THERMAL DEGRADATION STUDIES ON EDIBLE OILS DURING DEEP FAT FRYING PROCESS

Thesis submitted to
Cochin University of science and technology
in partial fulfillment of the requirement for the degree of

Doctor of Philosophy
in
Applied Chemistry
under the ***Faculty of Science***

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JUNE 2015

DECLARATION

I hereby declare that the thesis entitled **"THERMAL DEGRADATION STUDIES ON EDIBLE OILS DURING DEEP FAT FRYING PROCESS"** embodies the results of investigations carried out by me at Agroprocessing and Natural Products Division of National Institute for Interdisciplinary Science and Technology (NIIST), CSIR, Thiruvananthapuram, as a fulltime research scholar under the supervision of Dr. A. Sundaresan and the same has not been submitted elsewhere for any other degree. In keeping with the general practice of reporting scientific observations, due acknowledgement has been made wherever the work described is based on the findings of the other investigators.

Thiruvananthapuram
June, 2015

CHALLA RAVI KIRAN

CERTIFICATE

This is to certify that the work embodied in the thesis entitled **"THERMAL DEGRADATION STUDIES ON EDIBLE OILS DURING DEEP FAT FRYING PROCESS"** is an authentic record of research work carried out by **Mr. CHALLA RAVI KIRAN** under my supervision in partial fulfillment of the requirement for the Degree of Doctor of Philosophy in Applied Chemistry under the Faculty of Science, Cochin University of Science and Technology, Cochin and same has not been submitted elsewhere for any other degree. All the relevant corrections, modifications and recommendations suggested by the audience and the doctoral committee members during the pre-synopsis seminar of **Mr. CHALLA RAVI KIRAN** has been incorporated in the thesis.

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ACKNOWLEDGEMENTS

First and foremost, I express my sincere gratitude to Dr. A. Sundaresan, my thesis supervisor, for giving me an opportunity for performing the research, for his guidance, support and encouragement for the successful completion of this work.

I thank Dr. Suresh Das, Director of the National Institute for Interdisciplinary Science and Technology (NIIST) for providing the necessary facilities for carrying out this work.

My sincere thanks to Mr. Soban Kumar, Dr. L. Ravi Shankar, Mr. M.M. Sree kumar, Dr. Dileep Kumar, Dr. K.P. Padma Kumari Amma, Mrs. Prasanna Kumari, Dr. Beena Joy, Dr. P. Nisha, Mrs. M.V. Reshma, Dr. P. Jayamurthy, Mrs. Omana Kutty Amma, Mrs. Nirmala Menon, Mr. V.V Venugopalan, Dr. K.G. Raghu and Mrs. Sankarikutty Amma.

I gratefully acknowledge all the members of Knowledge Resource Centre, NIIST for their help during the course of this study and Mrs. Viji for her help in instrumental analysis.

I gratefully acknowledge the help and support from Indu Sasidharan and all the members of the Agroprocessing and Natural Products Division.

I am deeply indebted to my parents for their support that helped me to complete this work successfully.

My sincere thanks to Kamani oils, Mumbai; Kalesc Inc., Miami; Eastman chemical company, Tennessee for providing necessary samples for this work.

CONTENTS

ABBREVIATIONS

ANOVA	-	Analysis of Variance
AOAC	-	American Oil Chemists Society
AOCS	-	Association of Official Analytical Chemists
BHT	-	Butylated Hydroxy Toluene
CD	-	Conjugated Diene
CO	-	Coconut oil
DAG	-	Diacylglycerol
DTAG	-	Dimerized triacylglycerol
FFA	-	Free Fatty Acid
FSSAI	-	Food Safety and Standard Authorities of India
GC	-	Gas Chromatography
GNO	-	Groundnut oil
IUPAC	-	International Union of Pure and Applied Chemistry
MAG	-	Monoacylglycerol
MALDI	-	Matrix Assisted Laser Desorption/Ionization
MMT	-	Million Metric Tons
OTAG	-	Oxidized Triacylglycerol
*p*AV	-	*para*-Anisidine value
PO	-	Palmolein
PV	-	Peroxide Value
PTAG	-	Polymerized Triacylglycerol
RBO	-	Rice bran oil
SAIB	-	Sucrose acetate isobutyrate
SeO	-	Sesame oil
SNO	-	Sunflower oil
SO	-	Soybean oil
TAG	-	Triacylglycerol
TBHQ	-	*ter*- Butylhydroxyquinone
TLC	-	Thin Layer Chromatography
TOF-MS	-	Time-of-flight/Mass spectrometry
TPC	-	Total Polar Compounds

