristics of fruit seeds such as mango and jackfruit seed kernels which are abundantly available in most of the fruit processing industries and discarded as waste. The utilization of these fruit seeds as source of antioxidants could add value in the minimization of bio-wastes. Since the world of present time is heading towards the concept of naturalism, this work could be helpful in replacing the synthetic antioxidants. Use of natural antioxidants removes the chances of toxicity and health hazard cases of the user. Thus, this work is comprehensive to the desires of contemporary consumers.

1.5 Limitations

The total phenolic profile could not be evaluated due to the lack of HPLC. Similarly various other methods for accessing antioxidant activity could not be followed due to the lack of available instruments and laboratories facilities. Also the large number of seed kernels of different varieties of fruits could be studied due to the time constraints.

Part II

Literature review

2.1 Rancidity in fats and oils

Rancidity is defined as the subjective organoleptic appraisal of the off-flavor quality of food. It is subjective because the ability to perceive an off-flavor varies from person to person (Hamilton *et al.*, 1997). Dietary lipids, naturally occurring in raw food materials or added during food processing, play an important role in food nutrition and flavor. Meanwhile, lipid oxidation is a major cause of food quality deterioration. Oxidation may occur via different routes and includes autoxidation, photo-oxidation, thermal oxidation and hydrolytic processes in the presence of catalytic systems such as light, heat, enzymes, metals, metalloproteins, and micro-organisms, all of which lead to production of undesirable flavor and loss of essential amino acids, fat-soluble vitamins, and other bioactives harmful to health (Shahidi, 2005; Shahidi and Zhong, 2005).

2.1.1 Autoxidation or peroxidation

Autoxidation is the most common process leading to oxidative deterioration and is defined as the spontaneous reaction of atmospheric oxygen with lipids. It is a free-radical chain reaction, involving a complex series of reactions that initiate, propagate, and terminate the chain. The chain reaction is initiated by abstraction of an allylic hydrogen to give an allylic radical stabilized by delocalization over three or more carbons (Scrimgeour, 2005). This production of free radicals may take place by direct thermal dissociation (thermolysis), by hydroperoxide decomposition, by metal catalysis and by exposure to light (photolysis) with or without the intervention of photosensitizers (Frankel, 1980).

Autoxidation reactions commonly show an induction period, which is a period during which very little change occurs in the lipids. After the end of the induction period, oxidative deterioration of the lipids occurs much more rapidly. Off-flavours become most noticeable after the end of the induction period. One consequence of the sharp rise in the concentration of off-flavour components after the end of the induction period is that the rate of deterioration of foods is relatively insensitive to the precise fat content of the food (Gordon, 2001).

The induction period (IP) is very sensitive to small concentrations of components that shorten the IP, the pro-oxidants, or lengthen the IP, which are the antioxidants. Metal ions are the most important pro-oxidants in foods, whereas antioxidants include compounds that act by radical scavenging, metal chelation or other mechanisms. The presence of an induction period is characteristic of chemical reactions that proceed by a free-radical mechanism. The level of free radicals in oils is generally low, but in frying the rapid formation of free radicals can lead to the combination of free radicals to form triglyceride dimers.

2.1.1.1 Mechanism of autoxidation

As a free-radical reaction, autoxidation proceeds in three distinct steps (Fig.2.1). The first step is initiation in which lipid radicals are formed from lipid molecules.

a) Initiation

Initiation takes place by loss of a hydrogen radical in the presence of trace metals, light or heat. The resulting lipid free radicals ($R^{\bullet}$) react with oxygen to form peroxy radicals ($ROO^{\bullet}$) (Frankel, 1980). However, in oils there is often a trace of hydroperoxides, which may have been formed by lipoxygenase action in the plant prior to and during extraction of the oil. Secondary initiation by homolytic cleavage of hydroperoxides is a relatively low energy reaction, and it is normally the main initiation reaction in edible oils. This reaction is commonly catalysed by metal ions (Gordon, 2001).

Since initiation reaction is thermodynamically difficult (activation energy of about 35 kcal/mol), production of the first few radicals is necessary to begin the propagation reaction (Nawar, 1985). The reaction of a lipid with molecular oxygen in its excited singlet state ($^1O_2$), or by metal catalysis, or by exposure to light can form lipid peroxy radicals. The conversion of triplet oxygen to singlet oxygen may occur in many ways; the most important is via photosensitization of the natural pigments present in foods or by direct enzymatic production of singlet oxygen direct or indirect consequence of the action of certain microsomal oxidases, lipoxygenase, and prostaglandin synthetase (Erickson, 2002).

b) Propagation

After initiation, propagation reactions occur in which one lipid radical is converted into a different lipid radical (Gordon, 2001). In the propagation process, $ROO^{\bullet}$ react with more RH to form lipid hydroperoxides (ROOH), which are the fundamental primary products of autoxidation (Frankel, 1980). Conditions that determine the chain propagation length include initiation rate, structures of aggregates (increasing with increasing structure of the aggregates), temperature, presence of antioxidants, and chain branching. Chain branching involves the breakdown of fatty acid hydroperoxides to the lipid peroxyl or alkoxyl radical. Given the bond dissociation energies of ROO–H (about 90 kcal/mol) and RO–OH (about 44 kcal/mol), spontaneous decomposition is unlikely at refrigerated or freezing temperatures. Instead, breakdown of hydroperoxides would be dominated by one-electron transfers from metal ions during low temperature storage.

$$Fe^{2+} + ROOH \rightarrow Fe^{3+} + LO\bullet + OH^{-}$$

The major contributors to decomposition of lipid hydroperoxides in food and biological systems would be heme and nonheme iron, with reactions involving the ferrous ion occurring much more quickly than those involving ferric ion (Erickson, 2002).

c) Termination

To break the repeating sequence of propagating steps, two types of termination reactions are encountered: radical–radical coupling and radical–radical disproportionation, a process in which two stable products are formed from A• and B• by an atom or group transfer process. In both cases, nonradical products are formed (Erickson, 2002).

Initiation $X^{\bullet} + RH \longrightarrow R^{\bullet} + XH$

Propagation $R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$

$ROO^{\bullet} + R'H \longrightarrow ROOH + R^{\bullet}$

Termination $ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$

$ROO^{\bullet} + R^{\bullet} \longrightarrow ROOR$

$R^{\bullet} + R^{\bullet} \longrightarrow RR$

Secondary initiation

$$ROOH \longrightarrow RO^{\bullet} + {}^{\bullet}OH$$

$$2\ ROOH \longrightarrow RO^{\bullet} + ROO^{\bullet} + H_2O$$

Metal-catalysed initiation

$$M^{n+} + ROOH \longrightarrow RO^{\bullet} + {}^{-}OH + M^{(n+1)+}$$

$$M^{(n+1)+} + ROOH \longrightarrow ROO^{\bullet} + H^{+} + M^{(n)+}$$

Fig.2.1 Mechanism of lipid autoxidation

However, the termination reactions are not always efficient. When coupling gives rise to tertiary tetroxides, they decompose to peroxyl radicals at temperatures above -80°C and to alkoxyl radicals at temperatures above -30°C. Secondary and primary peroxyl radicals, on the other hand, terminate efficiently by a mechanism in which the tetroxide decomposes to give molecular oxygen, an alcohol, and a carbonyl compound (Erickson, 2002). In frying oils termination reactions are important, with dimers and higher polymers contributing to the increased viscosity of the oil (Gordon, 2001).

A generalized scheme for autoxidation of lipids is given in Fig.2.2.

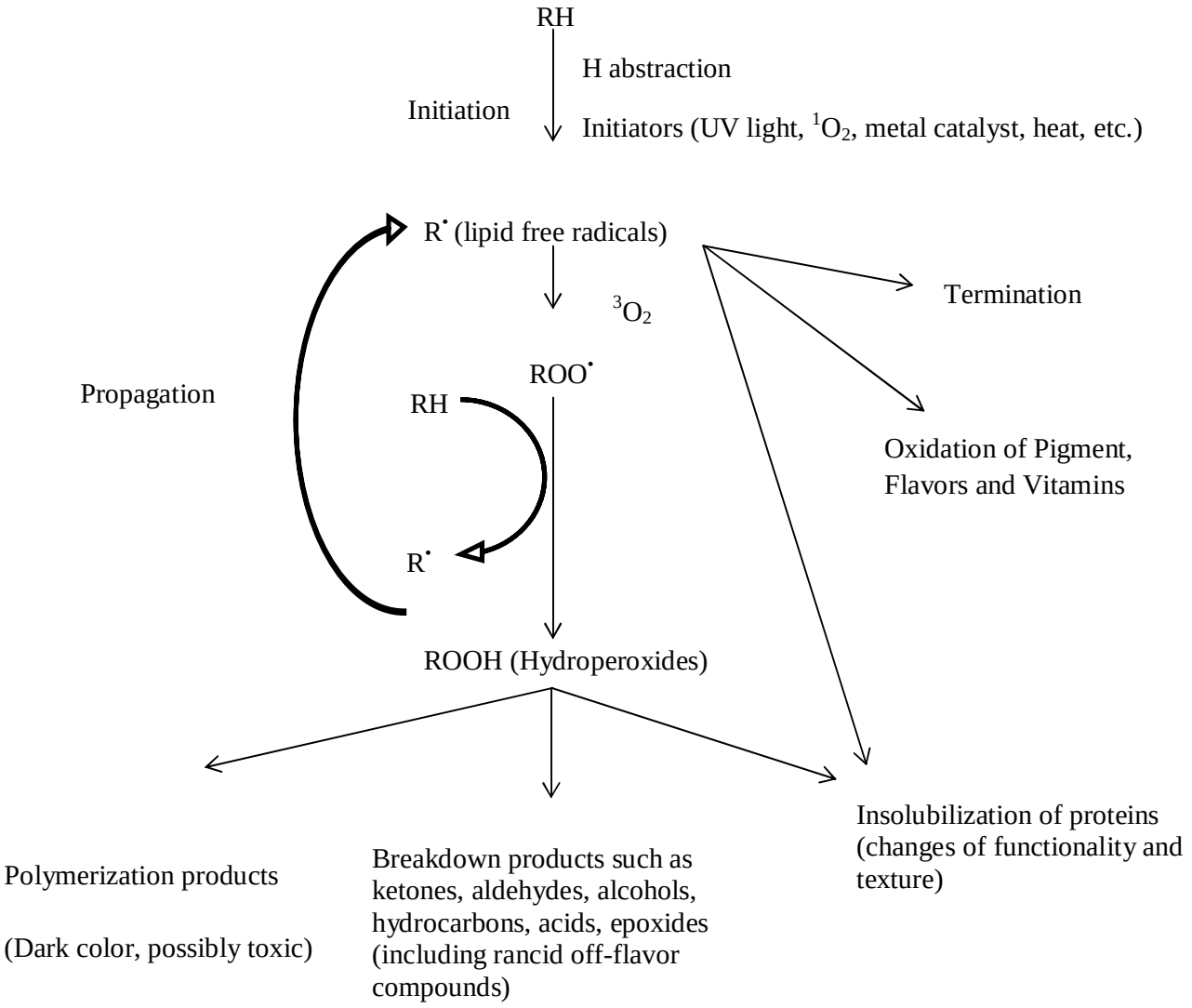


Fig.2.2 General scheme for autoxidation of polyunsaturated fatty acids of lipids and their consequences

Source: (Shahidi *et al.*, 1992)

2.1.2 Photo-oxidation

Photosynthesized oxidation (photooxidation) involves direct reaction of light-activated, singlet oxygen with unsaturated fatty acid and the subsequent formation of hydroperoxides (Reische *et al.*, 2002). Two pathways have been proposed for photosensitized oxidation. In

type I, as shown in Fig.2.3, sensitizer in the triplet state is excited by light energy to the singlet state followed by intersystem crossing to an activated triplet state. Energy is then transferred from the triplet sensitizer to oxygen to give excited singlet oxygen (Frankel, 1980).

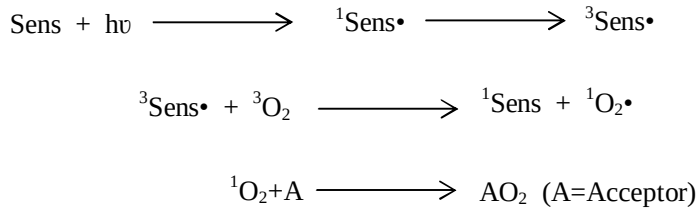


Fig.2.3 Mechanisms of photosensitized oxidation (Type I)

In type II, as shown in Fig.2.4, the triplet sensitizer forms a sensitizer-oxygen complex that reacts with a substrate acceptor to give a peroxide and regenerates the sensitizer (Frankel, 1980).

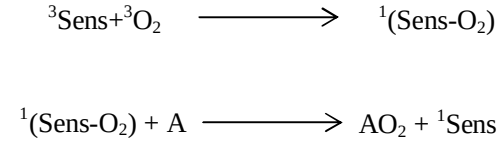
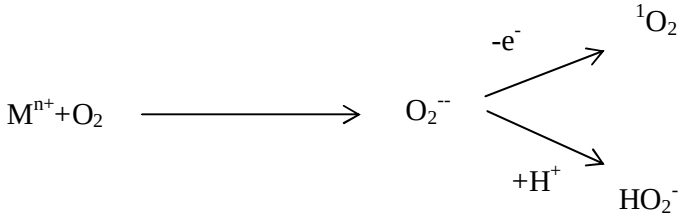


Fig.2.4 Mechanisms of photosensitized oxidation (Type II)

Several substances are commonly found in fat-containing foods that can act as a photosensitizer to produce $^1O_2$. These include natural pigments that are generally present in foods such as chlorophyll *a*, pheophytin *a*, myoglobin, hematoprophyrin, flavin, and riboflavin. The synthetic colorant, erythrosine, may also act as an active photosensitizer, and metal ions could be involved in activating molecular oxygen to produce singlet oxygen as shown in the reaction:



2.1.3 Thermal oxidation

High temperatures (e.g., frying temperatures) have sufficient energy to break covalent C–C or C–H bonds in the acyl backbone to form a variety of lipid alkyl radicals, which then start the radical chains of oxidation. Moderate temperatures have lower energy, so act primarily by breaking O-O bonds in traces of ROOH performed by other reactions, particularly metals, lipoxygenase, or photosensitizers. The RO• and •OH thus generated abstract hydrogens from neighboring lipids to form R• and initiate radical chains. As shown by the activation energies for the individual stages of lipid oxidation, ROOH decomposition and its subsequent contribution to propagation is the major catalytic effect of heat. Effects of increased ROOH decomposition are amplified by increased rates of subsequent H abstractions by RO• and ROO•, which is reflected in the doubling of oxidation rate for every 10°C rise in temperature (Schaich, 2005).

The chemical reactions that occur during the thermal oxidation process contribute to the formation of both volatile and nonvolatile decomposition products. The oxidation mechanism in frying oils is similar to autoxidation at 25°C; however, the unstable primary oxidation products—hydroperoxides—decompose rapidly at 190°C into secondary oxidation products such as aldehydes and ketones. Secondary oxidation products that are volatile significantly contribute to the odor of the oil and flavor of the fried food. If the secondary oxidation products are unsaturated aldehydes, such as 2,4-decadienal, 2,4-nonadienal, 2,4-octadienal, 2-heptenal, or 2-octenal, they contribute to the characteristic fried flavor in oils that are not deteriorated and can be considered desirable. However, saturated and unsaturated aldehydes, such as hexanal, heptanal, octanal, nonanal, and 2-decenal, have distinctive off-odors in olfactometry analyses of heated oil. Fruity and plastic are the predominate off-odors of heated high oleic oils and can be attributed primarily to heptanal, octanal, nonanal, and 2-decenal (Warner, 2005).

Analysis of primary oxidation products, such as hydroperoxides, at any point in the frying process provides little information because their formation and decomposition fluctuate quickly and are not easily predicted. During frying, oils with polyunsaturated fatty acids, such as linoleic acid, have a distinct induction period of hydroperoxides followed by a rapid increase in peroxide values, then a rapid destruction of peroxides. Measuring levels of polyunsaturated fatty acids, such as linoleic acid, can help determine

extent of thermal oxidation. Oxidative degradation produces oxidized triglycerides containing hydroperoxide, epoxy, hydroxy, and keto groups and dimeric fatty acids or dimeric triglycerides. Volatile degradation products are usually saturated and monounsaturated hydroxy, aldehydic, keto, and dicarboxylic acids; hydrocarbons; alcohols; aldehydes; ketones; and aromatic compounds.

2.1.4 Hydrolytic rancidity

Lipoxygenases are active lipid oxidation catalysts found in plants and some animal tissues. Lipoxygenases (LOX) catalyze the aerobic oxidation of fatty acids with cis-nonconjugated pentadiene structures to generate optically active conjugated LOOH without releasing a lipid free radical (Schaich, 2005). Lipoxygenase activity requires the presence of free polyunsaturated fatty acids. Linoleic acid is the most common substrate in plant foods. The enzyme occurs in a variety of isozymes, which often vary in optimum pH, as well as product and substrate specificity (Pokorny *et al.*, 2001). Soybean seed LOX was one of the first enzymes to be purified and crystallized and is the best characterized of all plant LOXs (Zhuang *et al*., 2005).

