1. INTRODUCTION

1.1. LIPID OXIDATION

Lipids are important in foods due to their contribution to palatability, satiety, and nutrition[1]. Therefore, lipid quality is significant to consumers and may have a link to many health problems. Lipid oxidation is a major problem in many sectors of the food industry. Retarding lipid oxidation not only extends product's shelf life but also reduces raw material waste, nutritional loss, and widens the range of lipids that can be used in specific products. Thus, control of lipid oxidation could allow food processors to use more available, less costly and/ more nutritionally favourable oils for product formulations.

Lipid oxidation can occur during oil extraction and refining, resulting in the formation of lipid hydro peroxides. Lipid hydro peroxides have no flavour or odour but break down rapidly to form secondary products, many of which have a strong flavour and odour. The lipid hydro peroxide concentration in oils is generally expressed as peroxide value (PV), and can range from < 1.0 to over 15.0 milli equivalents/kg in commercially available oils.

Lipid oxidation is one of the major causes of quality deterioration in natural and processed foods. Oxidative deterioration is a large economic concern in the food industry because it affects many quality parameters such as flavour (rancidity), colour, texture, and the nutritive value of foods. In addition, it produces potentially toxic compounds [2, 3, 4]. Lipid oxidation is important to food manufacturers especially when they increase unsaturated lipids in their products to improve nutritional profiles. Thus lipid oxidation is one of the major processes that limit the shelf life of foods. In addition, the oxidative instability of PUFA often limits their use as nutritionally beneficial lipids in functional foods.

1.2. OXIDATION PRODUCTS

Lipid oxidation in bulk oil has often been considered a homogeneous liquid phase reaction. However, edible oils contain polar lipids, such as mono- and diacylglycerols, phospholipids, sterols, tocopherols, and free fatty acids that are not completely removed by the refining process. Oils can also contain polar oxidation products (e.g. lipid hydroperoxides, aldehydes, ketones, and alcohols) that have higher polarity than their original lipid substrates due to the addition of oxygen. Bulk oils also contain small amounts of water. Since polar lipids are surface active and thus have an affinity for both polar and non-polar environments, they tend to form associated colloid structures. A good understanding of the impact of surface active compounds on lipid oxidation in bulk oils could lead to the development of new antioxidative techniques to control rancidity.

1.3. EFFECT OF UNSATURATED FATTY ACIDS

Most oils and some fats contain PUFA which are susceptible to oxidation. During the course of oxidation, the total unsaturated fatty acid content of lipids decreases with a concurrent increase in the amount of primary and secondary oxidation products such as lipid hydroperoxides, aldehydes, ketones, hydrocarbons, and alcohols. With the exception of hydrocarbons, these oxidation products are amphiphilic. Increasing levels of unsaturated fatty acids in foods increase lipid oxidation rates and thus decrease shelf-life of the foods. The autoxidation rate greatly depends on the rate of fatty acid or acylglycerol alkyl radical formation, and the radical formation rate depends mainly on the types of fatty acid or acylglycerol. The relative rate of autoxidation of oleate, linoleate, and linolenate is in the order of 1:40–50:100 on the basis of oxygen uptake and 1:12:25 on the basis of peroxide formation[5]. Therefore, oils that contain relatively high proportions of PUFA may experience stability problems. The breakdown products of hydroperoxides, such as alcohols, aldehydes, ketones, and hydrocarbons, generally possess offensive off-flavors. These compounds may also interact with other food components and change their functional and nutritional properties.

Rancidity in food occurs when unsaturated fatty acids decompose into volatile compounds. These volatile oxidation products are produced from the decomposition of fatty acid hydroperoxides. The homolytic cleavage of hydroperoxides between these two oxygen molecules is the most likely hydroperoxide decomposition pathway. Bleaching, deodorization, and physical refining can remove many oxidation products, but in reality most commercial fats and oils contain lipid oxidation products.

1.4. FACTORS INFLUENCING THE EDIBLE OIL QUALITY

Edible oil consists of mostly triacylglycerols, but it also contains minor components such as free fatty acids, mono- and di-acylglycerols, metals, phospholipids, peroxides, chlorophylls, carotenoids, phenolic compounds, and tocopherols. Some of them accelerate the oil oxidation and others act as antioxidants. Oxidation of edible oils is influenced by an energy input such as light or heat, composition of fatty acids, availability of oxygen, and minor compounds.