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CHAPTER 1
INTRODUCTION

1.1 Preamble

Deep fat frying process is one of the widely followed cooking practices throughout the world. Cooking oils serve as a medium for frying food for transferring heat and makes fried food tasty and palatable. Frying process is a most complex process involving numerous physicochemical changes which are complicated to understand. Frying leads to thermal degradation of oil through thermo-oxidation, hydrolysis, and polymerization. Hydrolysis results in formation of free fatty acids whereas oxidation process produces hydroperoxides and small molecular carbonyl compounds. This whole process leads to the formation of polar compounds and degradation of antioxidants that further degrades frying oil. Eventually, through mass transfer process these degradation products accumulate into fried food and reduce the nutritional quality of both oil and food. Thus, the frying process is of research interest calls for detailed systematic study which is chosen for the present study. The primary objective of this study is to understand the mechanism of degradation and characterization of degraded products which helps in arriving at the limits for frying oil utilization in terms of number of frying cycles. The mechanistic studies and the knowledge on the degraded products help to understand the way to retard the deterioration of oil for stability and enhancement of frying cycles. The study also explores the formation of the predominant polar compounds and their structural elucidation through mass spectrometry. Oxidation of oil is another important factor that ignites the degradation phenomena. One of the best ways to increase thermal stability of any oil is addition of potent antioxidants. But, most of the natural and synthetic antioxidants are unstable and ineffective at frying temperatures. Therefore, it is necessary to screen alternative antioxidants for their activity in the refined oils which are

devoid of any added antioxidants. In this context, this study discussed the efficacy of several natural and synthetic antioxidants to retard the formation of polar compounds and thermo-oxidation during prolonged frying conditions. Similarly, the advantage of blending of two different oils to improve the thermal stability was explored. The present study brings out the total picture on the type of degradation products formed during frying and the ways of retarding the determination to improve upon the stability of the oil and enhancement of frying cycles.

1.2 Fats and Oils in human health

Lipids are one of the largest groups of naturally occurring molecules that include phospholipids, glycerol glycolipids, sphingolipids, fatty acids, acylglycerols, sterol esters, waxes, etc. [1]. Lipids are insoluble in polar solvents such as water and methanol, but soluble in non-polar solvents such as chloroform and ether. Fats are esterified triglycerides, a major subset of lipids. The words oils and fats are used to refer to fats, where oils are liquids and fats are solids at room temperature [2]. Fats serve several important functions including, providing high energy value, imparting palatability and taste to food, acts as precursors to several biologically active compounds, required for absorption of fat soluble vitamins like A, D, E & K, to support body fluids and cell membranes, and also insulates the body through subcutaneous layer in skin [3]. Based on the presence of saturated and unsaturated fatty acids, fats are broadly classified into saturated fat and unsaturated fat respectively. Saturated fatty acids (SFA) do not have double bonds in the carbon atoms of the fatty acid chain while monounsaturated fatty acids (MUFA) contains at least one double bond and polyunsaturated

fatty acids (PUFA) contains multiple double bonds between carbon atoms of the fatty acid chain on triglycerides.

Many studies in the recent past have demonstrated that the consumption of diets with higher SFA (lauric, palmitic and stearic acids) increases the total cholesterol and low-density lipoprotein (LDL) cholesterol and further accelerates the risk of developing coronary heart disease (CHD). Moreover, influence of SFA on the cancer, insulin sensitivity, obesity and other disorders are widely studied [4-5]. In India, the major sources for SFA are ghee, vanaspati (hydrogenated fat), cheese and cooking oils such as coconut oil, palm kernel oils [6]. On the other hand, consumption of dietary sources which are rich in MUFA (oleic acid) and PUFA (linoleic and linolenic acids) lowers LDL cholesterol and increases high density lipoprotein (HDL) cholesterol, further to reduce health risk of CHD [7-8]. Cooking oils including groundnut, rice bran, mustard, olive and canola oils are rich in MUFA. Similarly, sunflower, corn, soybean, linseed oils are rich dietary sources of PUFA. Another class of fatty acids known as *trans* fatty acids (TFA) are formed during the production of partially hydrogenated vegetable oils and also occur naturally in ruminant fats. *Trans* fat comprises unsaturated fatty acids having one or more isolated double bonds in the trans geometric configuration [9]. Several clinical studies showed that a high intake of TFA was positively correlated with the risk of developing cardiovascular disease (CVD) by increasing serum LDL cholesterol relative to SFA [10]. Due to their higher deleterious effects on health, U.S. Food and Drug Administration (FDA) passed a labeling requirement for TFA in packaged food products, effective January 1, 2006, requiring it to be reported on the nutrition label if present at ≥0.5g/serving.