LOXs contain one atom of nonheme iron. The iron atom in lipoxygenase alternates between the Fe(II) and the Fe(III) states during catalysis. The native-resting LOX, a relatively inactive form, is in the high-spin Fe(II) state. Activation of the native E-Fe(II) requires oxidation of the iron atom from Fe(II) to Fe(III) by the reaction product, fatty acid hydroperoxides or H_2O_2. Because of the product activation requirement, the oxygenation reaction exhibits a characteristic initial lag period (Zhuang *et al.*, 2005). The LOX-catalyzed reaction is initiated by the stereospecific removal of hydrogen from the intervening methylene group of a polyunsaturated fatty acid used as a substrate (Gordon, 2001; Zhuang *et al.*, 2005). This occurs possibly by a basic amino acid, followed by the transfer of an electron back to the iron atom to reduce it to E-Fe(II) (Zhuang *et al.*, 2005).

The enzyme–alkyl radical complex is then oxidised by molecular oxygen to an enzyme–peroxy radical complex under aerobic conditions, before electron transfer from the ferrous atom to the peroxy group occurs. Protonation and dissociation from the enzyme allow the formation of the hydroperoxide. Under anaerobic conditions, the alkyl radical dissociates from the enzyme–alkyl radical complex, and a mixture of products including dimers, ketones and epoxides is produced by radical reactions (Gordon, 2001).

Lipoxygenase activity can be controlled by heat inactivation and plant breeding programs that decrease the concentrations of these enzymes (Decker, 2005). When lipoxygenase action needs to be inhibited without thermal inactivation, reduced oxygen pressures offer an excellent means of control (Schaich, 2005). Phenolics are capable of indirectly inhibiting lipoxygenase activity by acting as free radical inactivators, but also by reducing the iron in the active site of the enzyme to the catalytically inactive ferrous state (Decker, 2005).

2.2 Antioxidant

The term "antioxidant" must be carefully applied because some substances that retard lipid oxidation under one set of conditions actually promote it under a different set (Reische *et al.*, 2002). In a biological system, an antioxidant can be defined as ''any substance that when present at low concentrations compared to that of an oxidizable substrate would significantly delay or prevent oxidation of that substrate''(Halliwell and Gutteridge, 1995). The oxidizable substrate may be any molecule that is found in foods or biological materials, including carbohydrates, DNA, lipids, and proteins (Wanasundara and Shahidi, 2005).

However, regulatory bodies that overlook the food-supply categorize antioxidants under food additives and define them as ''substances used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation''(FDA, 2012).

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are very short lived, with half-lives in milli-, micro- or nanoseconds (Devasagayam *et al*., 2004). Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases as well as ageing (Devasagayam *et al.*, 2004; Halliwell *et al.*, 1995).

Antioxidants are substances that neutralize free radicals or their actions (Devasagayam *et al.*, 2004) . Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being (Percival, 1998).

It has been suggested that an ideal food grade antioxidant should be safe; not impart color, odor, or flavor; be effective at low concentrations; be easy to incorporate; survive after processing; and be stable in the finished product (carry-through) as well as being available at a low cost (Shahidi *et al.*, 1992).

2.2.1 History of antioxidants and their use

Use of substances to enhance quality of food by means of delaying lipid oxidation has been in practice for centuries, although it was not chemically defined or understood. The first recorded scientific observation on oxidation inhibitors came from Berthollet in 1797 and later from Davy. Their theory was described as ‘‘catalyst poisoning’’ in oxidative reactors, and this was well before the free radical theory of peroxidation had been proposed. Duclaux first demonstrated participation of atmospheric oxygen in oxidation of free fatty acids. Later, it was found that oxidation of unsaturated acylglycerols can generate rancid odors in fish oils (Wanasundara and Shahidi, 2005).

The earliest reported work on the use of antioxidants to retard lipid oxidation appeared in 1843, in which Deschamps showed that an ointment made of fresh lard containing gum benzoin (contains vanillin) or populin (from polar buds, contains saligenin and derivatives) did not become rancid as did the one with pure lard. Interestingly, the first reports on antioxidants employed for food lipids were about using natural sources; in 1852, Wright reported that elm bark was effective in preserving butterfat and lard. Moureu and Dufraise first reported the possibility of using synthetic chemicals, especially phenolic compounds, to retard oxidative decomposition of food lipids. Their work provided the basic information leading to theories of lipid oxidation and antioxidants, which they referred to as ‘‘inverse catalysis’’ (Wanasundara and Shahidi, 2005).

2.2.3 Classifications of antioxidants

2.2.3.1 Based on antioxidant mechanism

Antioxidants can inhibit or retard oxidation in two ways: either by scavenging free radicals, in which case the compound is described as a *primary antioxidant (*chain breaking antioxidants) , or by a mechanism that does not involve direct scavenging of free radicals, in which case the compound is a *secondary antioxidant (*preventive antioxidants) (Pokorny *et al.*, 2001; Wanasundara and Shahidi, 2005). The main difference with primary

antioxidants is that the secondary antioxidants do not convert free radicals into stable molecules (Wanasundara and Shahidi, 2005).

According to this classification, some antioxidants exhibit more than one mechanism of activity, therefore, referred to as multiple-function antioxidants. Another commonly used classification categorizes antioxidants into primary, oxygen scavenging, and secondary, enzymatic and chelating/sequestering antioxidants (Wanasundara and Shahidi, 2005).

a. Primary antioxidants

Primary antioxidants are also referred to as type 1 or chain-breaking antioxidants. Because of the chemical nature of these molecules, they can act as free radical acceptors/scavengers and delay or inhibit the initiation step or interrupt the propagation step of autoxidation. Primary antioxidants cannot inhibit photosensitized oxidation or scavenge singlet oxygen (Wanasundara and Shahidi, 2005). They are effective at extremely low concentrations of 0.01% or less and for some of them the effectiveness decreases as concentration is increased. At high concentrations they may become pro-oxidant due to their involvement in the initiation reactions (Bartosz *et al.*, 1997). The primary antioxidants (AH) react with lipid and peroxy radicals (ROO•) and convert them to more stable, non-radical products as shown in the Fig.2.5(Wanasundara and Shahidi, 2005).

$$ROO\bullet + AH \longrightarrow ROOH + A\bullet$$

$$R\bullet + AH \longrightarrow RH + A\bullet$$

$$ROO\bullet + A\bullet \longrightarrow ROOA$$

$$RO\bullet + AH \longrightarrow ROH + A\bullet$$

$$RO\bullet + A\bullet \longrightarrow ROA$$

$$A\bullet + A\bullet \longrightarrow AA$$

Fig.2.5 Mechanism of primary antioxidant activity (AH is an antioxidant molecule)

Source : (Wanasundara and Shahidi, 2005)

Most of the primary antioxidants that act as chain breakers or free radical interceptors are mono- or polyhydroxy phenols with various ring substitutions. The antioxidant effectiveness is influenced by the chemical properties of the compound including hydrogen

bond energies, resonance delocalization, and susceptibility to autoxidation. The ability of the primary antioxidant molecule to donate a hydrogen atom to the free radical is the initial requirement. When considering all of these, the primary antioxidants or free radical scavengers can inactivate at least two free radicals, the first one during the interaction with peroxy radical and the second in the termination reaction with another peroxy radical (Wanasundara and Shahidi, 2005).

The compounds that exhibit primary antioxidant activity include polyhydroxy phenolics as well as the hindered phenolics. There are several synthetic ring-substituted phenolics as well as naturally occurring phenolic compounds that may perform via the primary antioxidant mechanism. The common feature of all of these antioxidants is that they are mono or polyhydroxy phenols with various ring substitutes. Substitution with an electron-donating group/s ortho and/or para to the hydroxyl group of phenol increases the antioxidant activity of the compound by an inductive effect (e.g., BHA). Thus, the presence of a second hydroxyl group in the 2- (ortho) or the 4-position (para) of a phenol increases the antioxidant activity (e.g., TBHQ) (Wanasundara and Shahidi, 2005).

Table 2.1 Primary antioxidants that are commonly used in foods

Natural	Synthetic
Carotenoids	Butylated hydroxyanisole (BHA)
Flavonoids	Butylated hydroxytoluene (BHT)
Phenolic acids	Ethoxyquin
Tocopherols and tocotrienols	Propyl gallate (PG)
	Tertiary-butylhydroquinone (TBHQ)

Source: (Wanasundara and Shahidi, 2005)

To be most effective, primary antioxidants should be added during the induction or initiation stage of the autoxidation reaction cascade. Antioxidants can scavenge the formed free radicals, as the cyclical propagation steps have not occurred at this stage. Addition of primary antioxidants to a lipid that already contains substantial amounts of lipid peroxides may result in loss of antioxidant activity (Buck, 1984).

b. Secondary antioxidants

Secondary antioxidants are also classified as preventive or class II antioxidants. They offer their antioxidant activity through various mechanisms to slow the rate of oxidation reactions. The main difference with primary antioxidants is that the secondary antioxidants do not convert free radicals into stable molecules. They act as chelators for prooxidant or catalyst metal ions, provide H to primary antioxidants, decompose hydroperoxide to nonradical species, deactivate singlet oxygen, absorb ultraviolet radiation, or act as oxygen scavengers. They often enhance the antioxidant activity of primary antioxidants (Pokorny *et al.*, 2001; Wanasundara and Shahidi, 2005). Examples of these secondary antioxidants include dilauryl thiodipropionate and thiodipropionic acid (Shahidi and Naczk, 2004a).

Table 2.2 **Compounds that exhibit secondary antioxidant activity**

Mode of Activity	Compounds in use
Metal chelation	Cirtic, Malic, Succinic and Tartaric acids Ethylenediaminetetraacetic acid, Phosphates
Oxygen scavenging and reducing agents	Ascorbic acid, Ascorbyl palmitate, Erythorbic acid, Sodium erythorbate, Sulfites
Singlet oxygen quenching	Carotenoids (b-Carotene, Lycopene and Lutein)

Source: (Wanasundara and Shahidi, 2005)

Normally, secondary antioxidants only show antioxidant activity when a second minor component is present. This can be seen in the case of sequestering agents such as citric acid which are effective only in the presence of metal ions, and reducing agents such as ascorbic acid which are effective in the presence of tocopherols or other primary antioxidants (Pokorny *et al.*, 2001). Table 2.2 provides examples of some of these compounds that exhibit secondary antioxidant activity (Wanasundara and Shahidi, 2005).

c. Synergism and synergists

Synergism is the cooperative effect of antioxidants or an antioxidant with other compounds to produce enhanced activity than the sum of activities of the individual component when

used separately. Two types of synergism are observed, one involving primary antioxidants only and the other involving a combination of primary antioxidants with metal chelators or peroxy scavengers (Wanasundara and Shahidi, 2005).

Chelating antioxidants are also referred to as synergists because they enhance the activity of phenolic antioxidants. This synergism is sometimes referred to as acid synergism when the chelator is citric or other acids. Citric acid and TBHQ show excellent synergism in vegetable oils (Reische *et al.*, 2002).

In an antioxidant combination that contains compounds exhibiting different mechanisms of action and physical properties, inhibition of oxidation occurs in many different phases. This suggests that food antioxidants should be carefully selected considering such factors as the type of oxidation catalyst, physical state of lipid (bulk, emulsified), pH, temperature, and the ability to interact with other components in the food (Wanasundara and Shahidi, 2005).

2.2.3.2 Based on origin

a. Synthetic antioxidants

Synthetic antioxidants are manmade and are used to stabilize fats, oils, and lipid containing foods and are mostly phenolic-based. Many compounds are active as antioxidants, but only a few are incorporated into food because of strict safety regulations. These phenolic derivatives usually contain more than one hydroxyl or methoxy group. Ethoxyquin is the only heterocyclic, N-containing compound that is allowed for use in animal feeds (Wanasundara and Shahidi, 2005).

Synthetic phenolic antioxidants are p-substituted, whereas the natural phenolic compounds are mostly o-substituted. The p-substituted substances are preferred because of their lower toxicity. The m-substituted compounds are inactive. Synthetic phenolic antioxidants are always substituted with alkyl groups to improve their solubility in fats and oils and to reduce their toxicity (Pokorny, 2007).

The primary mechanism of activity of these antioxidants is similar to those of primary antioxidants. When antioxidants are present in excess, the reaction of antioxidant free radicals with oxygen may become important; even their reaction with polyunsaturated fatty

acids has some impact on the course of oxidation. Therefore, at high concentrations, phenolic antioxidants may act as pro-oxidants (Wanasundara and Shahidi, 2005).

Some of the more popular synthetic antioxidants used are phenolic compounds such as butylated hydroxyanisol (BHA), butylated hydroxy-toluene (BHT), tertiary butylhydroquinone (TBHQ), and esters of gallic acid, e.g. propyl gallate (PG). The physical properties of some of the synthetic antioxidants are shown in Appendix A. Synthetic phenolic antioxidants are always substituted by alkyls to improve their solubility in fats and oils (Hudson, 1990). The four major synthetic antioxidants in use are subjected to a 'good manufacturing practice' limit of 0.02 % of the fat or oil content of the food (Simic, 1981).

The most suitable antioxidant for vegetable oils is TBHQ. BHA and BHT are fairly stable to heat and are often used for stabilisation of fats in baked and fried products. The ability of an antioxidant to withstand thermal treatment (e.g., frying or baking) and to retain sufficient stabilizing activity for the food (fried or baked) is termed as ''carry through property.'' The disadvantage of gallates lie in their tendency to form dark participates with the iron ions and their heat sensitivity. Some antioxidants, such as BHA and BHT, are used in combination with resulting synergistic effects (Omura, 1995; Sherwin, 1972). BHA is also synergistic with PG (Angelo, 1996). Similarly, high degree of synergy exist between α-tocopherol and phospholipids (Bandarra *et al.*, 1999).

The synthetic antioxidants have been very thoroughly tested for their toxicological behaviours, but some of them are coming, after a long period of use, under heavy pressure as new toxicological data impose some caution in their use (Thompson and Moldeus, 1988). In this context, natural products appear as healthier and safer than synthetic antioxidants (Valenzuela and Nieto, 1996). Since about 1980 natural antioxidants have appeared as an alternative to synthetic antioxidants (Pokorny *et al.*, 2001).

Despite the superior efficacy, low cost, and high stability of synthetic antioxidants in foods, the suspicion that these compounds may act to promote carcinogenicity has led to a decrease in their use (Reische *et al.*, 2002). A summary of physical properties of some commonly used synthetic antioxidants is given in Appendix 1 (Wanasundara and Shahidi, 2005).

b. Natural antioxidants

Antioxidants in foods may originate from compounds that occur naturally in the foodstuff or from substances formed during its processing. Natural antioxidants are primarily plant polyphenolic compounds that may occur in all parts of the plant. They are divided into several classes, i.e. phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones, isoflavones, proanthocyanidins) stilbenes, and lignans, which are distributed in plants and food of plant origin (Gharras, 2009). Plant phenolics are multifunctional and can act as reducing agents (free radical terminators), metal chelators, and singlet oxygen quenchers. Examples of dietary antioxidants include flavonoid compounds, cinnamic acid derivatives, coumarins, ascorbic acids, carotenoids, tocopherols, and polyfunctional organic acids (Shahidi *et al.*, 1992).

Natural antioxidants can be divided into three main types: first line defence antioxidants, second line defence antioxidants and line defence antioxidants (Sharma and Gupta, 2006). The first defence line is to inhibit the formation of active oxygen species and free radicals by sequestering metal ions, reducing hydroperoxides and hydrogen peroxide and to quench superoxide and singlet oxygen (Pokorny *et al.*, 2001). Superoxide dismutase (SOD), Catalase (CAT), Glutathion peroxidase (GTx) , glutathione reductase and some minerals like Se, Mn, Cu, Zn come under first line defence antioxidants (Sharma and Gupta, 2006).

The radical-scavenging antioxidants function as the second line defence. Vitamin E (mainly α-tocopherol) and vitamin C are major lipophilic and hydrophilic radical-scavenging antioxidants (Pokorny *et al.*, 2001). Glutathion, uric acid, albumin, bilirubin, carotenoids, flavonoids, etc., also comes under second line defence antioxidants (Sharma and Gupta, 2006). They scavenge radicals and inhibit chain initiation or break chain propagation. Polyphenolic compounds may also work as important radical-scavenging antioxidants. Natural phenolic compounds with antioxidant properties can be classified into a lipophilic group (tocopherols mainly) or a hydrophilic group (phenolic acids and flavonoids) (Maestri *et al.*, 2006).