One of the major changes that occur during processing, distribution and final preparation of food is oxidation. Lipid autoxidation occurs by a free-radical chain reaction[6]. In foods, the oxidant is usually molecular oxygen from the air or singlet oxygen. Oxidation of lipids initiates other changes in the food system that affects its nutritional quality, wholesomeness, safety, colour, flavor and texture. Oxidation of oil also destroys essential fatty acids and produces toxic compounds and oxidized polymers. Several degradation reactions, both on heating and on long term storage, deteriorate fats, oils and lipid-based foods. Oxidation reactions and the decomposition of oxidation products are the main deterioration processes which result in decreased nutritional value and sensory quality.

1.5. MINOR CONSTITUENTS AND THEIR ROLE IN EDIBLE OILS

Edible oils naturally contain minor constituents such as tocopherols, tocotrienols, carotenoids, phenolic compounds, and sterols. In general, excluding free fatty acids, some of these minor constituents are referred to as the unsaponifiable fraction and consist of compounds such as phenols (e.g. tocopherols), sterols, carbohydrates, pesticides, proteins, trace metals, and pigments such as gossypol, carotenoids and chlorophyll. Some of the non-triacyglycerol compounds are deleterious to oil quality (e.g. free fatty acids and chlorophyll), however, some are desirable, such as carotenoids for color, and phenolic antioxidant compounds (e.g. tocopherols) that both protect the oil against oxidation as well as providing beneficial nutrients to consumers. Most consumers and food manufacturers want bland-flavored, light-colored, and physically and chemically stable oils. Thus, numerous non-triacylglycerol compounds in crude oils need to be removed through an operation known as refining. Some oils, such as olive, peanut, sesame, and fats like tallow, lard etc. are consumed with minimal refining.

Antioxidants are sometimes intentionally added to oil to improve oxidative stability. Antioxidants are compounds that extend the induction period (IP) of oxidation or slow down the oxidation rate. Antioxidants scavenge free radicals such as lipid alkyl radicals or lipid peroxy radicals, control transition metals, quench singlet oxygen, and inactivate sensitizers. Hence, antioxidants are one of the principal ingredients that protect food quality by preventing oxidative deterioration of lipids.

1.6. TYPES OF FOODS PRONE TO OXIDATION

Foods that may require stabilization include PUFA-rich edible oils and fats used for frying and in processed foods, margarines that contain animal fats, milk fat, fried foods such as potato chips, roasted nuts, dried soups, broths and seasonings, dried meat, frozen fish and fish oil, dried milk, potato powder, flakes, granules, cakes, chewing gum, concentrated vitamin preparations, flavourings and essential oils. Edible oils containing high proportions of linoleic or linolenic acids are more prone to oxidation than oils high in oleic acid.

1.7. CONSUMER AWARENESS

During the past two decades, the consumers have become suspicious about synthetic antioxidants; even though they have been proven by experiments to be safe, while natural alternatives are considered not to present significant health risks. Gradually, food safety legislation has developed to allow only the use of those synthetic antioxidants that have been tested for safety on animals, and more and more complex, time-consuming and expensive toxicological studies have been required to guarantee the safety of stabilized foods[7]. Manufacturers also prefer natural products, as synthetic antioxidants require expensive testing and their use might be objected by consumers[8].

1.8. OXIDATIVE STABILITY

Oxidative stability of lipids is the resistance to oxidation during processing and storage[9]. Resistance to oxidation can be expressed as the period of time necessary to attain the critical point of oxidation, whether it is a sensorial change or a sudden acceleration of the oxidative process. Oxidative stability is an important indicator to determine oil quality and shelf life because low-molecular-weight off-flavor compounds are produced during oxidation. The off-flavor compounds make oil less acceptable or unacceptable to consumers or for industrial use as a food ingredient. The prevention or retardation of these oxidation processes is essential for the food producer and for all persons involved in the value chain.