American Heart Association (AHA) recommended the maximum limit of fat consumption should be less than 30 -35% of total calories, among which SFA consumption should be limited to 10%, MUFA to 15% and PUFA to 10% of total calories. Similarly, TFA consumption is limited to 1% of total energy [11]. Hence, if maximum total fat intake is taken into consideration, ratio of SFA: MUFA: PUFA gets derived to 1:1.5:1. Since, consumption of single edible oil alone cannot satisfy the suggested guidelines, blending of two or more oils are practiced to provide health benefits.

1.3 Overview of edible oil production and consumption

Edible oils are one of the most consumed cooking ingredients across the world. Moreover, their applications are well known in the oleochemical industry, production of soaps, washing powders, cosmetics, and bio-fuels. Among all edible oils produced in the world, soybean oil and palm oil occupies majority (> 65%) in the production (Figure 1.1).

World's edible oil production increased gradually during past few years. A total of 126.02 MMT of major edible oils production from the year 2010/11 has reached 152.29 MMT by the year 2014/15 [12]. Figure 1 showed the worldwide production of edible oils by oil type from 2010/11 to 2014/15. This data clearly shows that palm oil (from 48.84 to 63.29 MMT) and soybean oil (41.29 to 46.95 MMT) are major oils produced compared to that canola oil (23.46 to 26.76 MMT) and sunflower oil (12.43 to 15.29 MMT).

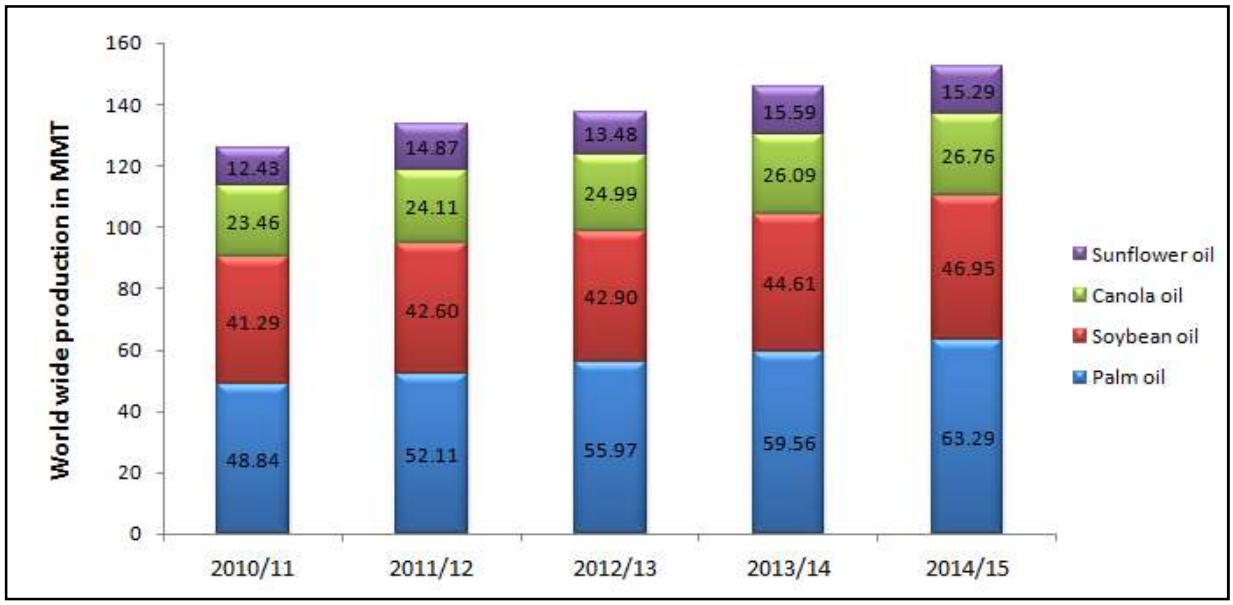


Figure 1.1 World production of edible oils from 2010/11 to 2014/15 by oil type.