The third-line of defence is the repair, *de novo* and clearance of oxidatively damaged DNA, damaged proteins, oxidized lipids and peroxides and also to stop chain propagation of peroxy lipid radical (Pokorny *et al.*, 2001; Sharma and Gupta, 2006). Various enzymes

such as lipases, proteases, tranferase, methionine sulphoxide reductase and DNA repair enzymes are responsible for such defence (Sharma and Gupta, 2006). There is another defence mechanism in which appropriate antioxidants are produced and transferred to the correct place at the correct time and in the correct amounts (Pokorny *et al.*, 2001).

Several natural antioxidants have already been isolated from different kinds of plant materials, such as oil seeds, cereal crops, vegetables, berries, fruits, leaves, roots, spices, and herbs (Dimitrios, 2006; Gharras, 2009; Heinonen and Meyer, 2002; Ramarathnam *et al.*, 1995). Antioxidant compounds have been identified in the seeds of citrus (Alessandra *et al.*, 1998), grape (Giamperi *et al.*, 2004; Jayaprakasha *et al.*, 2001), mango (Ashoush and Gadallah, 2011; Khammuang and Sarnthima, 2011; Maisuthisakul, 2011; Puravankara *et al.*, 2000; Schieber *et al.*, 2003), canola (Naczk *et al.*, 1998), papaya (Afolabi *et al.*, 2011; Zhou *et al.*, 2011), winter-melon (Mandana *et al.*, 2012), tamarind (Luzia and Jorge, 2011), pitanga (Bagetti *et al.*, 2009), cactus (Tlili *et al.*, 2011), jamun (Shahnawaz *et al.*, 2010), jackfruit (Gupta *et al.*, 2011), sunflower (Hamed *et al.*, 2012; Karamac´ *et al.*, 2012; Žilić *et al.*, 2010), sesame (Shahidi *et al.*, 1997), *Carya* (Zhiping *et al.*, 2011), millet (Asharani *et al.*, 2010; Karki and Kharel, 2012), flaxseeds (Oomah *et al.*, 1995), *Syzygium jambos (*Zheng *et al., 2011)* and lupin (S. Wang and Clements, 2008); yet, studies relating to the antioxidant activity of tropical and subtropical fruit seeds have been sparsely reported. Fruit seeds have not generally received much attention as antioxidant sources and this could be due to their lack of popularity and lack of commercial applications (unlike oil seeds). However, there are considerably higher ratios of by-products arising from fruit-processing plants as fruit juices and derived products have experienced growing worldwide popularity. It would be beneficial, in improving the complete utilization of the seeds, if they could be used as a source of natural food additives and ingredients (Soong and Barlow, 2004).

2.2.4 Biological effects of synthetic antioxidants

The biological effects can be grouped into the following major categories- modulation of growth, macromolecule synthesis and differentiation, modulation of immune response, interference with oxygen activation and miscellaneous. Not all of these effects have been observed with each of the synthetic antioxidant used (Schilderman, 1994).

Both Butylated hydroxyanisole (BHA) and Butylated hydroxytouluene (BHT) influence cellular metabolism through induction of various drug metabolizing enzymes such as cytochrome P450, cytochrome b, NADH reductase, glucose-6-phosphate dehydrogenase. BHA also has been shown to cause increase in organ weight like hypertrophy of liver, thyroid, adrenals, lungs and proliferation of endoplasmic reticulum in liver cells, whereas BHT has been shown to cause cell proliferation like alveolar cells and liver cells in addition to the proliferation of endoplasmic reticulum in liver cells (Gould, 1995). BHT may also cause increase in macromolecular synthesis in liver, lung cells & decrease in kidney cells and cell growth inhibition in kidney cells. Again, both BHA and BHT might cause induction of chromosomal & sperm abnormalities, cause inhibition of bacterial growth & toxin formation, lipid peroxidation in biological membranes and platelet aggregation, interfere with leukemia cell differentiation, prostaglandin synthesis and with immune response, enhance microsomal H_2O_2 formation, cause decrease in smooth muscle and heart muscle contractility and can have an influence on lipid metabolism and dietary levels of vitamin E (Blumenthal *et al.*, 1986; Gould, 1995). In addition, BHA might cause membrane labialization, cell lysis, alteration of lipid composition in liver, serum & platelets and might cause lesions in forestomach (Williams *et al.*, 1999).

On the other hand, BHT may cause inhibition of repair DNA synthesis in human lymphocytes, of drug cooxygenation during prostaglandin synthesis and of metabolic cooperation between V79 cells, might interfere with intracellular cGMP concentration and with prostaglandin synthesis, might cause shortening of life cycle in human leucocytes, membrane labialization leading to cell lysis and alteration of lipid composition in liver, serum & platelets. Moreover, BHT could also have antiviral activity, anti-inflammatory action and might play a role in the protection of sperm membrane from cold shock. BHT plays an important role in Vitamin K antagonism leading to a decrease in clotting factors (Williams *et al.*, 1999).

Propyl Gallate (PG), the most important gallate used as antioxidant preservative, may induce drug metabolizing enzymes like cytochrome b, NADH reductase, epoxide hydrolase, might inhibit mitosis in human cancer cells, lipid peroxidation in biological membranes and bacterial growth & toxin formation, may interfere with leukemia cell differentiation, with immune response and with prostaglandin synthesis, may enhance microsomal H_2O_2 formation and decrease smooth muscle and heart muscle contractility.

Furthermore, PG has been shown to have local anesthetic action and anti-inflammatory action (Venkatesh and Sood, 2011).

2.3 Free radicals

A free radical is any atom or molecule that contains one or more unpaired electron such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). However, in popular scientific/biomedical literature the term 'free radical' is used in a broad sense and also includes related reactive species such as 'excited states' that lead to free radical generation or those species that results from free radical reactions (Devasagayam *et al.*, 2004). The unpaired electrons alter the chemical reactivity of an atom or molecule, usually making it more reactive than the corresponding non-radical (Halliwell *et al.*, 1995). Details about some of the biologically important reactive species are presented as Appendix B (Devasagayam *et al.*, 2004). Radical formation in the body occurs by several mechanisms, involving both endogenous and environmental factors (Fig.2.6). Some internally generated sources of free radicals are Mitochondria, Xanthine oxidase, Peroxisomes, Inflammation, Phagocytosis, Arachidonate pathways, Exercise and Ischemia/reperfusion injury while Some externally generated sources of free radicals are: Cigarette smoke, Environmental pollutants, Radiation, Certain drugs, pesticides, Industrial solvents and Ozone (Lobo *et al.*, 2010).

It has to be emphasized that ROS and RNS are both produced in a well regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules (Yoshikawa *et al.*, 2000).

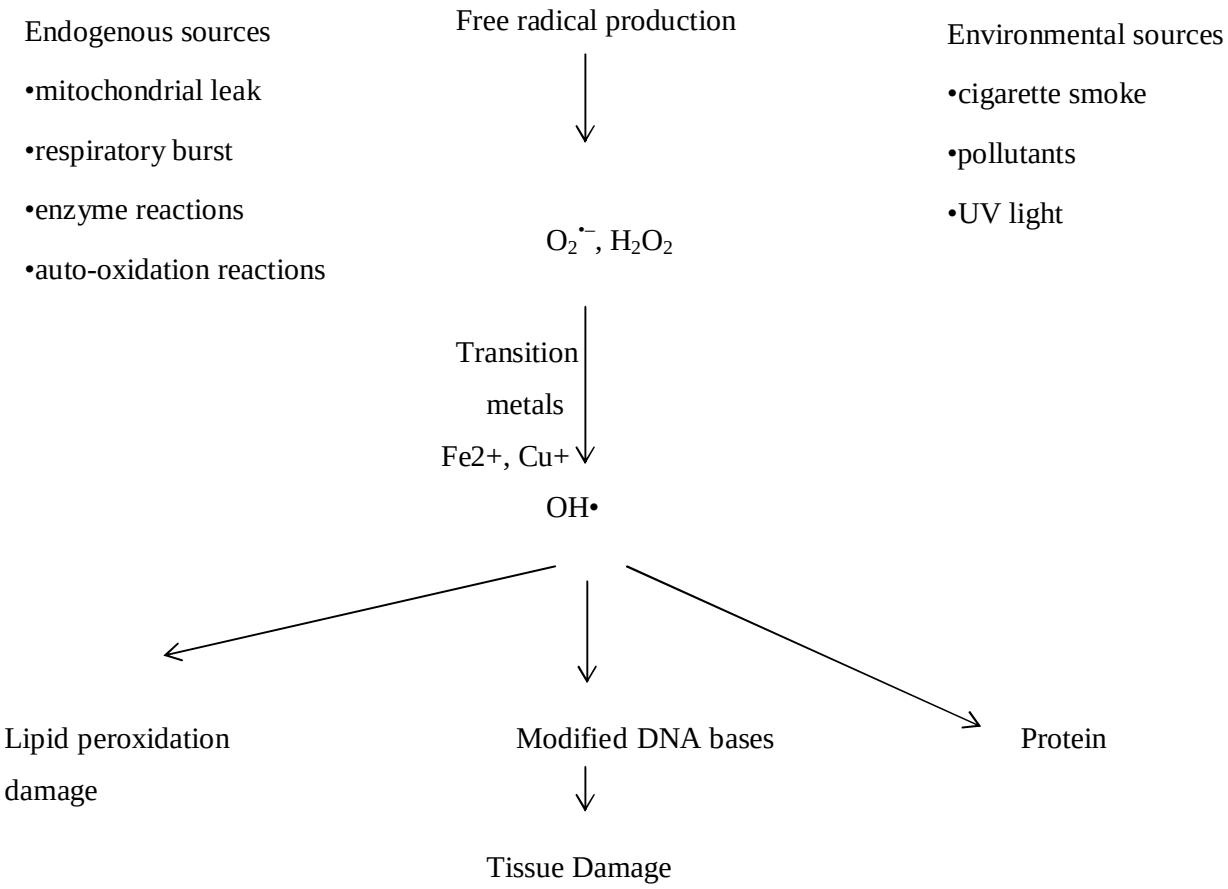


Fig.2.6 Major sources of free radicals in the body and the consequences of free radical damage

Source: (Young and Woodside, 2001)

2.4 Concept of oxidative stress

The relation between free radicals and disease can be explained by the concept of 'oxidative stress' (Sies, 1986). In a normal healthy human body, the generation of pro-oxidants in the form of ROS and RNS are effectively kept in check by the various levels of antioxidant defense. However, when it gets exposed to adverse physicochemical, environmental or pathological agents such as atmospheric pollutants, cigarette smoking, ultraviolet rays, radiation, toxic chemicals, over nutrition and advanced glycation end products (AGEs) in diabetes, this delicately maintained balance is shifted in favor of pro-

oxidants resulting in 'oxidative stress'. It has been implicated in the etiology of several (>100) of human diseases and in the process of ageing (Devasagayam *et al.*, 2004).

Some of the pathological conditions due to the 'oxidative stress' include AIDS, ageing, arthritis, asthama, atherosclerosis, autoimmune diseases, broncho-pulmonary dysplasia, carcinogenesis, cardiovascular dysfunction, cataract, diabetes, gastro-duodenal pathogenesis, genetic disordes, inflammatory diseases, ischemia reperfusion injury, liver disorders, muscular dystrophy, neurodegenerative diseases, parkinsons dementia, Alzheimer's disease, amyotropic lateral sclerosis, pulmonary fibrosis, radiation damage, retinopathy, rheumatism, skin disease porphyria and senile dementia stroke (Sharma and Gupta, 2006).

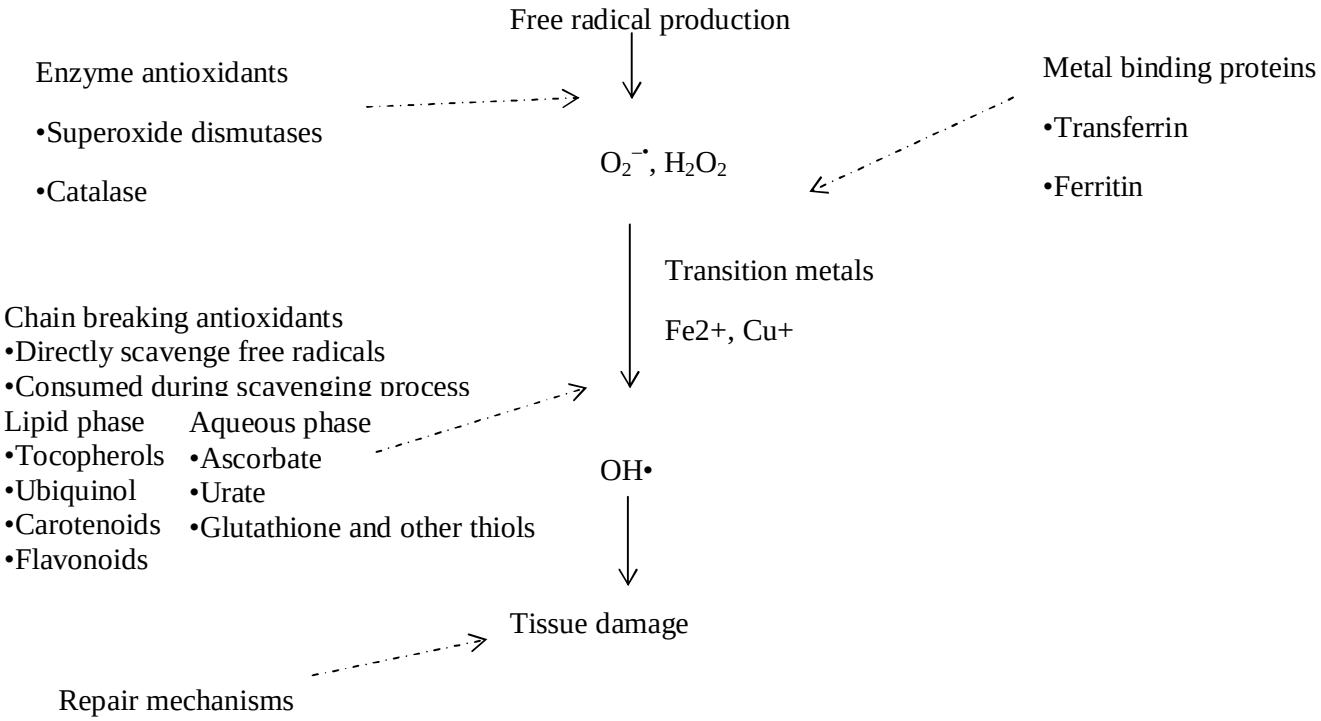


Fig.2.7 Antioxidant defenses against free radical attack

Source: (Young and Woodside, 2001)

Antioxidants may prevent and/or improve different diseased states (Knight, 2000). Because our endogenous antioxidant defenses are not completely effective, it seems reasonable to propose that dietary antioxidants are particularly important in diminishing the cumulative effects of oxidative damage over the long human lifespan, and that they

account for some of the beneficial effects of fruits, grains, and vegetables. For example, if continuous free-radical damage to DNA, perhaps not always efficiently repaired, is involved in the development of spontaneous cancers, more dietary antioxidants might help. An increased dietary intake of vitamin E seems to decrease death from myocardial infarction (Halliwell *et al.*, 1995). The defense mechanism of antioxidants against free radical attack is as shown in the Fig.2.7.

2.5 Deep-fat frying

Deep-fat frying is one of the most commonly used procedures for preparation and manufacture of foods throughout the world (Long, 1999). Nearly one-half of all lunch and dinner food orders in restaurants include one or more deep-fried items (Lalas and Dourtoglou, 2003). It is extensively used both at home and on a commercial scale to enhance the organoleptic characteristics of foods. In commercial deep-fat frying operations, a fat is exposed continously to heat, air, and light for hours per day at a temperature of around 180°C, and it may be used to cook a variety of foods (Jaswir and Che Man, 1999).

Deep-fat frying technology is considered to have originated and developed around the Mediterranean area due to the influence of olive oil. Other theories suggest that the technology developed in East Asia, mainly in a Chinese *wok* or in an Indian *kadhai* and migrated to Europe. Regardless of the actual origin, today, deep-fat fried foods are found in many countries around the world (Mallikarjunan *et al.*, 2010).

Deep-fat frying may be defined as the process of cooking foods by immersing them in an edible oil or fat maintained at a temperature of about 150–200 °C (Yamsaengsung and Moreira, 2002). In the first phase, within a few seconds, a thin crust forms, whose structure crucially affects the deep-frying process and the quality of the food with regards to fat absorption and crispness (Matthäus *et al.*, 2008). The simultaneous heat and mass transfer of oil, food and air during deep-fat frying produces the desirable quality of fried foods (Serjouie *et al.*, 2010).