Various methods may be used to inhibit oxidation, including prevention of oxygen access, use of lower temperature, inactivation of enzymes catalyzing oxidation, reduction of oxygen pressure, and the use of suitable packaging[10]. Another method of protection against oxidation is the use of specific additives, which inhibit or retard the reaction. These oxidation inhibitors are generally known as "antioxidants". Antioxidants represent a class of substances that vary widely in chemical structure and have varied mechanisms of action. Antioxidant activity depends on many factors, including lipid composition, antioxidant concentration, temperature, oxygen pressure, and the presence of other antioxidants and food constituents[11].

Traditionally, food manufacturers have increased the oxidative stability of their products by a variety of methods. Increased oxidative stability can be achieved by reducing PUFA concentrations. This can be accomplished by the replacement of PUFA with fats high in saturated fatty acids (e.g. tropical oils). However, this practice is contrary to current nutritional recommendations that advocate an increase in dietary unsaturated fatty acids for the purpose of decreasing the risk of coronary heart disease. Another method to decrease rancidity is to use partial hydrogenation of fats and oils to remove the most highly unsaturated fatty acids that are very susceptible to oxidation (e.g. linolenic acid). However, partial hydrogenation leads to the formation of *trans* fatty acids. Several studies have shown that *trans* fatty acids are more atherogenic than saturated fats because they both increase low density lipoprotein (LDL) and decrease high density lipoprotein (HDL). Besides alteration in fatty acid content, there are only a limited number of approaches that can be used to control lipid oxidation in foods. Exclusion of oxygen from products, while effective, is often not practical during processing and storage. Addition of antioxidants that scavenge free radicals and control prooxidative metals are the most common methods used to retard lipid oxidation[12] however, only a limited number of free radical scavengers and metal chelators are available for use in foods. Some of the most effective free radical scavengers and metal chelators are synthetic compounds which are often perceived negatively by consumers. Therefore, to overcome the challenge of developing consumer acceptable foods (e.g. no *trans* fatty acids or synthetic food additives) with nutritionally significant amounts of unsaturated fatty acids, the food industry will have to develop new antioxidant technologies.

Several antioxidant technologies have the potential to stabilize foods prone to oxidative rancidity. These technologies include control of prooxidants (e.g. reactive oxygen species and prooxidant metals) and reducing the damaging effects of free radicals (e.g. free radical scavengers)[13]. Unfortunately, very few new antioxidant ingredients have been introduced to the food industry over the past few decades. The efficiency of antioxidants can be increased by delivering them to the site of the oxidative reactions or to use them in combination with other technologies that can reduce oxidation rates. However, in order to be able to develop technologies that utilize antioxidant ingredients to their full potential, the mechanisms of lipid oxidation reactions in the food must be thoroughly understood.

1.9. MECHANISMS OF LIPID OXIDATION

An abundance of evidence indicates that the reaction between lipids and oxygen, termed autoxidation, is a free radical chain reaction .The mechanism of chain reaction can be discussed in terms of initiation, during which free radicals are formed, propagation, during which free radicals are converted into other radicals and termination during which two radicals combine with the formation of stable products[14].

Initiation $ROOH \leftrightarrow ROO\cdot + H$

$ROOH \leftrightarrow RO\cdot + OH^-$

$2ROOH \leftrightarrow RO\cdot + H_2O + ROO\cdot$ ----------1.1

Propagation $R\cdot + O2 \leftrightarrow ROO\cdot$

$ROO\cdot + RH \leftrightarrow ROOH + R\cdot$ ----------1.2

Termination $ROO\cdot + ROO \leftrightarrow ROOR + O_2$

$RO\cdot + R \leftrightarrow ROR$ ----------1.3

Reaction mechanism of phenolic antioxidant and hydroperoxide

A recent increase in serious research on the commercial application of radical scavengers and flavonoids as beneficial anti-aging and photo protection ingredients in cosmetic products[15] and the demand for non-toxic antioxidants that are active in hydrophilic and lipophilic systems, led to the additional focus on the tropical relevance of food derived extracts in the present study. As in food products two applications of phenolics antioxidants might be of interest: i) as a substitute for synthetic preservatives and ii) as active ingredients.

1.10. ANALYTICAL METHODS

Analytical methods play an important role in assessing the chemical composition of the extract of natural products. The accurate determination of their antioxidant activity, radical scavenging activity, the stability of PUFA-rich edible oils after the addition of these extracts has given added advantage to the food technologist to discover simple, speedy, precise methods which are amenable to automation.