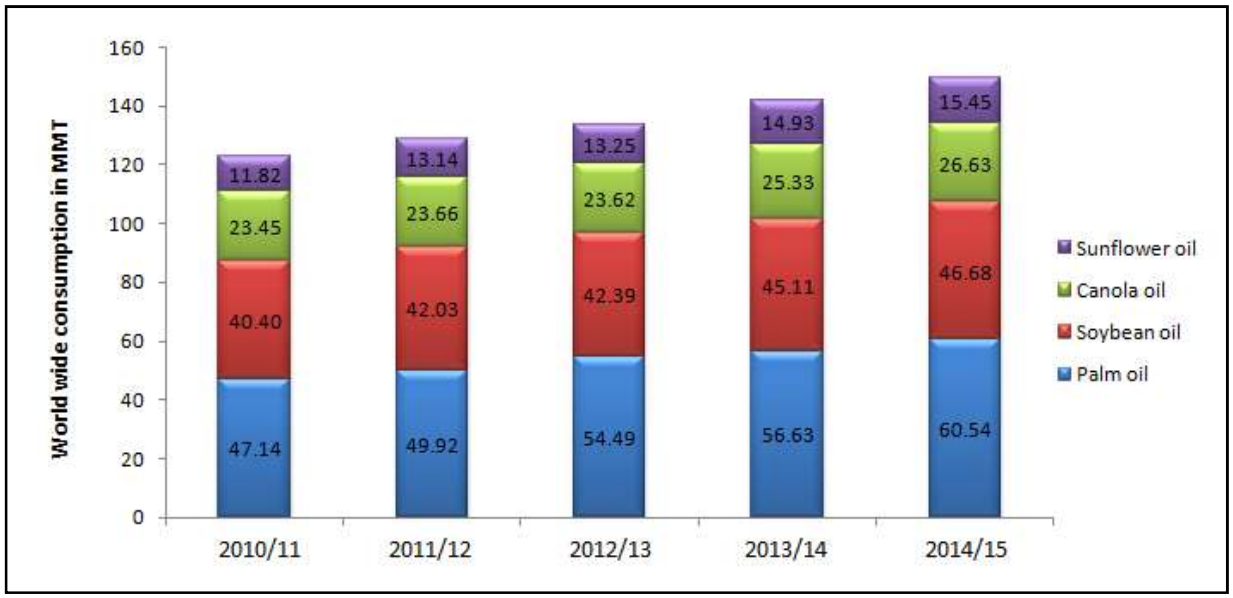


Figure 1.2 World consumption of edible oils from 2010/11 to 2014/15 by oil type.

Similarly, Figure 2 showed the worldwide consumption of edible oils by oil type from 2010/11 to 2014/15. The comparison pattern also reveals that palm oil (from 47.14 to 60.54 MMT) and soybean oil (40.40 to 46.58 MMT) are major oils consumed followed by canola oil (23.45 to 26.63 MMT) [12].

The Indian edible oil industry is highly fragmented with the extreme variation in the consumption pattern. The Indian edible oil market is the fourth largest in the world after China, USA and Brazil [13]. India's growing population, income levels, and different consumption patterns cause raise in the edible oil consumption by 10 percent every year. However, in India, edible oil consumption is highly diversified from region to region. Southern part of India consumes more palm oil, rice bran oil, and coconut oil while northern part of India highly depends on mustard oil, groundnut oil, and sunflower oils. Moreover, more than 60 % of India's vegetable oil demand is fulfilled by imports only.

