6. SUMMARY, CONCLUSION AND RECOMMENDATIONS

The search for cheap, renewable and abundant sources of antioxidant compounds is attracting worldwide interest. The natural antioxidants from residual sources may be used for increasing the shelf life of food by preventing lipid peroxidation and protecting from oxidative damage. Increasing the oxidation stability of PUFA rich edible oils is important for industrial practice and many antioxidant tests are based on this 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.

The investigations of the present study are concluded as follows:

Methanol proved to be the best among five solvents selected in extracting polyphenols from the plant materials under study. Ethanol showed the maximum efficiency for TP and BP. Ethyl acetate showed the least efficiency in extracting mango peel and kernels whereas pomegranate peel, areca nut and different leaves (except guava) were extracted least with chloroform. Only guava leaves gave significant yields with chloroform. All other solvents also gave significant yields with four mango kernels except chloroform with TK. Statistical analysis of extraction efficiency of solvents showed that there was significant difference between each solvent.

It would appear that liquid phenolic extracts have good stability at a temperature range of 4-37^{0}C when stored in the dark, while significant degradation occurs when these extracts are exposed to light. Degradation of the phenolic compounds in the presence of light is generally accompanied by reduction in antioxidant potency. Based on this observed effect of light on phenolic acids, it could hence also be expected that flavonoids may similarly be degraded under the influence of light.

Stability of phenolic compounds, however, also appears to be greatly affected at temperatures higher than 50^{0}C and may lead to decrease in AA. Rates of chemical reactions increase as temperature increases; the reduction in the AA of phenoilc extracts at these high temperatures can be observed. Handling or storing the phenolic compounds at low temperatures may, therefore, prevent the loss of their AA.

Different solvent extracts of all plant materials under study were tested for the TPC by FCR method. Methanol extract of all the plant materials was highest in TPC followed by ethanol. Though, methanol was not efficient in terms of extracting TP and BP, the TPC was more in methanol extract for these plant materials. As the TPC of methanolic extract of all the raw materials selected was highest, it was taken to study the different antioxidant assays and oxidative stability tests.

In the conventional solvent extractions, methanol showed the greatest capability in extracting phenolic antioxidants and inhibiting the free radicals produced by DPPH among the five solvents studied. All the extracts of raw materials used in the study showed higher DPPH activity based on the concentration. PPE showed highest DPPH activity than all mango peel extracts. All the mango kernel extracts were having higher DPPH activity than BHT and BHA. The scavenging activities of phenolic substances are attributed to active hydrogen donating ability of hydroxyl substitutes. Presence of phenolic groups in the phytochemicals could be responsible for free radical scavenging activity. GLE showed the lowest and BLE showed the highest DPPH activity among the leaves. DPPH activity of leaf extracts are in correlation with concentration. At all concentrations, the activity of CLE and BLE are higher than GLE, BHT and BHA. Among all the extracts, BLE had highest DPPH activity.

All the extracts of raw materials under study showed a correlation between the concentration and RRP. PPE when tested for RRP showed that it was higher than all the four mango peel extracts, BHT and BHA. The presence of RRP indicates that the extracts have electron donors and can react with free radicals to convert them to more stable products and terminate radical chain reaction. The highest RRP of the MKE is in correlation with its TPC. Among all the leaves, BLE showed the highest RRP. The RRP of GLE was lower than BHT. There was an increase in RRP of leaves as the concentration increased.

The ability of extracts to prevent peroxide formation was tested by FTC method. Among all mango peels, TPE is efficient in inhibiting the peroxide formation. PPE showed a lower value than BHT and BHA. When the absorbance of PPE and mango peels were compared, PPE was more efficient. MKE was most effective inhibitor of linoleic acid peroxidation when compared with other kernel extracts. From the results, it can be concluded that the AE had capacity to hinder the oxidation better than BHA and BHT. The BLE had a high activity in preventing peroxide formation which can be shown by lower absorbance value of BLE than other leaf extracts. The inhibition rate of compounds by thiocyanate method is proportional to phenolic content and other antioxidant assays. Among all the extracts of selected plant materials, BLE had highest ability to inhibit the peroxidation of linoleic acid in FTC method.