Among all, spectrophotometric methods are very informative and widely used for both quantitative and qualitative analyses. Spectrophotometric methods based on absorption or emission of radiation in the Ultraviolet (UV), Visible (Vis), Infrared (IR) and Radio Nuclear Magnetic Resonance (NMR) frequency ranges are most commonly encountered in traditional food laboratories. Spectroscopy in the Ultra-Violet-Visible (UV-Vis) range is one of the most commonly encountered laboratory techniques in food analysis.

Similarly, chromatography is one of the most powerful techniques available to the analyst for the separation of the mixtures. It is a group of techniques which work on the principle of separation of components of a mixture, depending on their affinities for the solutes, between two immiscible phases. Chromatography encompasses a diverse but related group of methods that permit the separation, isolation, identification and quantification of components in a sample[16]. All of the methods involve application of the sample in a narrow (small) initial zone to a stationary phase by the flow of a mobile phase. Separations are based on differences in migration rates among sample components during multistage distribution process. Thin-Layer chromatography (TLC), paper chromatography (PC), column chromatography (CC), High performance liquid chromatography (HPLC) and Liquid Chromatography-Mass Spectroscopy (LCMS) techniques were employed during the separation, isolation, identification and quantification of components from the test samples.

TLC is a simple, quick, and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate). Generally, a solvent or a solvent mixture of the lowest polarity consistent with a good separation is employed.

CC is often carried out technique which is adaptable to all the major types of chromatography. Diverse operations as adsorption, partition, ion exchange, exclusion and affinity chromatography techniques can be carried out in column chromatography.

HPLC is a popular method of analysis as it provides both quantitative and qualitative data in a single operation and is easy to learn and use. It is not limited by the volatility or stability of the sample compound. HPLC involves the separation of the compounds on the basis of their polarity. It is used to analyze, identify, purify and quantify the compounds of various organic and inorganic solutes in any sample especially biological, pharmaceutical, food, environmental, industrial, etc.

Oxidative rancidity is one of the most critical factors affecting the shelf-life of processed foods. PUFA are attacked by oxygen to yield peroxides, which then decompose further. A rapid test to predict resistance of oils to oxidation has long been sought. The schaal oven test and the active oxygen method[17] are the oldest and the most widely used. These tests are of great utility when comparing oils for their general stability towards oxidation. Their major disadvantage is that they fail to predict stability, particularly the onset of or nature of off-flavours in lipid containing products.

Recently, the Metrohm Rancimat (Metrohm Instruments, Herisau, Switzerland) has been advanced as a method to determine resistance of oil toward oxidation. Several studies in the literature, [18, 19 & 20] have claimed a correlation between Active Oxygen Method (AOM) and the Oil Stability Index (OSI) determined by the Rancimat. Advantages of the Rancimat technique are that it is a continuous measurement, which requires no periodic analytical determinations and therefore, requires no organic solvents for titrations. The Rancimat method is based on automatically determining the time elapsed for the maximum rate change of oxidation by measurement of the increase in the conductivity of deionized water caused by dry air bubbled through heated sample carrying the volatile acids into a separate container with the deionized water[20].

1.11. PHENOLIC ANTIOXIDANTS AND THEIR IMPACT ON OXIDATION

Phenolic antioxidants functions as free radical terminators and sometimes also as metal chelators. Phenolic compounds and some of their derivatives are very efficient in preventing auto oxidation. The major considerations for acceptability of such antioxidants are their activity and potential toxicity. Antioxidants in foods may originate from compounds that occur naturally in the foodstuff or from substances formed during its processing. Examples of common plant phenolic antioxidants include flavonoid compounds, cinnamic derivatives, coumarins, tocopherols and polyfunctional organic acids. Several studies have been carried out in order to identify natural phenolics that possess AA. Phenolic antioxidants play a very important role of protecting organisms against harmful effects of oxygen radicals and other highly reactive oxygen species. The formation of these oxygen species in human organisms is closely connected with the development of a wide range of degenerative and non degenerative diseases, mainly arteriosclerosis and other associated complications, cancer, indispositions, and last but not least with the accelerated aging of organisms.