Fats and oils have a high heat capacity, thereby enabling heat transfer at temperatures far above that of the boiling point of water. Due to the evaporation in the boundary zone between food and oil, the water bound in the food is gradually transported from thes inside to the boundary layer into the surrounding oil (mass transfer). The speed of transfer

depends more or less on the structure of the outer crust. As soon as the transfer of water ends, the temperature inside the food starts to rise above 100 °C. At this point the typical deep-frying aromas and flavors as well as the gold-yellow color begin to develop. With the rise of temperature in the boundary layer to more than 120°C, the formation of acrylamide begins, in particular in the presence of reducing sugars and asparagine like in grain- or potato products (Matthäus *et al.*, 2008).

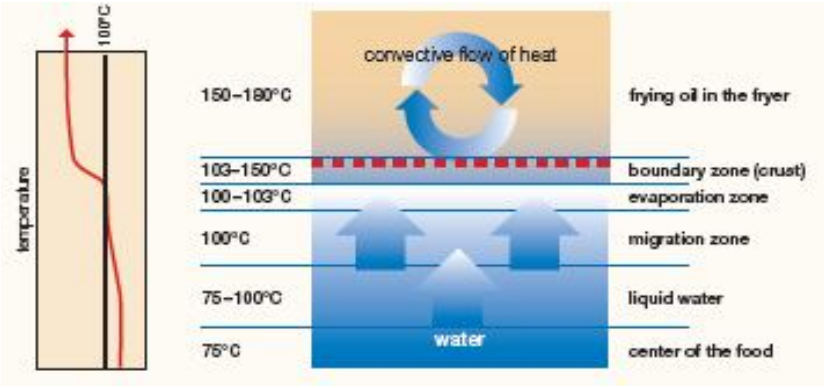


Fig.2.8 Heat and Mass Transfer during deep-frying

Source: (Matthäus *et al.*, 2008)

The moisture released from the food acts as a protective shield, preventing direct contact of oxygen to the fat surface. Consequently, frying fats that are constantly used for the preparation of meals deteriorate more slowly as if being heated up frequently without food (Matthäus *et al.*, 2008). The heat and mass transfer during deep frying is shown in Fig.2.8.

In the presence of oxygen, moisture, trace elements and free radicals, physiochemical reactions such as thermoxidation, hydrolysis, polymerisation, isomerization or cyclization take place at high temperatures of frying process, thus leading to the decomposition of frying oil and formation of monomeric, polymeric, primary and secondary oxidative compounds, therby affecting the quality of oil and fried product (Andrikopoulous *et al.*, 2002). At high temperatures, the formation of new compounds is very rapid, the oxygen pressure is reduced, and the hydroperoxides decompose rapidly and are practically absent above 150°C, indicating that the decomposition of hydroperoxides becomes faster than their formation (Marmesat *et al.*, 2010). As a result, dimeric and oligomeric triglycerides form from the very early stages of heating and a significant part of the new compounds

formed are non-oxygenated compounds due to the combination of two variables: high temperature and low oxygen pressure (Marmesat *et al.*, 2007). Physical and chemical changes in oils that occur during heating and frying are presented in Table 2.3.

Table 2.3 Effects of physical and chemical reactions during deep-fat frying

Physical Changes
Increased density, refractive index, conductivity, specific heat, viscosity, color, and foaming
Decreased surface tension, dielectric coefficient and smoke point
Chemical Changes
Increased free fatty acids, anisidine value, polar compounds, polymerised triacylglycerols, carbonyl compounds, high molecular weight products
Decreased unsaturation, iodine value, flavor quality, nutritive value (essential fatty acids)

Source: (Matthäus *et al.*, 2008; Warner, 2005)

Excessive heating of oils or fats can cause the formation of compounds with anti-nutritional properties, such as enzyme inhibitors, and accelerated loss of the antioxidant vitamins, such as vitamin E leading to growth and histologic changes in the gastrointestinal tissues. Moreover, oxidized lipids enhance peroxidation of the membrane macromolecules, contributing to their mutagenicity, genotoxicity and angiotoxicity. These cellular aberrations induced by thermally oxidized oils have been linked to growth retardation, colon carcinogenesis and reproductive disorders. Notwithstanding these potential adverse health effects, lipid oxidation also decreases the acceptability of fried products. Thus, to prevent undesirable changes in oil during storage and frying, antioxidants are required (Jaswir *et al.*, 2005). The physical and chemical reactions that occur during frying is as shown in Fig.2.9.

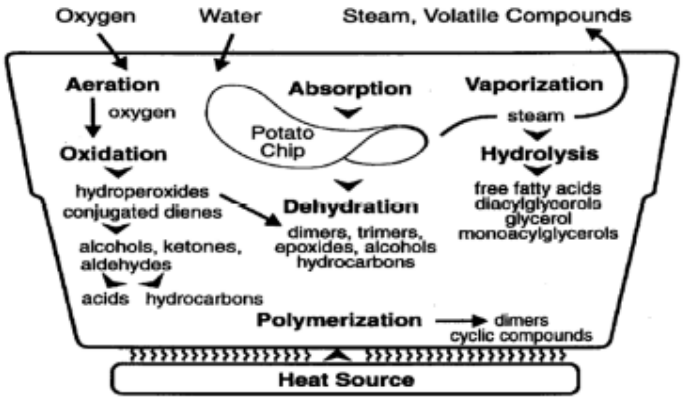


Fig.2.9 Physical and chemical reactions that occur during frying

Source: (Warner, 2005)

The reactions in deep-fat frying depend on factors such as replacement with fresh oil, frying conditions, orginal quality of frying oil, food materials, type of fryer, type and concentration of antioxidants and oxygen concentration. Other factors such as frying temperature, quantity of frying, initial content of free fatty acids, polyvalent metals and unsaturated fatty acid content of the oil also affect the oxidative stability and overall quality of oil during frying process. In general, deep-fat frying increases foaming, color, viscosity, density, the amount of polymeric and polar compounds and the free fatty acid content of frying oils (Serjouie *et al.*, 2010).

The activity to inhibit the oxidation and hydrolysis, as expressed by changes of polar products, is not a sufficiently reliable criterion of deterioration as polymerization reactions are also important. The concentration of polymers should not exceed 10% (Reblova *et al.*, 1999). As quality of fried foods is affected by that of the frying oil, the used frying oil should be replaced when the total content of polar compounds, representative of the new compounds formed during frying, is higher than 25% expressed on oil weight (Marmesat *et al.*, 2007).

Several factors affect the overall performance of frying oils (Andrikopoulous *et al.*, 2002). Some of the minor components of the crude vegetable oil, e.g. tocopherols (particularly c-tocopherol), phospholipids (at less than 100 mg/ kg), carotenoids (at low levels) and certain sterols, are beneficial to oil stability during frying. The stability of frying oils may be further enhanced by addition of nonpolar substances, such ashigher

alkanes, squalene or silicone oils. They form a thin layer on the surface of frying oil, thus preventing the diffusion of air oxygen (Reblova *et al.*, 1999).

2.5.1 Selection of deep-frying oils and fats

The products used for frying range from unhydrogenated fully refined fats and oils to specially hydrogenated products designed for frying. Fry- life, mouthfeel, product appearance, specific product requirements, ease of handling and cost are the major criteria for selecting a frying fat for a given application (Dunford, 2000).

From the nutritionist point of view, the mono-unsaturated oleic acid, which can be found in olive oil and rapeseed oil, is to be preferred. The polyunsaturated fatty acids, linoleic – and *alpha-linoleneic* acids are essential, but should be consumed in a ratio not higher than 5:1, the linoleic acid uptake within the diet being in general too high. Both fatty acids, are basic material for different hormone-similar substances in the metabolism which are effective against cardiovascular diseases. In addition *alpha-linolenic* acids help against certain inflammatory processes (Matthäus *et al.*, 2008).

In terms of temperature stability and oxidation stability against atmospheric oxygen, saturated long chain fatty acids are more stable than the corresponding unsaturated fatty acids. The important essential fatty acids, linoleic – and linolenic acids are less stable with longer hours and/or days of continuously heating over 175/180°C. For sensory reasons the linolenic acid concentration of the frying oil should be less than 3%, otherwise it could contribute to a fishy flavor within the product and the deep-frying medium (Matthäus *et al.*, 2008).

Solid fats which are rich in saturated fatty acids (e.g. stearic acid), offer a higher temperature- and oxidation stability than oils with a high content of unsaturated fatty acids. However, high contents of short and middle chain saturated fatty acids (e.g. in coconut and palm kernel oil) increases the formation of smoke and foam. A semi-liquid deep-frying medium usually combines the stability advantages of a solid fat with the positive nutritional-physiological characteristics of liquid oil. The semi liquid consistency also allows favourable handling by the user (Matthäus *et al.*, 2008).

Table 2.4 Inherent stability of common fats and oils

Oil	Inherent Stability
Safflower	7.6
Soybean	7.0
Sunflower	6.8
Corn	6.2
Rapeseed (Low Erucic Acid)	5.5
Cottonseed	5.4
Rapeseed (High Erucic Acid)	4.1
Peanut	3.7
Lard	1.7
Olive	1.5
Palm	1.3
Tallow	0.86
Palm Kernel	0.27
Coconut	0.24

Source: (Dunford, 2000)

A useful way to determine the suitability of an oil for frying is to consider its inherent stability to oxidation. Inherent stability numbers relate to relative reaction rates of unsaturated fatty acids with oxygen. Therefore, an oil with a low inherent stability number is less susceptible to oxidation during frying. Calculated inherent stabilities of common fats and oils are shown in Table 2.4. The inherent stability calculation assumes that all oils are refined, bleached and deodorized from reasonably good quality crude oil (Dunford, 2000).

2.6 Palm oil

Palm oil is extracted from the fleshy orange-red mesocarp of the fruit of the oil palm (*Elaeis guineensis)*. This plant, indigenous to West Africa, has spread to the tropical and subtropical zones of the world, particularly Malaysia and Indonesia (Edem, 2002). It is extracted in the oil mill and then fractionated, bleached and deodorized in the refinery (Pimpa *et al.*, 2009). It is a highly viscous semi-solid fat, orange-red in colour and has 45-56 iodine value and 31.38°C melting point.

Table 2.5 Fatty acid composition of palm oil and its fractions

Oil Source	16:0 Palmitic	18:0 Stearic	18:1 Oleic	18:2 Linoleic
Palm Oil	44	4	39	11
Palm Olein	41	4	41	12
Palm Stearin	47-74	4-6	16-37	3-10

Source: (Preeti *et al.*, 2007)

Palm oil, melting in the range 21–27°C, can be fractionated to give solid (palm stearin, 30–35%, mp 48–50°C) and liquid fractions (palm olein, 65–70%, mp 18– 20°C), thereby extending the range of usefulness of this oil. With improved filtration procedures, the yield of olein has been increased to 71–78%. This olein has a cloud point of 7–10°C and can be fractionated further to give even more unsaturated oleins and palm mid fraction as shown in the table. Palm olein is a high-quality, highly stable frying oil. Palm stearin is the less valuable commodity, but it can be used as a hard fat in the production of spreads and as a vegetable alternative to tallow in the oleo- chemical industry (Preeti *et al.*, 2007).

Palm oil consists mainly of glycerides (9%) and about 0.5 per cent non-glyceride materials. The nonglyceride components include free fatty acids (FFA), trace metals, moisture, impurities, and minor components. Crude palm oil contains approximately 1% minor components, which include carotenoids, vitamin E, sterols, phospholipids, glycolipids, terpenic, and aliphatic hydrocarbons. The carotenoids, tocopherols and tocotrienols are the most important of these minor components. They contribute to the stability and nutritional properties of palm oil (Gunstone, 2008; Liu *et al*., 2008).

The oil contains almost equal proportions of saturated (palmitic 48% and stearic 4%) and unsaturated acids (oleic 37% and linoleic 10%) (Wu and Ng, 2007). The major triacylglycerol species are POP (29%), POO (23%), PLO (10%), and PLP (10%). Palm oil contains 5% of diacylglycerols and low phospholipids (5–130 ppm) (Gunstone, 2008).

Carotenoids impart the characteristic orange-red color to palm oil. Oil from the *tenera* variety, which is widely planted in Malaysia, has a carotenoid content of about 500–700 mg/L. Alpha and β-carotenes are the major components present (ca. 36 and 54%,

respectively), and the rest are γ-carotene, lycopene, and xanthophylls. Carotenes, particularly β-carotene, are converted into vitamin A *in vivo*. Unfortunately, most of the carotenoids are degraded during the refining, bleaching and deodorization processes, which traditionally produce the light colored oils preferred by most consumers (Goh *et al.*, 1985).

Palm oil is potentially one of the best sources of vitamin E. The vitamin E content in palm oil is unique in that it is composed of tocotrienols rather than tocopherols. Palm oil normally contains 600–1,000 mg/L, of which 43% is γ-tocotrienol, 24% is α-tocotrienol, 11% is δ-tocotrienol, and 21% is α-tocopherol. Tocopherols and tocotrienols are potent natural antioxidants that play an important role in the stabilization of oils and fats. They extend the induction period and delay the time when oxidation produces off-flavors and/or odors (Edem, 2002). Palm tocotrienols may also have beneficial health impacts as they have been reported to lower plasma cholesterol by inhibiting the activity of HMG-CoA reductase, which regulates cholesterol synthesis in the liver. Tocotrienols may also play an important role in suppressing the progression of certain types of cancer particularly breast cancer (Liu *et al.*, 2008).

Recent studies have shown that palm oil and/or its antioxidant constituents are effective in controlling anthrosclerosis and certain types of cancer (Goh *et al.*, 1985). Tocotrienols, which are active components of palm oil, are shown to be effective in preventing oxidative damage to lipids *in vitro* and *in vivo*). Furthermore, tocotrienolrich fraction (TRF) from palm oil was reported to be more potent than a-tocopherol against oxidative damage in brain mitochondria and more effective in inhibiting protein oxidation and lipid peroxidation in liver microsomes (Wu and Ng, 2007).

The advantages of the palm oil are not only economic. The high content in mono-unsaturated acids drop rates of LDL – " bad " cholesterol – all while maintaining the HDL or " good " cholesterol. The uniqueness of palm oil from other vegetable oils lies in its fatty acid composition and their position in the triglyceride structure. Inspite of its higher palmitic acid content, red palm oil does not behave like animal fats that are rich in saturated fatty acids. This is because, in palm oil the middle (2nd) position of triglyceride structure is occupied mainly by unsaturated fatty acid (oleic), which are absorbed into the intestine after the fatty acid at 1 and 3 positions are split off during digestion. Thus, more of oleic acid is available to the body from palm oil. It could be blended with other

vegetable oils rich in polyunsaturated fatty acids such as sunflower, groundnut, coconut etc. so that crude palm oil blend contains recommended ideal fatty acid composition which is required for the maintenance of good health (Preeti *et al.*, 2007). Palm Oil also has balanced fatty acid content with equal ratio of saturated to unsaturated fatty acids. The presence of natural antioxidants, tocopherol and tocotrienol further contribute to the superior oxidative stability of palm oil. Unlike the unstable polyunsaturated edible oils, palm oil does not have to be hydrogenated to impart stability. Hence, it is naturally free of trans fatty acid. Another important attributes of palm oil, which help to distinct it from others, is its bland taste. This helps to carry the natural flavor of the food during frying process (Hashim, 2011).

Palm olein (IV 56–59) is used mainly as frying oil, palm stearin (IV 40–42) as hardstock, and palm mid-fraction (range of iodine values between 32 and 47) in confectionery fats. (Gunstone, 2008).

2.7 Mango

Mango (*Mangifera indica* L), commonly referred to as the king of fruits, is an important fruit crop cultivated in tropical regions of Asia and Africa and belongs to the family Anacardiaceae (Schieber *et al.*, 2003). It is consumed throughout the world, due to its excellent eating quality and nutritional composition (Maisuthisakul, 2011). Mango and mango products such as puree, nectar, leather, chutneys, pickles, and canned slices have experienced worldwide popularity and also gained increasing importance all over (Puravankara *et al.*, 2000).

During processing of mango, by-products such as peel and kernel are generated. Kernel constitutes about 17–22% of the fruit (Soong and Barlow, 2004). The disposal of mango bio- waste is a growing problem due to increasing production of this material (estimated to be around 75000 mT worldwide). From an environmental perspective, it is vital to reuse the plant by-products produced by the agro-food industry (Dorta *et al.*, 2012).

Mango pulp has been reported to have antilithiatic and free radical scavenging properties, which reduce lipid peroxidation and enhance antioxidant enzymes (superoxide dismutase and catalase) against isoproterenol. Mango pulp contains vitamins, organic acids, carbohydrates, amino acids, polyphenols and certain volatile compounds. Several studieshave reported polyphenolic compounds in mango flesh and peel, including various

ascorbic acids, dehydroascorbic acids, flavonoids, xanthones, phenolic acids, and gallotannins (Khammuang and Sarnthima, 2011; Schieber *et al.*, 2003).