There was a correlation between the phenolic compounds and discoloration of β- carotene i.e., the extracts which showed higher β- carotene bleaching activities, had high phenolic content than other extracts. In the present study, it is observed from the results that the β-carotene bleaching activity of TPE and PPE was higher than other peel extracts and synthetic antioxidants. Further, it was observed that the kernel extracts including AE have the potential to hinder the extent of β- carotene bleaching to varying degree by neutralizing the linoleate free radical and other free radical formed in this system. Similarly, the BLE has more potential to hinder the extent of discoloration than other leaf extracts, BHT and BHA. GLE was having least activity than BHA at all stages of the assay. The highest activity of BLE is correlating with TPC.

Among the four *in vitro* antioxidant assays, a correlation between the TPC and the activities was observed. BLE which had a highest TPC showed high activity than other extracts under study. Though, GLE had high TPC, it was not effective than BHT in different methods of testing AA.

The extracts were tested for individual phenolic component by HPLC and LCMS. All the extracts showed the presence of phenolic compounds belonging to flavonols, flavan-ols, cinnamic and benzoic acid derivatives and xanthones. Mango peel extracts showed the presence of mangiferin a unique xanthone in *Mangifera* family.

HPLC analysis shows that most of the extracts contain tocopherol, kaempferol, rutin and gallic acid in higher concentration. Tocopherol has been proved to be one of the best natural antioxidants used to prevent oxidation and this may be the main reason for high AA of extracts.

LCMS analysis shows the phenolic compounds like gallic acid which is present in the form of esters. Cinnamic acid and benzoic acid derivatives, flavonols, xanthones are identified in most of the extracts. Due to the variation in phenolic compounds, the extracts showed variation in AA.

All the extracts were incorporated in RBD SFO and SBO and tested for their ability to prevent peroxide formation and IP. RBD SFO with PPE showed higher stability than four different mango peel extracts.

The study provides the data about some of the naturally occurring phenolic compounds and their antioxidative activity and they can be used as natural antioxidants to retard oxidation of PUFA-rich edible oil, which make them attractive candidates for commercial exploitation. The results of the present study revealed that the PPE is free radical inhibitor and primary antioxidant that react with free radicals. In conclusion, the AA of PPE as a potent source of natural antioxidant for beverage industry and is suitable for extraction and purification of natural antioxidants.

Statistical analysis of the data showed that all the kernel extracts were more effective than BHT in preventing the rancidity of RBD SFO and SBO. MKE and TKE gave the best stability and NKE gave the least stability of RBD SFO and SBO. There was no significant difference between RBD SBO with MKE and TKE at all temperatures in rancimat. Statistical analysis of the data showed that RBD SFO and SBO with MKE showed the best stability by preventing the formation of peroxides than other extracts, BHT and control.

Lower PV and higher IP were observed in RBD SFO and SBO with mango kernel extracts. There was no significant difference between the PVs of RBD SFO with NKE and BKE. MKE showed a lower PV than all other kernel extracts. IP of RBD SFO with MKE was higher than other kernel extracts. RBD SBO showed a lower PV with MKE than with other kernel extracts.

When the leaves were tested for oxidative stability tests, RBD SFO and SBO showed low PV and high IP with BLE and CLE than BHT and control samples. GLE showed a low stability in both oils than BHT.

The results of the present study conclude that all the selected raw materials had AA at different rates. Among all the extracts BLE was showing high activity in all the methods followed by mango kernel extracts. CLE and AE were higher in activity than mango peel extracts but less than PPE. Further studies can be undertaken to investigate the effect of these extracts in different foods so that toxicity due to synthetic antioxidants can be reduced by alternative natural antioxidants. GLE showed potential antioxidant activity and can be used to extend the shelf life of foodstuffs at a higher concentrations than BHT. However, antioxidant cannot improve the quality of an already oxidized food product.