1.12. ESSENTIAL REQUIREMENTS FOR ANTIOXIDANTS

The most readily acceptable oxidation inhibitors are common food ingredients, as their use is not limited by legislation. Many foods contain compounds that possess AA, but some of such foods are of limited use as additives, as they impart a specific flavour, aroma or colour to the finished product; furthermore, those that have low AA or low solubility in lipids are of limited use in the stabilization of edible oils and fats, although they may be used in fatty foods. Purified ‘nature-identical’ substances or extracts obtained from foods that have AA may be used as well. Manufactures also prefer natural products, as synthetic antioxidants require expensive testing and their use might be objected by consumers. Of the hundreds of compounds that have been proposed to inhibit oxidation deterioration of oxidizable substance, only a few can be used in the products for human consumption. Antioxidants are required to be approved for the intended use. It has been suggested that an ideal food grade antioxidant should be safe; absence of undesirable effects on colour, odor or flavor, be effective at low concentrations (0.001-0.01%); Compatibility with the food and ease of application; survive after processing; and be stable in the finished product (carry-through) as well as being available at low cost. The compound and its oxidation products must be nontoxic, even at doses much larger than those that normally would be ingested in food. The usage levels of antioxidants are generally defined on the basis of the fat content of the recipient food and are limited to 0.02% total antioxidants.

1.13. PRIMARY ANTIOXIDANTS

Primary antioxidants interfere with autoxidation by interrupting the chain propagation mechanism. In turn, they are oxidized slowly in the process and normal autoxidation proceeds when the antioxidant has been destroyed completely. The best known and most effective primary antioxidant substances are polyphenols. AA, however, is not restricted to the phenols; other compounds, usually containing nitrogen or sulfur functional groups with electronic configuration similar to that of the polyphenols may be potent antioxidants.

No single antioxidant offers a panacea to oxidative deterioration for all food products. The selection of the appropriate antioxidant appears to be determined by compatibility with and resulting effectiveness in certain fats i.e., animal fats or vegetable oils; its application; its solubility in the fat or water phase of the product; good dispersibility throughout the food - a factor of even greater importance in foods with low fat content and its stability or "carry through" potential post processing.

Antioxidants have possibly been used to preserve fats long before recorded history. In prehistoric times, it is conceivable that aromatic plants were used to give fats, to be used for medicinal purposes and foods, pleasant odors and flavors.

Antioxidants were first used prior to World War II as food preservatives[10]. These early antioxidants were natural substances but soon were replaced by synthetic ones. Synthetic antioxidants were not only cheaper, but also exhibited more consistent purity and more uniform antioxidant properties than did early natural antioxidants. However, consumers challenged the increased use of various synthetic food additives. Consequently, a growing demand for the use of natural compounds to preserve food was shown by consumers as natural materials were considered to be more acceptable as dietary components. Industrial producers have tried to act in accordance with consumers' wishes, and have moved towards the increased use of natural antioxidants. Antioxidants are the compounds that when added to food products, especially to lipids and lipid-containing foods, can increase the shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage. Synthetic antioxidants, such as BHA and BHT, have restricted use in foods as these synthetic antioxidants are suspected to be carcinogenic[21]. Therefore, the importance of the search for and exploitation of natural antioxidants, especially of plant origin, has greatly increased in recent years[22].

1.14. PHYTOCHEMICALS AND NATURAL ANTIOXIDANTS

Hundreds of phytochemicals are currently being studied. Many are believed to have a major positive impact on human health. The antioxidants are usually phenolic or polyphenolic compounds. Plant phenolic compounds are secondary metabolites which have been isolated over a period of time from natural sources. The widespread interest in the potential benefits of phytochemicals as phenolic antioxidants has led to considerable commercialization activities surrounding several commodity extracts.