Mango Seed Kernels, depending on the variety, contain on average 5.7% protein, 9.3% fat, 79.9% carbohydrate, 2.0% crude fibre and 3.11% ash. In times of scarcity and famine, mango seed kernels are consumed, after boiling, by poor people. The fat from mango seed kernel is also a promising source of edible oil. In addition, this fat from mango seeds has attracted the attention of scientists in recent years as a cocoa butter substitute, because the former has a fatty acid and triglyceride profile similar to that of cocoa butter. There was a significant increase in the shelf-life of ghee on the addition of mango seed kernel to buffalo ghee and thus mango seed kernel can be a good source of natural antioxidants (Puravankara *et al.*, 2000). This could be attributed to the phospholipids and phenolic compounds in the MSK extract. Gallotannins and condensed tannin-related polyphenols detected by thin-layer chromatography were reported to be present in MSK. In addition, phenolics from dry MSK meal were reported to contain tannic acid, gallic acid, and epicatechin in the ratio 17: 10: 1, respectively (Maisuthisakul, 2008; Puravankara *et al.*, 2000).

Mango seed kernel was also shown to be a good source of phytosterols such as campesterol, β-sitosterols, stigmasterol and also contains tocopherols (Soong and Barlow, 2004). The antioxidant effect of mango seed kernel is due to their high content of polyphenols, sesquiterpenoids, phytosterols and microelements like selenium, copper and zinc (Schieber *et al.*, 2003). Similarly, various phenolic compounds such as tannins, gallic acid, coumarin, caffeic acid, vanillin, mangiferin, ferulic acid, cinamic acid and unknown compounds have been characterized in MSK (Abdalla *et al.*, 2006). In addition, mango seed kernel showed potent tyrosinase inhibitor, antioxidant activity and chelating activity. Moreover, the extraction conditions affected the content of phenolic compounds and the activities of mango seed kernel extracts (Maisuthisakul, 2009).

Recently, mango seed kernels has also reported to have hepatoprotective activities (Nithitanakool *et al.*, 2009) along with anti-hemorrhagic and anti-dermonecrotic activities against snake venoms (Leanpolchareanchai *et al.*, 2009). Besides, MSK extract has also exhibited potent antibacterial activity (Kabuki *et al.*, 2000) and has been used as an immunomodulating agent in animals (Khammuang and Sarnthima, 2011; Sahu *et al.*,

2007). The MKE had a broad antimicrobial spectrum, and was more active against gram-positive than gram-negative bacteria with a few exceptions. The antimicrobial activity of the MKE was stable against heat (121°C, 15 min), freezing (−20°C, 16 h) and pH treatment (pH 3–9) normally used in food processing. Chemical analysis showed that the MKE was composed of 79.5% polyphenol and 21.7% carbohydrate (Kabuki *et al.,* 2000).

2.8 Jackfruit

The jackfruit (*Artocarpus heterophyllus* Lam.) belonging to family Moraceae has been cultivated since prehistoric times and has naturalized in many parts of the tropics, particularly in Southeast Asia, where it is today an important crop of India, Burma, China, Sri Lanka, Malaysia, Indonesia, Thailand, and the Philippines. It is also grown in parts of Africa, Brazil, Suriname, the Caribbean, Florida, and Australia (Elevitch and Manner, 2006).

Jackfruit is a medium-size, evergreen tree that typically attains a height of 8–25 m (26–82 ft.) and a stem diameter of 30–80 cm (12–32 in). The canopy shape is usually conical or pyramidal in young trees and becomes spreading and domed in older trees. The canopy diameter at 5 years old ranges from 3.5–6.7 m (11–22 ft.) and can reach 10 m or more in older trees (Elevitch and Manner, 2006). The leaves are simple, alternate, coriaceous, entire dark, shiny green above oblong, oval or elliptic in form, 4 to 6 inches in length, glabrous, hairless with smooth skinned surface. The leaves show the presence of sapogenin, cycloaratenone, cycloartenol, β-sitosterols and tannins. The leaves are reported to be used in skin diseases and ash of leaves is useful in healing ulcer. The leaves are also useful in fever, boils, wound, skin diseases and vitiated condition of pitta and vata (Jain *et al.,* 2009).

Jackfruit has a compound or multiple fruit (syncarp) with a green to yellow-brown exterior rind that is composed of hexagonal, bluntly conical carpel apices that cover a thick, rubbery, whitish to yellowish wall. The acid to sweetish (when ripe) banana-flavored flesh (aril) surrounds each seed. The heavy fruit is held together by a central fibrous core. Fruits are oblong-cylindrical in shape, typically 30–40 cm (12–16 in) in length but sometimes up to 90 cm (35 in). They usually weigh 4.5–30 kg (10–66 lb.), although a weight of 50 kg (110 lb.) has been reported. The heavy fruit is borne primarily on the trunk

and interior part of main branches. Fruits take 90–180 days to reach maturity (Elevitch and Manner, 2006).

Seeds are light brown to brown, rounded, 2–3 cm (0.8–1.2 in) in length by 1–1.5 cm (0.4–0.6 in) in diameter, and enclosed in a thin, whitish membrane. Up to 500 seeds can be found in each fruit. Seeds are recalcitrant and can be stored up to a month in cool, humid conditions (Elevitch and Manner, 2006).

Jackfruit pulp is eaten afresh and used in fruit salads and possesses high nutritional value. Jackfruit also has been reported to contain antioxidant prenyl flavones (Ko *et al.*, 1998). Recently, antioxidant capacity of fruit pulp has been evaluated (Jagtap *et al.*, 2010). However, jackfruit seeds are less popular as vegetable and are eaten when boiled or roasted. These are believed to be digested with difficulty. The composition of jackfruit seeds has been reported and found to contain similar compositions as that of grains. The seeds are also rich source of carbohydrates and proteins and good source of fibre and vitamins. A major protein, Jacalin has been isolated from jackfruit seeds and possessed immunological properties (Kumar *et al.*, 1982).Jackfruit seeds are not much explored in terms of nutrition and antioxidant properties. Chemical composition and mineral content of jackfruit seeds have been studied (Gupta *et al.*, 2011).

Part III

Materials and methods

3.1 Materials

3.1.1 Fruit seed

The fruits of mangoes (*Mangifera indica* L.) and jackfruit (*Artocarpus heterophyllus*) varieties were obtained from a local area near Dharan and Biratnagar from June to July, 2012 and the seed kernels were obtained from the respective fruits. Jackfruit seeds were sampled from equally ripened Jackfruits from a single plant cultivated at Vijaypur, Dharan while mango seeds were sampled from equally ripened mangoes from a single plant cultivated on farm at Pokhariya, Biratnagar.

3.1.2 Palm oil

Refined Bleached and Deodorized (RBD) Palm oil of AV and PV less than 0.3 and 3 respectively used for the frying purpose was obtained from Asian Thai Foods Pvt. Ltd., Sonapur, Sunsari, Nepal.

3.1.3 Potato

Even-sized good-quality potatoes, used for the preparation of chips, were purchased from the local market of Dharan.

3.1.4 Chemicals

2, 2-Diphenyl-1-Picryl-Hydrazyl (DPPH) free radical were purchased from Sigma-Aldrich Company while Folin-ciocalteau reagents from Merck. BHA (Butylated Hydroxy Anisole), BHT (Butylated Hydroxy Toluene), Ethanol, Methanol, Acetic acid, Chloroform, Sodium hydroxide, Sodium nitrite, Sodium Carbonate, Sodium thioisulphate, Alumunium chloride, Potassium iodide, Potassium hydroxide, Sulphuric acid, Starch, Phenolphthalein and Bromophenol-blue of analytical grade reagents were used in laboratory.

3.2 Method

3.2.1 Preparation of extracts

The seeds of mangoes (*Mangifera indica* L.) and jackfruit (*Artocarpus heterophyllus*) varieties were sampled, washed and then sun dried. The kernels and kernel sheathes were removed manually from the seeds. The seed kernel was then chopped and extraction of phenolics was done using the method described by Maisuthisakul (2011). Fresh kernel seeds were chopped and refluxed with ethanol at a ratio of sample/ethanol of 1:3 (w/v) for 3 hours. The residue was then removed by filtering through Whatman No 4 filter paper. The extracts were collected individually and the solvent was evaporated under vacuum at 40°C using a rotary vacuum evaporator (Buchi, Switzerland). All the ethanol solvent was evaporated and the flask was made completely dry. The stock solution of the extract was made at concentration of 10 mg/mL using ethanol. The extracts were stored at 4°C until use. The extraction yield of each sample was calculated and reported as a percentage (g dwt extract/100 g dwt sample). The dried extracts and reference samples were used to estimate the antioxidant properties, total phenolic content and total flavonoid content.

3.2.2 Frying operations

Fresh potatoes were peeled and sliced to a thickness of about 2 mm using a knife manually. The sliced potatoes were then soaked in 2.5% salt (NaCl) solution for 5 min at room temperature and dried before weighing into 100 g batch for frying (Jaswir and Che Man, 1999).

Frying experiments were carried out in various systems that contained: RBD palm oil without antioxidant or control; RBD palm oil with 200 ppm BHT; RBD palm oil with 200 ppm BHA; RBD palm oil with 200 ppm MSK extract; and RBD palm oil with 200 ppm JSK extract.

RBD palm oil (0.5 kg) was put into a Pan-fryer. The temperature was brought up to 60°C, then 200 ppm of BHT, BHA, MSK extract, or JSK extract was added in oil respectively. The oil was stirred for 10 min to ensure dissolution of antioxidant. In the case of control, the oil also was held for 10 min at 60°C, although no antioxidant was added. The temperature was then raised to 180±5°C during 20 min. Frying started 20 min after the temperature had reached 180±5°C. A batch of 100 g raw potato chips was fried for 2.5 min

at 17.5 min intervals for a period of 3.5 h per day for seven consecutive days. This is equivalent to 10 fryings per day and 70 fryings for seven consecutive days. The frying pan was left uncovered during the frying period. At the end of the tenth frying, the frying was stopped and the temperature was allowed to drop to room temperature. Oil samples for analysis (about 100-120 g) were collected in amber bottles for further analyses. All samples were stored under refrigeration at nearly 4°C for further analysis. The lid was then put on the frying pan and the oil was allowed to cool overnight. The frying was continued the next day. Fresh oil was not added to the frying pan.

3.2.3 Determination of moisture content of fruit seed powder

The moisture content of the fruit seed powder was determined by Hot-air oven method as per AOAC (2005).

3.2.4 Determination of yield of extracts

The dried extracted sample was weighed to calculate the yield by the following equation;

$$\text{Yield (\%, dry weight basis)} = (W_1/W_{2)}\ x100$$

Where W_1 is the weight of extracts after vacuum drying and W_2 is the weight of dry powder of the seed kernel samples.

3.2.5 Determination of the amount of total flavonoids

The amount of total flavonoids was measured using the method described by Jia *et al*. (1999). Briefly, sample fractions or standard solution of rutin (1 mL) was mixed with distilled H_2O (4 mL) in a 10 mL volumetric flask, followed by the addition of 5% $NaNO_2$ solution (0.3 mL). After 5 min, 10% $AlCl_3$ solution (0.3 mL) was added. At 6 min, 1 M NaOH solution (2 mL) was added to the mixture. Immediately, distilled H_2O (2.4 mL) was added to the reaction flask and the contents mixed well. The absorbance at various concentration was measured at 510 nm with spectrophotometer. Measurements were calibrated to a standard curve of prepared rutin standard solution (0–0.5 mg/L). The total flavonoids of the extract fractions were expressed on an extract weight basis as mg/g rutin equivalents (RE). All samples were analyzed in three replications.

3.2.6 Determination of the total phenol contents

The amounts of phenolic compounds in the extracts were determined according to the Folin-Ciocalteu colorimetric method based on the procedure described by Khammuang and Sarnthima (2011) with some modifications, using Folin- Ciocalteu reagent. Gallic acid was used as the standard phenol compound. The extracted solution, in the appropriate dilution, (0.5 mL) was transferred to a test tube containing 0.5 mL of Folin-Ciocalteu reagent. After three minutes, 0.5 mL of 35% w/v sodium carbonate solution was added. The mixture was allowed to stand for 90 min at room temperature in the dark and then 3.5 mL distilled water was added to adjust the total volume to 5.0 mL. The sample was shaken well before being measured at 725 nm with spectrophotometer. The experiment was carried out in triplicate and the total phenol content was expressed as milligrams of Gallic acid equivalents (GAE) per gram of dried weight of extracts.

3.2.7 DPPH radical scavenging assay

The DPPH radical scavenging activity assay used by Chan *et al.* (2007) was adopted with slight modification. DPPH solution was prepared by dissolving 6 mg of DPPH in 100ml of methanol. To 1mL of various concentrations of the extracts (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml), 2 mL of DPPH solution (0.1 mM) was added. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and was left to stand in dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The experiments were performed in triplicate and the percentage scavenging activity of each extract on DPPH radical was calculated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of the control- Absorbance of the sample}}{\text{Absorbance of the control}} \times 100\%$$

DPPH radical scavenging activities of the extracts were expressed as “efficient concentration” or EC_{50} values (otherwise called the IC_{50} value). This is defined as the concentration of substrate that causes 50% scavenging (loss) of the DPPH activity (color) which was calculated from the graph of scavenging activity plotted against sample concentration using Microsoft Excel software (Marxen *et al.*, 2007; Molyneux, 2004).

Antiradical activity was reported in the form of 1/IC50 which directly correlated with the antioxidant properties (Maisuthisakul, 2011).

3.2.8 Estimated of peroxide value

The frying oil was analyzed for Peroxide Value as per AOAC (2005).

3.2.9 Estimation of acid value

Acid Value of frying oil was determined by titration method as per AOAC (2005).

3.2.10 Estimation of iodine value

Iodine Value of frying oil was determined by titration method as per AOAC (2005).

3.3.11 Data analysis

Data for chemical analysis were plotted for comparison and were graphically represented using Microsoft excel 2010 program ©Microsoft corporation 1985-2010. Data were statistically processed by GenStat Discovery Edition 3, GenStat procedure library release PL15.2, version 7.22 DE (copyright 2008, VSN international ltd) for analysis of variance (ANOVA). Means of the data were separated whether they are significant or not by using LSD (Least Square difference) method at 5% level of significance.

Part IV

Result and discussions

The seed of mango and jackfruit were sampled, washed and then sun-dried. The kernel sheaths were removed from the seed of the mango and jackfruit and the kernels were chopped and powdered. The extracts of mango and jackfruit seed kernel were prepared by solvent extraction technique using ethanol as solvent and their antioxidant potential were investigated. Thereafter, the extracts were incorporated in palm oil used for deep frying along with other synthetic antioxidants such as BHT and BHA. The efficacy of these extracts on oxidative stability of palm oil on deep frying was then compared with the efficacy of BHA and BHT. To study the oxidative stability of palm oil, peroxide value, free fatty acid content and iodine value were analyzed and interpreted.

4.1 Analysis of seed extract

4.1.1 Determination of the moisture content of the seed powder

The moisture content of the powder of the seed kernel is shown in Table 4.1.

Table 4.1 Moisture content of seed powder

Fruit Seed Powder	Moisture Content
Mango Seed	4.16 ± 0.08^{a}
Jackfruit Seed	4.72 ± 0.06^{b}

Each value in the table represents mean ± standard deviation of three replications. Means within each column with same superscripts were not significantly ($p > 0.05$) different.

The moisture content of the mango and jackfruit seed powder, after sun drying, were found to be 4.16 % and 4.72 % respectively. Statistical analysis (one way ANOVA) was done for the % moisture content of the seed powder which showed a significant difference ($p \leq 0.05$) between the two fruits used (Appendix C).

4.1.2 Determination of the yield

The yield of the MSK extract and JSK extract is shown in Table 4. 2.

Table 4. 2 Yield of MSK extract and JSK extract

Fruit Seed Extract	% Yield (g dwt extract/100 g dwt sample)
MSK Extract	11.28 ± 0.11^{a}
JSK Extract	10.37 ± 0.08^{b}

Each value in the table represents mean ± standard deviation of three replications. Means within each column with same superscripts were not significantly ($p > 0.05$) different.

The average yields were found to be 11.28% and 10.37 % for MSK and JSK extract respectively. Statistical analysis (one way ANOVA) was done for the yield percentage of two extracts which showed a significant difference ($p \leq 0.05$) between the extracts (Appendix C).

The yield of the extract is mainly dependent on the method of the extraction. Different methods could be adopted for the extraction such as shaking, acid hydrolysis (refluxing in suitable solvent in acidified condition) and refluxing (refluxing in suitable solvent). Maisuthisakul (2009) found that the yield was maximum for refluxing and slightly lesser for acid hydrolysis whereas the yield was too low for shaking. Thus, refluxing was used as technique for the preparation of extract using ethanol as solvent for three hours.