This research is mainly used to optimize the usage of wastes generated during the processing of fruits and leafy vegetables by implementing the design of its extraction for prospective applications that will be beneficial to people. Also, it will help to play a role in minimizing waste generation worldwide.

These results provide us with valuable information on the capability of extracting antioxidants and phenolic compounds from food products, and may provide insight for future extraction analysis techniques for determining antioxidants and phenolic compounds in food products.

The information of this study would be helpful in the development and utilization of all these extracts as food antioxidants or as antioxidant-rich nutritional supplements. Much research is needed in order to select raw materials; those of residual origin are especially promising due to their lower costs. However, extensive research on potential sources, optimization of extraction processes, knowledge of the mechanisms of the *in vivo* action and assimilation are still required.

The results confirmed that agricultural and agro industrial wastes contain high amounts of phenolics and suggest the antioxidant recycling of the wastes of fruits and leafy vegetables. If the phenolic content of waste-derived raw extracts is compared to that of the original fruits and vegetables, an advantage of waste and by products use for further enrichment of phenolics is also apparent. Maintenance of the AA can be assumed but this should be confirmed in further investigations involving longer stability tests and higher temperatures during the production. More nutritional studies, however, are needed to measure the necessary level of extract to observe the beneficial effect. They should not only consider the influence of different conditions, including temperature, moisture, oxygen and added ingredients on these bioactive compounds, but specifically by products and by product derived extracts, as apparently main desired constituents are at higher concentrations in the residuals. Further studies on the effective antioxidants contained in the pomegranate peel fractions and the mechanisms by which they protect against disease development are highly recommended.

Antioxidants for use in food, however, undergo strenuous evaluation to ensure that they satisfy economical, technological and toxicological requirements such as effectiveness at low concentrations, ease of incorporation in the food, should not impart flavour, odour or colour to the food, should be inexpensive, safe and non-toxic, and should not affect physical properties of the food. Antioxidants from natural sources would therefore have to satisfy these requirements for their acceptability and use in lipid foods.

Edible oil undergoes autoxidation and photosensitized oxidation during processing and storage. The oxidation of edible oil produces off-flavor compounds and decreases oil quality. Free fatty acids, mono- and diacylglycerols, metals, chlorophylls, carotenoids, tocopherols, phospholipids, temperature, light, oxygen, oil processing methods, and fatty acid composition affect the oxidative stability of edible oil. To minimize the oxidation of edible oil during processing and storage, it is recommended to decrease temperature, exclude light and oxygen, remove metals and oxidized compounds, reducing their surface-to-volume ratio and use appropriate concentrations of antioxidants such as tocopherols and phenolic compounds preferably from natural sources that can prevent their oxidation. Removal of residual oxygen by degassing, gas flushing with nitrogen or vacuum packaging may also be employed to reduce lipid oxidation.

Food processing technology should be optimized in order to minimize the amounts of waste arising. Methods for complete utilization of waste and by products resulting from food processing on a large scale and at affordable levels should be developed. Active participation of the food and allied industries with respect to sustainable production and waste management is required.

Many sources of naturally occurring antioxidants from plants have been discovered in recent years. Full structural identification of the active components of plant foods is therefore required. These novel antioxidants must undergo toxicological studies at reasonable concentrations, such as those present in their natural environment. The activity of these individual antioxidants must be tested in different food systems. Furthermore, breakdown products of these novel antioxidants, under different conditions and their antioxidant and toxicological properties must be investigated.

The exploitation of waste and by products of fruit and vegetable processing as a source of functional compounds and their application in food is a promising field which requires interdisciplinary research of food technologists, food chemists, nutritionists and toxicologists. It is recommended that the bioactivity, bioavailability and toxicology of phytochemicals need to be carefully assessed by *in vitro* and *in vivo* studies. Under certain conditions, e.g. in the presence of some transition metal ions, antioxidants such as phenolics may behave as prooxidants. Further, there is a need for specific analytical methods for the characterization and quantification of organic micronutrients and other functional compounds.