Natural antioxidants may be found in any plant parts, viz., fruits, vegetables, spices, nuts, seeds, leaves, roots stem and bark have been considered as potential sources of natural antioxidants. The same compound or group of compounds is not always present throughout the plant. Plants have the ability to synthesize mixtures of structurally diverse bioactive compounds with multiple and mutually potential therapeutic effects. Some of the plant materials are rich in antioxidants, which make them excellent sources for increased health benefits. Health advantages of diets rich in antioxidant plant compounds include lowering the risk of cardiovascular disease, certain cancers and the natural degeneration of the body associated with the aging process. Phytochemicals with antioxidant properties tend to be brightly colored because they contain chromophores, i.e., a series of alternate single and double bonded carbons. Isoprene is the building block of such units. The phytochemicals may belong to the following categories; Terpenoids, Phenolic compounds, Alkaloids, Glycosides, Carbohydrates, Lipids, Proteins, Nucleic acids, etc. Antioxidant compounds are usually present in rather low amounts in natural materials. Therefore, large additions of antioxidant-containing material would be required to obtain a significant improvement in stability against oxidation, which may be accompanied by a negative effect on the flavour or functional properties of the product.

Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The phytochemicals in plant foods are believed to exert health beneficial effects, as they combat oxidative stress in the body by maintaining a balance between oxidants and antioxidants. Therefore, food industry is concentrating on plant phenolics, as they retard oxidative degradation of biomolecules like lipids, DNA and proteins[23]. Leafy and green leafy vegetables are most commonly consumed and are accessible throughout the year in India and other parts of world. They are also rich in bioactive molecules such as carotenoids and polyphenols with health promoting potential[24]. The AA of plants is mainly contributed by active compounds present in them. The amount of such compounds deposited in each part of plant is usually different. Spices and herbs, in addition to contributing taste and aroma to foods, also contain a variety of bioactive substances which are of considerable use from the standpoint of food science and technology[25]. These may be used singly or in combination and some act synergistically to control spoilage of foods [26].

1.15. FRUIT PEEL AND KERNELS

Fruits from the temperate zone are usually characterized by a large portion and moderate amounts of waste material such as peel, seeds and stones. In contrast, considerably higher ratios of waste and by products arise from tropical and sub-tropical fruit processing. Due to increase production, disposal represents a growing problem since the plant material is usually prone to microbial spoilage, thus limiting further exploitation. On the other hand, cost of drying, storage and shipment of byproducts are economically limiting factors. Therefore, agro-industrial waste is often utilized as feed, fertilizer or firewood. However, demand for feed may be varying and dependent on agricultural yields. The problem of disposing waste and byproducts is further aggravated by legal restrictions. Thus, efficient, inexpensive and environmentally sound utilization of these materials is becoming more important especially since profitability and jobs may suffer[27].

Mango (*Mangifera indica* Linn.) is one of the most important tropical fruits in the world and currently ranked 5th in total world production among the major fruit crops[28]. As mango is a seasonal fruit, about 20% of fruits are processed for products such as puree, nectar, leather, pickles, canned slices and chutney and these products experience worldwide popularity and have also gained increased importance in the International markets[29]. During processing, huge amounts of peel and kernels are generated as a waste and by-product and their disposal is a major problem. Waste and by products of mango processing amount to 35–60% of the total fruit weight. Their complete exploitation for further product recovery is a promising measure from both an environmental and economic point of view. The peel constitutes about 10–20% and kernel contributed about 17-25% of the fresh fruit[30]. Both the peel and kernel contains various bioactive compounds and their extracts exhibited potential antioxidant properties.

Pomegranate (*Punica granatum* L.), also known as Granada, Grenade, Grenadier, and Granatum, is a native of Persia, but is now cultivated around the world. Pomegranate, a rich source of antioxidants, has been linked to improve heart health, but a growing body of science indicates the fruit protect against prostate cancer and slowing cartilage loss in arthritis. The many compounds contained in pomegranate rind often work synergistically to achieve its many benefits, however each compound can also be potent separately. It is these antioxidants, and particularly ellagitannin compounds like punicalagins and punicalins, which accounts for about half of the fruit's antioxidant ability, that are reportedly behind the proposed health benefits. Pomegranate peel is underestimated as an agricultural waste. Pomegranate juice has proved to be very strong antioxidant; however the peel is also in possession of life saving chemical properties[31]. The most poignant health benefits of this fruit stem from the many active phytochemicals that it contains particularly the tannins and flavanols. Tannins are polyphenol complexes derived from plants that bind and precipitate proteins. Studies show that some tannins exhibit moderate antimicrobial activities. Plants containing tannins are generally astringent in nature and have been used in the treatment of gastrointestinal disorders such as diarrhea and dysentery. The peel is populated by these substances as well as several others. Studies show that it contains tannins, anthocyanins, flavonoids, and pectins. Pomegranate juice was found to exhibit three times more antioxidant activity than red wine or green tea. The active constituent that appears to be responsible is ellagic acid, a naturally occurring polyphenolic compound in pomegranates.