Solvent extraction is frequently used for isolation of antioxidants. The antioxidant activities of the extracts are strongly dependent on the solvent, due to the different antioxidant potentials of compounds with different polarity (Soong *et al*., 2004). The ethanol was used as extraction solution in the present study as it is the most widely used solvent for hygiene, widely available and compatible with food (Maisuthisakul, 2011).

The yield of the extract is also dependent on the different cultivars/variety of same plant. Among the four different Thai varieties of mango analyzed by Khammuang and Sarnthima (2011), the extract yield was different for each of the cultivar. The same was true among eleven different Thai varieties of mango analyzed by Maisuthisakul (2008).

The yield of the seed extract for a same variety of mango also depend on different other factors such as environmental condition, the soil in which the plant is grown, agronomic

parameters (irrigation and pest management), maturity level at harvest and likewise. The yield of the seed extract for Thai mango varieties *Chok-a-nan, Fah-lun, Kaew and Namdok- mai* as analyzed by Maisuthisakul (2008) were found to be 3.33%, 3.27%, 3.25% and 3.09% whereas the yield for same Thai varieties as determined by Khammuang and Sarnthima (2011) were found to be 3.92%, 5.36%, 2.53% and 11.9%.The above determined yield for the extract of mango seed (*Malda* variety) was in close agreement to that for Thai mango variety *Namdok-mai* as determined by Khammuang and Sarnthima (2011).

4.1.3 Determination of total phenol content and total flavonoid content

Total phenol content (mg GAE/gdry extract) and total flavonoid content (mg RE/mg dry extract) of the mango and jackfruit seed extract is shown Table 4.3. The total phenol content for Mango seed kernel extract was found to be 117 mg GAE/ g and that for Jackfruit seed kernel extract was found to be 27.7 mg GAE/ g of the dried extract whereas the total flavonoid content for Mango seed kernel extract was found to be 0.372 mg RE/mg dry extract and that for Jackfruit seed kernel extract was found to be 0.195 mg RE/mg dry extract.

Table 4. 3 Total phenol content and total flavonoid content in MSK extract and JSK extract

Fruit Seed	Total Phenol Content (mg GAE/g dry extract)	Total Flavonoid Content (mg RE/mg dry extract)
MSK Extract	116.43 ± 3.79^{a}	0.372 ± 0.02^{a}
JSK Extract	27.7 ± 2.55^{b}	0.196 ± 0.01^{b}

Each value in the table represents mean ± standard deviation of three replications. Means within each column with same superscripts are not significantly ($p > 0.05$) different.

Calibration curves were constructed by linear regression of total phenol content (*Y*), versus the concentration (*X*) as shown in Appendix D. For linearity validation, Gallic acid standard solutions at a concentration range of 0 to 600.0 mg/L was used for which the regression equation was $Y = 0.001X + 0.0041$ ($R^2 = 0.9859$). The total phenol content of

Seed extract was expressed as gallic acid equivalents (GAE) in mg/g of dried extracts. Similarly, Calibration curves were constructed by linear regression of total flavonoids content (*Y*), versus the concentration (*X*) as shown in Appendix D. For linearity validation, Rutin standard solutions at a concentration range of 0.1 to 1.0 mg/L was used for which the regression equation obtained was $Y = 0.5406X + 0.0333$ ($R^2 = 0.9863$). The total flavonoid content of seed extract was expressed as rutin equivalents in mg/mg of dried extracts.

Statistical analysis (one way ANOVA) was done for the total phenol content of two extracts which showed a significant difference ($p \leq 0.05$) between both of the extracts (Appendix E). Similarly, the statistical analysis (one way ANOVA) was done for the flavonoid content of two extracts which showed a significant difference ($p \leq 0.05$) between both of the extracts (Appendix E).

The similarities and discrepancies could be related, among other factors, to similar or dissimilar extraction conditions used to obtain these compounds from the plant matrices. However, it is necessary to be careful when comparing data described by different authors, because the phytochemical composition of seed extracts can vary depending on cultivar or other preharvest factors such as climate (temperatures, rainfalls, and light hours), soil type, and fertilization (Gonz´alez and Gonz´alez, 2010).

The phenolics of mango seed extract are extracted using different methods such as shaking, acid hydrolysis and refluxing by Maisuthisakul (2009) and found that the total phenol content of the extract was highest in acid hydrolysis, intermediate in refluxing and lowest in shaking. Acid hydrolysis release and degrade phenolic compounds, which was in agreement with the work of Krygier *et al.* (1982) and Chiang *et al.* (2001). Most phenolic compounds in plant seeds occur primarily in the bound form as conjugates with sugars, fatty acids or proteins (Krygier *et al.*, 1982). Therefore, it is important that a hydrolysis process is adopted in order to obtain the maximum yield of the phenolic content of MSK. Moreover, during acid hydrolysis at elevated heating temperatures, high molecular weight phenolics can degrade to low molecular weight phenolics (Chiang *et al.*, 2001). Structurally different phenolics show different rates of hydrolysis (Nuutila *et al.*, 2002). Degradation of some flavonoids, such as quercetin and myricetin, due to a reaction temperature of more than 95°C, has also been reported by(Hertog *et al.*, 1992). Thus,

refluxing was adopted for extraction of phenolics to prevent the degradation of the flavonoid with phenolic content comparable to the acid hydrolysis method.

Dorta *et al.* (2012) observed the significant effect ofextraction solventon the antioxidant properties of the fruit seed extracts. The total phenolics in mango seed kernel extracts are a mixture of tannins, gallic acid and epicatechin. Tannins are the predominant phenolic constituents of mango kernel; hydrolyzable tannins comprise 75% of total tannins present (Arogba, 2000). For the extraction of condensed tannins, several solvent systems have been used. Absolute methanol, ethanol, acidified methanol, acetone, water, and their combinations are among the most commonly used solvent systems. The polarity of the solventand of the different phenolic compounds affect extraction efficiency and the activity of the obtained extracts (Gonz´alez and Gonz´alez, 2010) and, in general, highly hydroxylated aglycone forms of phenolic compounds are soluble in alcohols such as methanol or ethanol and their mixtures with water. The alcoholic solvents rupture cell membranes and enhance the extraction of endocellular materials (Robards, 2003). The most widely reported extracting solvents are mixtures (between 50% and 95%) of methanol, ethanol, and acetone with water (Abdalla *et al.*, 2007; Ajila *et al.*, 2007; Berardini *et al.*, 2005; Kabuki *et al.*, 2000; Soong and Barlow, 2004). These solvent are specifically effective in the extraction of polyphenols from protein matrices, since they appear to degrade polyphenol-protein complexes (Kallithraka *et al.*, 1995). This could explain why it is so efficient when extracting antioxidants from mango seed, as this material has a high protein content (Abdalla *et al.*, 2007).

Dorta *et al.* (2012) also observed that the extraction of the phenolics in the mixtures (1:1) of organic solvents and water was more than in sole organic solvents like methanol, ethanol and water. Although mango seed extracts obtained with methanol (not a food-grade solvent) have high antioxidant activity, the conditions under which methanol can be used are strict and maximum residue values permitted in food and food ingredients are limited. Therefore, from the perspective of food security, it would be preferable to choose solvents such as ethanol (also with notable antioxidant activity and phytochemical compound content), ethanol:water or acetone: water, as they can all be used in compliance with good manufacturing practice (Lapornik *et al.*, 2005). Thus, the ethanol: water (1:1) solvent system was used for the extraction of the phenolics from the seed extracts.

Gupta *et al.* (2011) observed that the total phenol content of the jackfruit seed extract using dichloromethane: methanol (1:1) and acetone as solvent were 1.45 mg GAE/g extract and 2.12 mg GAE/g dry extract which was found to be quite lower than the value obtained using ethanol:water (1:1) as solvent system. However, the flavonoid content of the JSK extract was found to be lower than value obtained by Gupta *et al.* (2011) which could be probably due to the degradation of the flavonoid on using refluxing as extraction technique unlike Gupta *et al.* (2011) who used shaking for extraction. However, the result was close to the value obtained by Soong and Barlow (2004) using ethanol: water as solvent. The obtained value of total phenol content of the mango seed extract was found to be higher than the value obtained by Norshazila *et al.* (2010) but was closely related to the value obtained by Maisuthisakul and Thepkunya (2011) and Soong and Barlow (2004).

The optimization of the antioxidant extraction from murta leaves (Rubilar *et al.*, 2006) and from apple pomace (Wijngaard and Brunton, 2010) showed that increasing temperature improved extraction; however, temperature could not be increased indefinitely, because the stability of phenolic compounds decreased. Although the effect of temperature was not as important as that of the solvent, it is important to highlight that tannin content increased 50% to 70% in extracts obtained with ethanol, acetone, or ethanol: water when the extraction temperature was increased from 25 to 75°C. Thus, the extraction of phenolics was done at temperature of 75°C. The result of total phenol content and total flavonoid content was similar to the value obtained by Soong and Barlow (2004) using 70°C as extraction temperature.

The extraction period is another factor that affects the recovery of polyphenolics. Extraction periods varying from 1 min to 24 h have been reported. However, longer extraction times increase the possibility of oxidation of phenolics unless reducing agents are added to the solvent system (Shahidi and Naczk, 2004b).

The recovery of polyphenols from food products is also influenced by the ratioof sample to solvent (R)(Naczk and Shahidi, 1991)found that changing R from 1:5 to 1:10 increases the extraction of condensed tannins from commercial canola. Thus, the ratio of sample to solvent was used as 1:50 similar to that used by Soong and Barlow (2004) and Dorta *et al.* (2012).

Deshpande and Cheryan (1985) demonstrated that the yield of tannin recovery from dry beans is strongly influenced by variations in the sample particle size. They found that tannins decrease by about 25 to 49% as the minimum size is reduced from 820 to 250 µ. Thus, the sample particle size was adjusted 600µthe maximum extraction of the phenolics.

The total phenolics of the extract are strongly dependent on variety/cultivar of the fruit. Khammuang and Sarnthima (2011) and Maisuthisakul (2008) studied the total phenol content of the seed extract obtained from the various Thai varieties of mango and found that the total phenolics of the extracts vary on variety of the mango. The seed extracts of four Thai mango varieties such as *Chok-a-nan, Fah-lun, Kaew* and *Nam-dok-mai* were analyzed in common by Maisuthisakul (2008) and Khammuang and Sarnthima (2011) but the phenol content and antioxidant activity were different for each varieties. It illustrates that variety along with environmental conditions and agronomic parameters also affect the recovery of phenolics in the extract. The total phenolics of the mango seed extract from Malaysian varieties (Norshazila *et al.*, 2010) is lower than the total phenolics obtained from the Nepalese varieties of mango whereas the obtained value closely resembled to the total phenolics obtained from Thai varieties (Khammuang and Sarnthima, 2011; Maisuthisakul, 2008) and mango varieties from Singapore (Soong and Barlow, 2004). The seed extract from Nepalese jackfruit varieties was found to have higher phenolics than Indian varieties (Gupta *et al.*, 2011) whereas the value closely resemble to the varieties from Singapore (Soong and Barlow, 2004).

4.1.4 DPPH radical scavenging activities of selected seeds of fruits

The DPPH assay has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants. DPPH is one of the compounds that have a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers. The DPPH radical scavenging by antioxidants is attributable to their hydrogen donating ability. The antioxidant activity of seed extracts was calculated according to the reduction in absorbance of DPPH radicals at 517nm. The DPPH scavenging activity of extracts during 30 min was shown inAppendix D.

The quality of the antioxidants in the extract/fractions was determined by the IC_{50} values (the concentration with scavenging activity of 50%) (Table 4.5). A low IC_{50} value indicates strong antioxidant activity in a sample. The lowest IC_{50} value of the MSK extract

(0.112mg/mL) indicated that this fraction exhibited the highest radical scavenging effect. Statistical analysis (one way ANOVA) was done for theIC_{50} values of four different antioxidants which showed a significant difference ($p \leq 0.05$) between the antioxidants (AppendixF).

Table 4. 5 Antioxidant characteristics of fruit seed extracts and reference compounds determined by DPPH method

Antioxidant	IC_{50}	Antiradical activity (A_{AR})
BHA	0.2108 ± 0.008^{a}	4.75 ± 0.178^{a}
BHT	0.1558 ± 0.0005^{b}	6.42 ± 0.019^{b}
MSK extract	0.1117 ± 0.005^{c}	8.97 ± 0.404^{c}
JSK extract	0.2884 ± 0.006^{d}	3.47 ± 0.073^{d}

Each value in the table represents mean ± standard deviation of three replications. Means within each column with same superscripts are not significantly ($p > 0.05$) different.

The IC_{50} values were changed to antiradical activity (A_{AR}) defined as $1/IC_{50}$, since this parameter increases with antioxidant activity. The highest A_{AR} was found to beof MSK extract (8.97) and the lowest was for JSK extract (3.47). Statistical analysis (one way ANOVA) was done for the A_{AR} values of four different antioxidants which showed a significant difference ($p \leq 0.05$) between the antioxidants (Appendix F). The DPPH radical scavenging activity was found to be in the order: MSK extract > BHT > BHA > JSK extract.

Mango seed kernel extract showed higher phenol and flavonoid content than the Jackfruit seed kernel extract. Similarly the antioxidant activity of the extract was shown to be greater for MSK extract than the JSK extract. The higher antioxidant activity for the MSK extract may be due to the higher level of phenol and flavonoids present in the extract as the antioxidant activity has been found to be positively correlated with the total phenol and flavonoid content in mango (Khammuang and Sarnthima, 2011; Soong and Barlow, 2004) and jackfruit (Gupta *et al.*, 2011). Mostly the factors affecting the concentration of phenolics in the seed extract contribute to the antioxidant activity of the extract. Method of

extraction, solvent system for extraction, time of extraction, temperature, ratio of sample to solvent, sample particle size and most importantly cultivar/variety affect the concentration of phenolics in the seed extract and therefore affect the antioxidant activity (Gonz´alez and Gonz´alez, 2010).

Khammuang and Sarnthima (2011) also observed the IC_{50} for seeds of four mangoes (*Mangifera indica* L.) Thai varieties including *Chok-a-nan, Fah-lun, Kaew and Namdok-mai* for which the yield was 2.45, 4.06, 9.31 and 7.74 µg/ml respectively. Maisuthisakul (2008) also observed the IC_{50} for seed kernel extracts of eleven Thai mango varieties including *Kaew, Nam Dokmai, Khiew Sawoey, Pimsaen, Chok-Anan, Rad, Phalun, Hua Chang, Mun Duan Kao, Okrong* and *Maha Chanok* where the IC_{50} were in the range between 13.06-20.54 µg/ml. Similarly, the IC_{50} value for Malaysian mango varieties was found to be 0.11 mg/ml (Norshazila *et al.*, 2010). The IC_{50} value for JSK extract as determined by Gupta *et al.* (2011) was found to be 0.64-0.78 mg/ml. The IC_{50} value for the MSK extract was found to be higher than that determined by Maisuthisakul (2008) and Khammuang and Sarnthima (2011) whereas the IC_{50} value for the JSK extract was found to be lower than that determined by Gupta *et al.* (2011). Thus, the difference in antioxidant activities may be primarily due to the different cultivar and the preharvest factors such as climate (temperatures, rainfalls, and light hours), soil type, and fertilization among the fruits grown in different countries.

The time of extraction also affect the extraction of the phenolics in the seed extract. Maisuthisakul (2009) obtained a mango seed extract using ethanol refluxing for 3 hours whose IC_{50} value was 0.38 whereasIC_{50} of the seed extract obtained by Norshazila *et al.* (2010) using same methods for 8 hours was 0.11. Using the soxhlet extraction for longer time could have extracted the phenolics completely and the antioxidant activity of the extracts could have been higher.

One of the limiting factors for applying conventional solvent extraction is extraction of thermo-labile compounds due to their sensitivity to high temperature(L. Wang and Weller, 2006). Thermo-labile compounds could be extracted by using other extraction techniques with lower temperature and shorter extraction time as these compounds could be protected against thermo degradation.

4.2 Analysis of palm oil on deep-frying

Palm oil (0.5 kg) was put into a Pan-fryer. A batch of 100 g raw potato chips was deep fried for 2.5 min at temperature of 180±5°C. After the interval of 17.5 min, another batch of potato chips was fried again at same temperature for same time. The process was repeated for 10 batch of frying per day. At the end of tenth frying, the temperature was allowed to drop down and the oil samples were analyzed for PV, FFA content and IV. The frying was continued similarly on the next day with no fresh oil added to the pan. At the end of tenth frying on second day, the oil samples were again analyzed for PV, FFA content and IV. Similar frying operation followed by the analysis of oil samples were repeated till seven days.

Five different oil systems were designated following exactly the same frying procedure as described earlier with four of the system having different antioxidants viz., BHA, BHT, Mango seed extract and Jackfruit seed extract and one system without antioxidants i.e., control.

4.2.1 Changes in peroxide value

Changes in the peroxide value during seven consecutive days of frying in all systems are given in Table 4.6.

The results of this study showed that in system without antioxidants, the formation of peroxides seemed to increase rapidly till3rd day of frying, and then dropped over the last 4 days of frying. In system with BHT, results showed that there was a marked increase till the first 4 days of frying but a decrease after 5thday of frying. Statistical analysis (one way ANOVA) was done for the PV of different oil system which showed a significant difference ($p \leq 0.05$) between the different antioxidants used. Similarly, Statistical analysis (one way ANOVA) was done for the PV of different oil system which showed a significant difference ($p \leq 0.05$) between the PV of different days.

The PV rose and fell during frying, which is the same pattern observed for peroxides in most deep-fat frying studies (Gwo *et al.*, 1985; Rady and Madkour, 1995). Since high heat (180 ±5°C) was used on these systems, peroxides formed during oxidation may have decomposed to secondary oxidation products (Robards *et al.*, 1988). Peroxides under deep fat frying conditions are unstable and can break down to carbonyl and aldehydic

compounds under conditions of high heat, air, and light, as present in deep fat frying operations (Perkins, 1967).

Table 4.6 Changes in peroxide value of oil

Characteristics	Day	Control	BHT	BHA	MSK extract	JSK extract	LSD
Peroxide Value (meq hydroperoxide/ Kg oil)	0	$1.53^{a}{}_{Z}$ (0.04)	$1.55^{a}{}_{Z}$ (0.02)	$1.51^{a}{}_{Z}$ (0.03)	$1.57^{a}{}_{Z}$ (0.01)	$1.57^{a}{}_{Z}$ (0.05)	0.061
	1	$13.74^{b}{}_{Z}$ (0.24)	$5.55^{b}{}_{Y}$ (0.30)	$6.93^{b}{}_{X}$ (0.04)	$5.75^{b}{}_{Y}$ (0.08)	$7.13^{b}{}_{X}$ (0.48)	0.537
	2	$19.23^{c}{}_{Z}$ (0.11)	$9.68^{c}{}_{Y}$ (0.73)	$11.05^{c}{}_{X}$ (0.14)	$8.53^{c}{}_{W}$ (0.60)	$11.17^{c}{}_{X}$ (0.39)	0.918
	3	$21.45^{d}{}_{Z}$ (0.12)	$10.68^{d}{}_{Y}$ (0.19)	$12.26^{d}{}_{X}$ (0.02)	$10.43^{d}{}_{Y}$ (0.69)	$12.16^{d}{}_{X}$ (0.21)	0.735
	4	$20.82^{e}{}_{Z}$ (0.37)	$11.86^{e}{}_{Y}$ (0.09)	$12.72^{e}{}_{X}$ (0.20)	$11.16^{d}{}_{W}$ (0.07)	$12.57^{d}{}_{X}$ (0.29)	0.443
	5	$18.67^{f}{}_{Z}$ (0.21)	$9.69^{c}{}_{Y}$ (0.80)	$11.07^{c}{}_{X}$ (0.18)	$9.05^{e}{}_{Y}$ (0.85)	$11.96^{d}{}_{X}$ (0.47)	1.160
	6	$15.74^{g}{}_{Z}$ (0.21)	$8.32^{f}{}_{Y}$ (0.28)	$9.36^{f}{}_{X}$ (0.10)	$7.53^{c}{}_{Y}$ (0.89)	$9.22^{e}{}_{X}$ (0.45)	1.009
	7	$12.82^{h}{}_{Z}$ (0.61)	$6.47^{g}{}_{Y}$ (0.24)	$8.44^{g}{}_{X}$ (0.16)	$7.28^{c}{}_{Y}$ (0.86)	$8.80^{e}{}_{X}$ (0.67)	1.159
LSD		0.502	0.744	0.278	1.326	0.717	

Each value in the table represents mean of three replications. Figures in parenthesis are standard deviation. Mean within each row with same subscripts are not significantly ($p > 0.05$) different. Means within each column with same superscripts are not significantly ($p > 0.05$) different.

RBD palm oil with the addition of antioxidants (BHA, BHT, MSK extract and JSK Extract) had PV that were significantly ($p \leq 0.05$) lower than those of the control throughout the duration of the study. Within the system with antioxidants, the 200 ppm BHA and JSK extract were not significantly ($p > 0.05$) different from each other, and the 200 ppm BHT and MSK extract were also not significantly ($p > 0.05$) different from each other. The PV of all the oil system increased till 4^{th} day of frying and then decreased on the later days. With same palm oil, the oil system with no antioxidant had highest PV after 4^{th} day of frying i.e. 20.82 while the oil system with antioxidants had relatively lower PV values. Thus, it was clear that antioxidant added attributed the oxidative stability of palm oil on frying. On comparing the PV values among the oil system with antioxidants, it was observed that the antioxidant efficacy was in the range MSK extract >BHT > JSK extract > BHA with corresponding values of PV as 11.16, 11.86, 12.57 and 12.72 respectively. In general, these results indicated that BHT and MSK extract were more effective in reducing formation of peroxides in RBD palm oil during frying than JSK extract and BHA. Judging from the PV, the oxidative stability was decreased in the order MSK extract ≈ BHT > JSK extract ≈ BHA > control.

With reference to PV in Table 4.5, it was clear that in the case of MSK extract, the rates of increase in PV were lower than in the four other systems. The effectiveness of MSK and JSK extracts as antioxidants has been attributed mainly to their ability to remain stable at high temperature (Dorta *et al.*, 2012; Gupta *et al.*, 2011; Schieber *et al.*, 2003). Phenolic antioxidants compounds present in the seed extract react with lipid or hydroxyl radicals and convert them into stable products (Gordon, 2001). The major antioxidants in these extracts may be protected by other substances such as flavonoids, found in smaller amounts, which provide thermal stability and enhanced antioxidative activity (Tapas *et al.*, 2008). BHA and BHT are commercially available synthetic phenolic antioxidants with similar molecular structures, and BHT was found to be slight more effective than BHA in performance relative to PV.

4.2.2 Changes in free fatty acid values

Changes in FFA content during seven consecutive days of frying in all systems are given in Table 4.7.

Table 4.7 Changes in free fatty acid values

Characteristics	Day	Control	BHT	BHA	MSK extract	JSK extract	LSD
FFA Content (%)	0	$0.10^{a}{}_{Z}$ (0.03)	$0.10^{a}{}_{Z}$ (0.10)	$0.10^{a}{}_{Z}$ (0.01)	$0.10^{a}{}_{Z}$ (0.01)	$0.10^{a}{}_{Z}$ (0.02)	0.03123
	1	$0.16^{b}{}_{Z}$ (0.02)	$0.14^{b}{}_{Z}$ (0.02)	$0.15^{b}{}_{Z}$ (0.01)	$0.15^{b}{}_{Z}$ (0.005)	$0.15^{b}{}_{Z}$ (0.01)	0.02152
	2	$0.24^{c}{}_{Z}$ (0.03)	$0.20^{c}{}_{Z}$ (0.02)	$0.21^{c}{}_{Z}$ (0.03)	$0.21^{c}{}_{Z}$ (0.02)	$0.20^{c}{}_{Z}$ (0.02)	0.04549
	3	$0.35^{d}{}_{Z}$ (0.03)	$0.32^{d}{}_{Z}$ (0.04)	$0.34^{d}{}_{Z}$ (0.01)	$0.29^{d}{}_{Z}$ (0.03)	$0.32^{d}{}_{Z}$ (0.02)	0.05054
	4	$0.49^{e}{}_{Z}$ (0.04)	$0.42^{e}{}_{Y}$ (0.03)	$0.44^{e}{}_{Y}$ (0.01)	$0.41^{e}{}_{Y}$ (0.01)	$0.45^{e}{}_{Y}$ (0.01)	0.04394
	5	$0.57^{f}{}_{Z}$ (0.03)	$0.55^{f}{}_{Z}$ (0.03)	$0.56^{f}{}_{Z}$ (0.01)	$0.54^{f}{}_{Z}$ (0.01)	$0.57^{f}{}_{Z}$ (0.01)	0.03457
	6	$0.70^{g}{}_{Z}$ (0.02)	$0.66^{g}{}_{Z}$ (0.02)	$0.68^{g}{}_{Z}$ (0.01)	$0.65^{g}{}_{Z}$ (0.03)	$0.68^{g}{}_{Z}$ (0.01)	0.03471
	7	$0.81^{h}{}_{Z}$ (0.02)	$0.77^{h}{}_{XY}$ (0.01)	$0.79^{h}{}_{X}$ (0.005)	$0.77^{h}{}_{XY}$ (0.02)	$0.79^{h}{}_{X}$ (0.02)	0.03307
LSD		0.04525	0.03918	0.02710	0.04071	0.02290	

Each value in the table represents mean of three replications. Figure in the parenthesis are the standard deviation. Mean within each row with same subscripts are not significantly ($p > 0.05$) different. Means within each column with same superscripts are significantly ($p \leq 0.05$) different.

Statistical analysis (one way ANOVA) was done for the FFA values of different oil system which showed a significant difference ($p \leq 0.05$) between the different antioxidants used. Similarly, Statistical analysis (one way ANOVA) was done for the FFA of different oil system which showed a significant difference ($p \leq 0.05$) between the FFA of different days.

Free fatty acid content is the measure of the amount of acylglycerol hydrolysis (Haas, 2005). Free fatty acids act as prooxidants in bulk oils. The prooxidant activity of free fatty acids is due to the ability of the carboxylic acid group of free fatty acids to form complexes with transition metals and or the ability of the acid group to directly promote hydroperoxide decomposition (Frega *et al.*, 1999). Free fatty acids could be important prooxidants in oil-in-water emulsions because they are surface active compounds since they are more polar than triacylglycerols due to the presence of an unesterified carboxylic acid groups (Waraho, 2011). Free fatty acids can therefore reduce the palatability, acceptability, and performance of a lipid and are hence considered a negative trait (Haas, 2005). Thus, the oxidative stability of oil-in-water emulsions could be greatly improved by maintaining low levels of free fatty acids (Waraho, 2011).

The increment of FFA content in oil systems with antioxidants was in the order: MSK extract ≈ BHT < JSK extract ≈ BHA. However, the higher FFA contents of the oil system without antioxidants compared to those with antioxidants cannot be credited to direct antioxidant action. This is because phenolic antioxidants act by inhibiting oxidation reactions and have no direct effect on hydrolytic reactions (Coppen, 1989).

4.2.3 Changes in iodine value

Changes in IV content during seven consecutive days of frying in all systems are given in Table 4.8. Statistical analysis (one way ANOVA) was done for the IV of different oil system which showed a significant difference ($p \leq 0.05$) between the different antioxidants used. Similarly, Statistical analysis (one way ANOVA) was done for the IV of different oil system which showed a significant difference ($p \leq 0.05$) between the IV of different days.

Iodine value is a measure of overall unsaturation and is widely used to characterize oils and fats. Thus, a decrease in iodine value is consistent with the decreasing number of double bonds in oil as it becomes oxidized. Indeed, the tendency of an oil to combine with oxygen of the air and become gummy (known as drying) is measured with the iodine number, which in fact is merely a measure of the level of unsaturation of the oil in question (a higher iodine number will indicate higher unsaturation seeing that iodine is absorbed primarily by the mechanism of addition to the double bonds characteristic of unsaturation) (Aluyor and Ori-Jesu, 2008). Iodine value can be used to estimate oxidative stability of a fat or oil; products with highest unsaturation are most likely to experience autoxidation

(Jana *et al.*, 2011). Changes in iodine value due to loss of unsaturation during accelerated oxidation studies may be used as an index of lipid oxidation (Shahidi and Wanasundara, 2002). As fatty acid oxidation occurs at double bonds, a high IV can indicate a fat sample that will have marginal oxidative stability (Haas, 2005).

Table 4.8 **Changes in iodine value**

Characteristics	Day	Control	BHT	BHA	MSK extract	JSK extract	LSD
Iodine Value	0	$56.96^{a}{}_{Z}$	$56.53^{a}{}_{Z}$	$56.47^{a}{}_{Z}$	$56.58^{a}{}_{Z}$	$56.47^{a}{}_{Z}$	0.992
(g of Iodine/		(0.49)	(0.76)	(0.20)	(0.56)	(0.39)	
100 g oil)	1	$55.31^{b}{}_{Z}$	$55.70^{a}{}_{Z}$	$55.73^{a}{}_{Z}$	$55.62^{ab}{}_{Z}$	$55.51^{b}{}_{Z}$	0.751
		(0.42)	(0.49)	(0.35)	(0.29)	(0.37)	
	2	$53.02^{c}{}_{Z}$	$54.63^{b}{}_{Y}$	$54.10^{b}{}_{Y}$	$53.89^{b}{}_{YZ}$	$54.53^{c}{}_{Y}$	1.016
		(0.41)	(0.13)	(0.31)	(0.88)	(0.29)	
	3	$51.48^{d}{}_{Z}$	$52.26^{c}{}_{Z}$	$52.54^{c}{}_{Z}$	$53.19^{bc}{}_{Z}$	$52.55^{d}{}_{Z}$	1.204
		(0.57)	(0.18)	(0.66)	(0.78)	(0.42)	
	4	$50.11^{e}{}_{Z}$	$51.28^{d}{}_{Y}$	$51.77^{c}{}_{Y}$	$51.40^{c}{}_{Y}$	$50.79^{e}{}_{Y}$	1.031
		(0.43)	(0.13)	(0.31)	(0.87)	(0.38)	
	5	$48.63^{f}{}_{YZ}$	$49.79^{e}{}_{XYZ}$	$50.25^{d}{}_{XY}$	$50.71^{cd}{}_{X}$	$49.23^{f}{}_{YZ}$	1.295
		(0.52)	(0.61)	(0.66)	(0.84)	(0.44)	
	6	$43.47^{g}{}_{Z}$	$44.13^{f}{}_{Z}$	$45.44^{e}{}_{Y}$	$45.89^{e}{}_{Y}$	$44.21^{g}{}_{Z}$	0.972
		(0.33)	(0.30)	(0.78)	(0.08)	(0.57)	
	7	$41.83^{h}{}_{Z}$	$42.45^{g}{}_{Z}$	$43.38^{f}{}_{Z}$	$43.59^{e}{}_{Z}$	$42.74^{h}{}_{Z}$	2.062
		(0.37)	(0.84)	(0.54)	(0.86)	(0.55)	
LSD		0.7721	0.873	1.151	0.873	0.7474	

Each value in the table represents mean of three replications. Figure in the parenthesis are the standard deviation. Mean within each row with same subscripts are not significantly ($p > 0.05$) different. Means within each column with same superscripts are not significantly ($p > 0.05$) different.

Iodine value of all the oil system continuously decreased from the first day of frying to the end of frying. The changes in iodine value of oil system over the 7 days of frying were used as factor for comparing the efficacy of antioxidants in reducing the iodine value. The changes in iodine value over 7 days of frying were 15.13, 14.08, 13.09, 12.99 and 13.73 g of I_2/100 g oil for systems without antioxidants, system with BHT, system with BHA, system with MSK extract and system with JSK extract respectively. A significantly ($p \leq 0.05$) larger change in iodine value in the control (system without antioxidants) compared to the other four systems indicated that the rate of oxidation of unsaturated fatty acids was reduced in the presence of antioxidants. The significantly ($p \leq 0.05$) smaller change in iodine value in systems with BHA and MSK extract compared to that of Systems with BHT and JSK extract showed that less oxidation of unsaturated fatty acids was taking place in systems with BHA and MSK extract at a greater rate. Therefore, the changes in IV showed that the effectiveness in protecting oxidation of unsaturated fatty acid was in the order MSK extract > BHA > JSK extract > BHT > Control.

Part V

Conclusions and recommendations

Based on result & discussion, following conclusions were drawn:

a. Mango & Jack fruit seed extract possess antioxidant activity due to the recognized phenol & flavonoid content that can be effectively used for the oxidative stability of palm oil on deep-fat frying in various industrial applications for the preparation of potato chips, noodles, etc.
b. Among the various natural (mango & jackfruit seed extract) and synthetic (BHA & BHT) antioxidants used in palm oil on deep frying the order of antioxidant potential was found in the order of MSK extract>BHT>JSK extract>BHA.
c. Like various other natural antioxidant sources such as herbs & spices, fruit seed can also be explored as source of antioxidants to minimize the by-products of processing industries.

Following recommendations can be made after the research for further study:

a. Various other fruit seeds can be investigated for antioxidant potential.
b. Sensory quality & storage stability of the potato chips fried in oil incorporated with various antioxidants can be evaluated.
c. The effectiveness of the extract at various other concentrations than 200 ppm can be investigated.
d. The research can be extended to other sample of oil used in culinary purposes & for the deep frying of food products other than potato chips.