Areca catechu Linn. (Palmae, Arecaceae) commonly known as Betel palm or Betel nut tree is a species of palm, which grows in the tropical Pacific, Asia and parts of east Africa for their economically important seed crop. The activities of areca nut are antihelminthic, antifungal, antibacterial, anti-inflammatory, antioxidant, insecticide and lavicide[32]. The main constituents of the areca nut are carbohydrate, fats, fibre, polyphenol including flavonoids and tannins, alkaloids and minerals[33]. Areca nut is already made available in the market with different varieties such as powdered quality.

1.16. PLANT LEAVES

Curry leaf (*Murraya koenigii)* is a tropical to sub-tropical tree in the family Rutaceae, which is native to India[34]. The leaves of *Murraya koenigii* have a slightly pungent, bitter and feebly acidulous taste and these characteristics are retained after drying. Its leaves are commonly used in Indian cooking as a flavoring agent. The leaves are also used as herb in Ayurvedic medicine.

Guava (*Psidium guajava* L.) is widely cultivated and its fruit is popular in most of the countries. Guava was also used as a hypoglycemic agent in folk medicine.

The leaves and skin of the fruit have greater effects. Guava tea, the infusion of dried guava fruit and leaves, has recently become popular as a drink (health tea) in certain parts of the world. Its leaf contains copious amounts of phenolic phytochemicals which inhibit peroxidation reaction in the living body and therefore, can be expected to prevent various chronic diseases. There is an important role of oxidative stress in the development of cancer and diabetes. As noted above, the infusion of guava leaves has potential as a functional drink[34].

Betel leaves (*Piper betel*) need no introduction in India except in North Western parts as its main use in 'pan' throughout the country is well known. Betel belongs to the genus Piper of the family *Piperaceae*. The plant is a slender, aromatic creeper, rooting at the nodes[34]. The branches of the plant are swollen at the nodes. The plant has alternate, heart shaped smooth, shinning and long stalked leaves with pointed apex. Betel leaves are used as masticatory in Asian countries. Recent studies have shown that betel leaves contain tannins, sugars, diastases and an essential oil. The essential oil contains a phenol called chavicol which has powerful antiseptic and antioxidant properties. The betel leaf chewed along with betel nut, which contains eugenol, also a vasoconstrictor. It is often chewed in combination with the betel nut (*Areca catechu*), as a stimulatory. Some evidence suggests that betel leaves have immune boosting properties as well as anti-cancer properties. The essential oil is produced by steam distillation from the leaves of *Piper betel*. Betel leaf oil is yellow to brown with a distinctly phenolic, almost tar-like or smoky.

The natural antioxidants from residual sources may be used for increasing the shelf life of food by preventing lipid per oxidation and protecting from oxidative damage. Increasing the oxidation stability of vegetable oils is important for industrial practice and many antioxidant tests are based on their ability to retard or inhibit the oil rancidity. Many tests are carried out under extreme conditions; however, more practical assays have been used for food during storage or processing.

1.17. OBJECTIVES

(1) To concentrate and extract natural antioxidants from different plant materials using organic solvents such as methanol, ethanol, acetone, chloroform and ethyl acetate.

(2) To evaluate the antioxidant capacities of the extracts obtained from plant material by organic solvents using the total phenolic assay, the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging method, Reducing Power(RP), Ferric Thiocyanate (FTC) method and β-carotene-linoleic acid (linoleate) model system.

(3) To isolate and identify the bio active phytochemicals from the extracts of the plant materials using appropriate chromatographic techniques and spectral studies.

(4) To study the oxidative inhibition efficiency of the extracts by incorporating them in PUFA-rich edible oils such as RBD SFO, RBD SBO etc.

Therefore, this study directly compares typical byproducts from fruit and vegetable residues with interesting phenolic content but no reported detailed study of their antioxidant potential with regard to two aspects; i) the general potential of plant residues as a source of antioxidants compared to already established ones; ii) whether further investigation might be worthwhile in view of practical and economic limitations on their production and effective utilization.

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