Part VI

Summary

Fats & Oils play important role in the industry of deep fried foods such as noodles, potato chips, etc. and hence their deterioration is the major cause of food quality deterioration. Most of the industry uses palm oil for deep frying of food products because of the noted advantage of palm oil. Almost all of the industry use synthetic antioxidants such as BHA, BHT, TBHQ, etc., for the oxidative stability of palm oil during deep frying. However, several health hazards have been researched for all of the synthetic antioxidants so that the consumer now approaches for naturalism to avoid the deterioration of fats & oils. Most of oilseeds, cereal crops, leaves, berries, fruits, spices & herbs were explored by various researchers for processing the antioxidant properties & some obtained positive results when they incorporated these antioxidants source to the fats & oils. However, fruit seeds have received much less attention in comparison to various fruits, herbs & spices. Fruit seeds are thus investigated for their antioxidant capacities to incorporate in fats & oils during deep frying as they are by-product in fruits processing industries and if could be successfully used, it could be assets to the environment protection & make a worthless material useful.

In this study, the extract of mango & jackfruit seed kernel obtained from the fruits cultivated in eastern Nepal were prepared. Fresh kernel seeds were chopped and refluxed with ethanol at a ratio of sample/ethanol of 1:3 (w/v). The residue was then removed by filtering through Whatman No 4 filter paper. The extracts were collected individually and the solvent was evaporated under vacuum at 40°C using a rotary vacuum evaporator (Buchi, Switzerland). All the ethanol solvent was evaporated and the flask was made completely dry. The stock solution of the extract was made at concentration of 10 mg/mL using ethanol. The extracts were stored at 4°C until use. Phenol content, flavonoid content & the radical scavenging activity of the extract were analyzed along with the moisture & yield.

Different frying experiments were conducted for frying of potato chips in various systems: Palm oil with no antioxidants (control), Palm oil with 200 ppm BHT, Palm oil with 200 ppm BHA, Palm oil with 200 ppm MSK extract and Palm oil with 200 ppm JSK

extract. Frying was performed for 100 g of raw potatoes chips for 2.5 min at 180±5℃. Frying were repeated at the interval of 17.5 min and hence 10 frying experiments were accomplished in a day for a period of 3.5 hours. Similar frying experiments were repeated for seven consecutive days and the oil at the end of the day were analyzed for peroxide value, free fatty acids & iodine value. The analytical parameters of the oil were statistically interpreted to know the effect of antioxidants added to the oil for its oxidative stability.

MSK powder had moisture content of 4.16±0.085 whereas that for JSK powder was found to be 4.72±0.06%. One way ANOVA was done for the moisture content of two extracts which showed a significant difference ($p \leq 0.05$) between the extracts.The yield of MSK extract was found to 11.28±0.11 g dry weight extract / 100 g dry sample and that for JSK extract was 10.37±0.08 g dust extract / 100 g dry sample. Statistical analysis (one way ANOVA) was done for the % yield of two extracts which showed a significant difference ($p \leq 0.05$) between the extracts.

The flavonoid content of the extract were determined as per Jia *et al.* (1999)& expressed as MSK extract had significantly ($p \leq 0.05$) higher flavonoid content than JSK extract. The flavonoid content for MSK extract was 0.372±0.02 mg RE/mg dry extract whereas that for JSK extract was 0.196±0.01 mg RE/mg dry extract. Similarly, the phenol content was determined as per Khammuang and Sarnthima (2011) and expressed as mg Gallic Acid equivalents (GAE) per gram of dry extract. MSK extract had significantly higher phenol content than JSK extract. The phenol content for MSK extract was formed to be 116.43±3.79 mg GAE/g dry extract while that for JSK extract was 27.7±2.55 mg GAE/g dry extract.

DPPH assay was used as a tool for estimating from radical scavenging activities of antioxidants. The quantity of antioxidants in the extract was determined by IC_{50} value (concentration with scavenging activity of 50%). A law IC_{50} value indicates strong antioxidant activity in sample. The IC_{50} value for BHA was found to be 0.2108, for BHT to be 0.1558, for MSK extract to be 0.1117 and for JSK extract to be 0.2884. Statistical analysis (one way ANOVA) was done for IC_{50} values of four different samples which showed significant difference ($p \leq 0.05$) between each of the antioxidant source. MSK extract had the highest antioxidant activities, JSK the lowest & BHA and BHT had the antioxidant activities in between the MSK extract & JSK extract.

To study the oxidative stability of palm oil, various analytical parameters such as PV, FFA content and IV were analysed. In the system without antioxidants, the formation of peroxides seems to increase rapidly from day 0 to the day 3 of frying, and then dropped were the last 4 day of frying. In system with antioxidants, PV increased till first 4 day of frying but decreased after 5 day of frying. Judging the PV, the oxidative stability was decreased in the order MSK extract ≈ BHT>JSK extract ≈ BHA>control.

The FFA content of all the five oil system was not significantly ($p>0.05$) different from each other till 3 days of frying. At 4 day of frying, the system with antioxidants had lower FFA value than the system without antioxidants. Judging the FFA at the end of frying operation, the increment of FFA content in oil system was in the order MSK extract<BHT<JSK extract ≈ BHA.

The change in IV was 7 days of frying were 15.13, 14.08, 13.09, 12.99 & 13.73 g of I_2/100 g oil for system without antioxidants, system with BHT, system with BHA, system with MSK extract & system with JSK extract respectively. Thus, the change in IV showed that the effectiveness in protecting oxidation of unsaturated fatty acid was in the order: MSK extract>BHA>JSK extract>BHT>control.

Thus, in conclusion, mango & jackfruit seed kernel can be utilized for the preparation of extract which can be used effectively to replace the synthetic antioxidants incorporated in palm oil for deep frying. Mango seed extract has shown higher potential than the synthetic antioxidants & hence, essential steps have to be further progressed for its effective utilization.

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Appendices

Appendix A

Table A.1 Physical properties of synthetic antioxidants used in foods

Property/Characteristic	BHA	BHT	Dodecyl Gallates	Propyl Gallates	TBHQ
Appearance	Waxy solid	White crystals	White crystals	White crystals	White-tan crystals
Carry through properties	Very good	Fair–Good	Fair–Good	Poor	Good
Boiling point	264–270	265	—	Decompose above 148	300
Melting point	50-52	69-70	146-148	146-148	126-128
Solubility (%, w/w) in					
Corn oil	30	40	0	0	5-10
Glycerol	1	0	—	25	<1
Lard	30-40	50	—	1	5-10
Methyl linoleate	very soluble	very soluble	1	1	>10
Propylene glycol	50	0	6.5	4	30
Water	0	0	<1	<1	<1
Synergism	BHT & gallates	BHA	BHA	BHA	—

Source:(Wanasundara and Shahidi, 2005)

Appendix B

Table B.1 Reactive oxygen and nitrogen species of biological interest

Reactive Species	Symbol	Half-life (in Sec)	Reactivity/Remarks
Reactive oxygen species			
Superoxide	$O_2^{\bullet -}$	10^{-6} s	Generated in mitochondria, in cardiovascular system and others
Hydroxyl	•OH	10^{-9} s	Very highly reactive, generated radical during iron overload and such conditions in our body
Hydrogen Peroxide	H_2O_2	Stable	Formed in our body by large number of reactions and yields potent species like _OH
Peroxyl radical	ROO•	S	Reactive and formed from lipids, proteins, DNA, sugars etc. during oxidative damage
Organic hydroperoxide	ROOH	Stable	Reacts with transient metal ions to yield reactive species
Singlet oxygen	$^1O_2$	10^{-6} s	Highly reactive, formed during photosensitization and chemical reactions
Ozone	O_3	S	Present as an atmospheric pollutant, can react with various molecules, yielding $^1O_2$
Reactive nitrogen species			
Nitric oxide	NO•	S	Neurotransmitter and blood pressure regulator, can yield potent oxidants during pathological states
Peroxynitrite	$ONOO^-$	10^{-3} s	Formed from NO. and superoxide, highly reactive
Peroxynitrous acid	ONOOH	Fairly stable	Protonated form of ONOO
Nitrogen dioxide	NO_2	S	Formed during atmospheric pollution

Source:(Devasagayam *et al.*, 2004)

Appendix C

Appendix for change in moisture and yield of the MSK and JSK extract

Table C.1 ANOVA for change in moisture content (% dry weight basis) for different natural antioxidants, one way analysis at least significant differences of means (5% level)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Extract	1	0.470400	0.470400	94.08	<.001
Residual	4	0.020000	0.005000		
Total	5	0.490400			

Significant difference observed between the samples. LSD for samples = 0.1603

Table C.2 ANOVA for change in Yield (g dwt extract/100 g dwt)for different natural antioxidants, one way analysis at least significant differences of means (5% level)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Extract	1	1.260417	1.260417	141.09	<.001
Residual	4	0.035733	0.008933		
Total	5	1.296150			

Significant difference observed between the samples. LSD for samples = 0.2143

Appendix D

List of figures

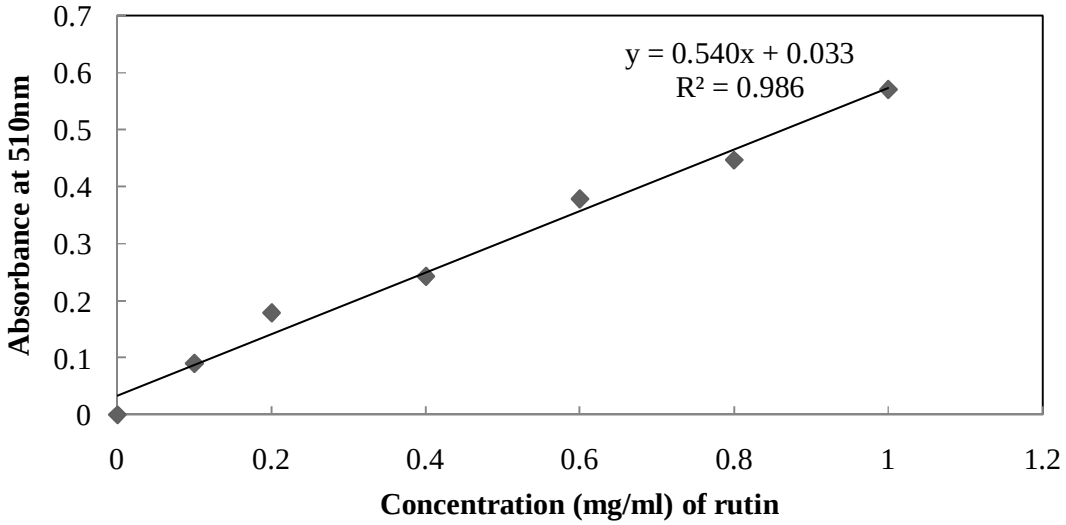

Fig.D.1 Standard curve of determination of Rutin Equivalent (RE) for total flavonoids assay

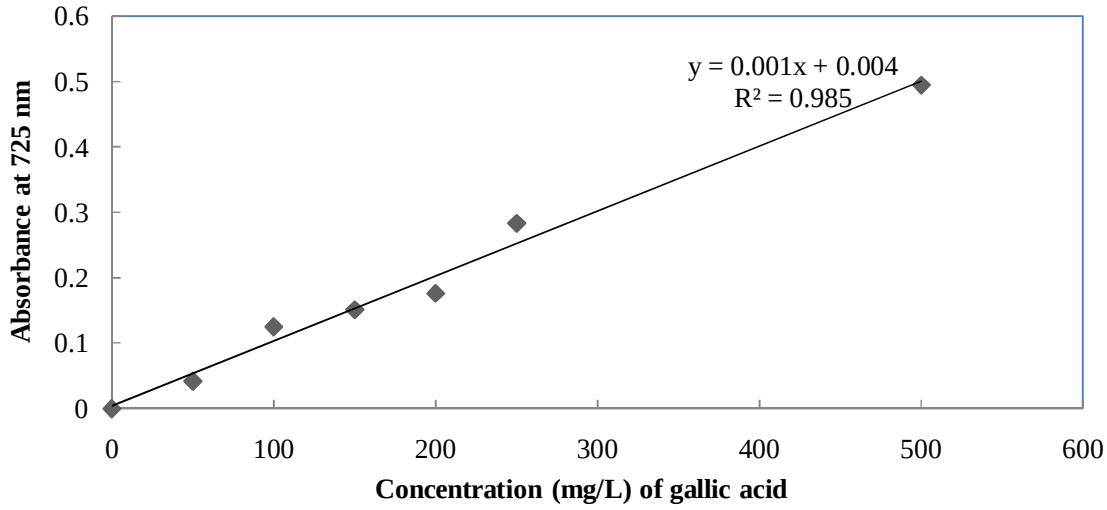

Fig.D.2 Standard curve of determination of Gallic Acid Equivalent (GAE) for total phenolic assay

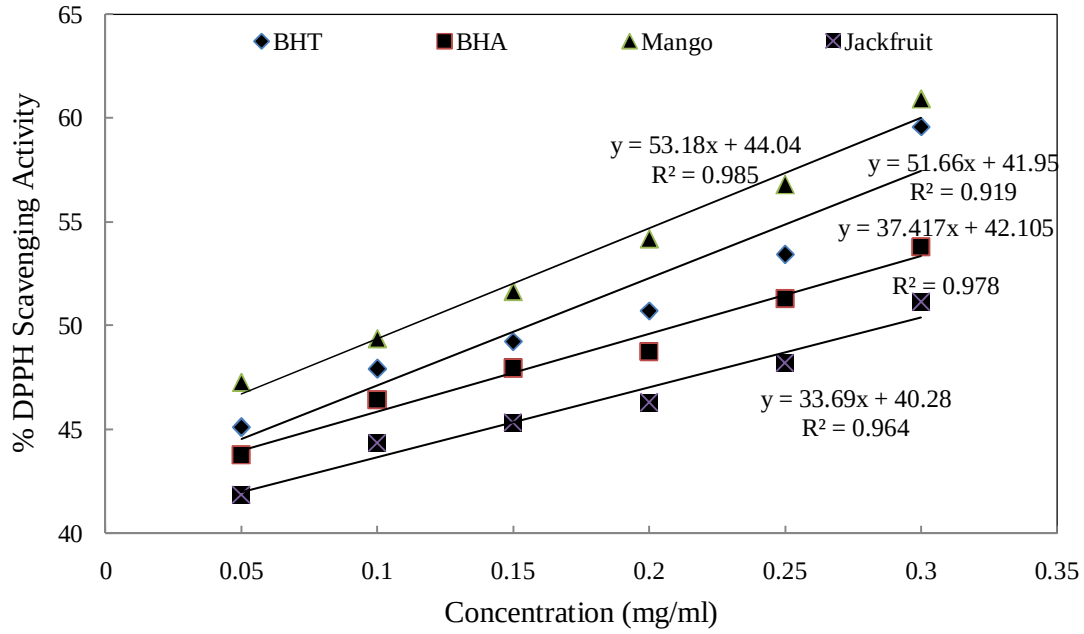

Fig.D.3 Antioxidant activities at different concentration of MSK and JSK extracts in comparison with reference antioxidant compounds, BHA and BHT using DPPH free radical scavenging assay

Appendix E

ANOVA for the flavonoid and phenol content of the MSK and JSK extract

Table E.1ANOVA for change in flavonoid content (mg RE/mg dry extract) for different natural antioxidants, one way analysis at least significant differences of means (5% level)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Extract	1	0.0464640	0.0464640	272.78	<.001
Residual	4	0.0006813	0.0001703		
Total	5	0.0471453			

Significant difference observed between the samples. LSD for antioxidants = 0.02959

Table E.2 ANOVA for change in phenol content (mg GAE/mg dry extract)for different natural antioxidants, one way analysis at least significant differences of means (5% level)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Extract	1	11806.86	11806.86	1131.62	<.001
Residual	4	41.73	10.43		
Total	5	11848.59			

Significant difference observed between the samples. LSD for antioxidants = 7.32

Appendix F

ANOVA for comparing the antioxidant activity of different antioxidants including BHA, BHT, MSK extract and JSK extract

Table F.1 ANOVA for change in % DPPH Scavenging Activity for different antioxidant, two way analysis at least significant differences of means (5% level)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antioxidants	3	511.728	170.576	168.03	<.001
Concentration (mg/ml)	5	1047.917	209.583	206.45	<.001
Residual	63	63.956	1.015		
Total	71	1623.601			

Significant difference observed between the samples.

LSD for antioxidants = 0.671; LSD for concentration (mg/ml) = 0.822

Table F.2 ANOVA for change in Antiradical Activity for different antioxidant, one way analysis at least significant differences of means (5% level)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antioxidants	3	50.75822	16.91941	336.68	<.001
Residual	8	0.40203	0.05025		
Total	11	51.16024			

Significant difference observed between the samples. LSD for antioxidants = 0.4221

Table F.3 ANOVA for change in $IC_{50}$for different antioxidant, one way analysis at least significant differences of means (5% level)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Antioxidants	3	0.05224053	0.01741351	560.10	<.001
Residual	8	0.00024872	0.00003109		
Total	11	0.05248925			

Significant difference observed between the samples. LSD for antioxidants = 0.01050