1.4 Deep fat frying process

Deep fat frying is one of the oldest methods of cooking process, with which water containing foodstuff is immersed in edible oils or fats at temperatures between 140°C –180 °C [14]. The general advantages of deep fat frying are that heat destroys bacteria and toxins and the frying medium also provides certain important nutritional elements, and renders an appealing texture and taste which makes fried food more palatable and therefore readily accepted by the consumer [15]. During the recent years, a rapid growth in fast food processing sector with several commercial advantages make the deep fat frying as a most popular cooking method used in the food industry [16-17]. More than 20 million tons of

edible oil consumed for industrial frying in a year necessitates a better understanding of the deep frying process for the optimum production of fried food [18].

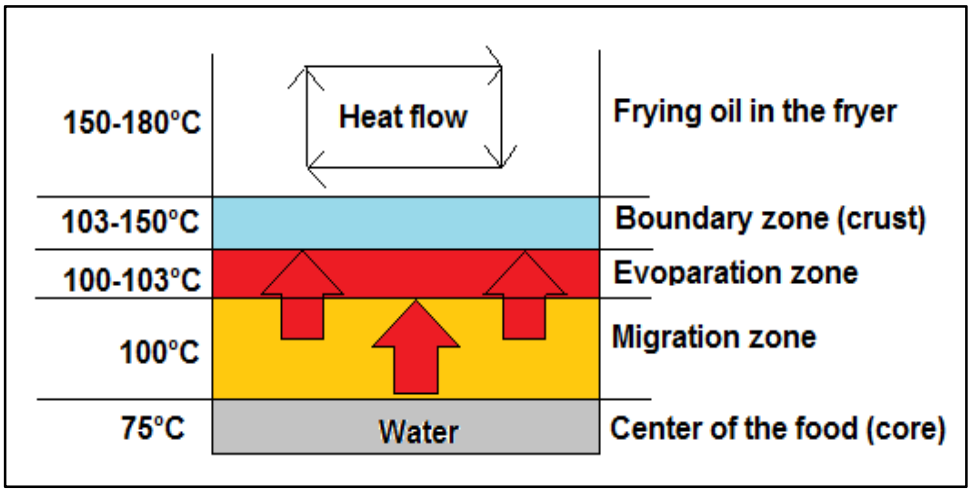


Figure 1.3 Heat and mass transfer during deep fat frying.

Deep fat frying involves simultaneous heat and mass transfer process, where oil transfers heat to the food material and loss of moisture, carbohydrate, protein and other components of fried material transfers into frying oil [19-20]. Figure 1.3 illustrates the heat and mass transfer process during frying in progress. In this process, heat transfer is initially due to combined conduction and convection. After immersion of the moist food in hot oil, free water boiling at the surface also plays a major role in this process [21]. During this process, escape of moisture from center of the food material to crust part through vaporization results in the creation of capillary pores through which hot oil enters the food [22]. In this process, both fried material and frying oil impact on each other with numerous chemical reactions which eventually lead to degradation of cooking oil quality that makes oil unhealthy for consumption [23]. Potatoes, conventional frying snack food are low in protein and high in

starch. On the other hand, Meat, fish, and chicken nuggets are different in composition, being mainly composed of protein. So, there is an enormous difference in the frying progression of one material to another [24].

The total process of thermal degradation of frying oil is referred to as thermal-oxidative decomposition, which involves a set of physicochemical reactions such as oxidation, hydrolysis and polymerization [25]. These degenerative reactions increase the viscosity of the frying oils on repeated usage and their by-products are potential health risk factors due to their links with cardiovascular diseases, obesity, diabetes, etc. [26]. New compounds formation depends on the type of oil, frying temperature and also accessibility of oxygen. Inevitably, these degradation products transfer into fried food due to intense mass transfer, which reduces the nutritional quality of both [27]. This whole decomposition phenomenon has to be observed in detail for better understanding of the formation of new compounds during the frying process and absorption of frying oils into foods.

1.5 Major reactions and degraded products formed during deep fat frying

Several reviews discussed the reactions in edible oils while frying process. Choe *et al.,* (2007) described the major chemical alterations that take place while frying in depth. They include Hydrolysis, Thermo-oxidation, and Polymerization [25].

A. Hydrolysis:

Fats and oils composed of esters of triglycerides. These triglycerides are much prone to hydrolysis at glycerol backbone in the presence of moisture. When triacylglycerols (TAG)

undergoes hydrolysis, they yield free fatty acids (FFA), diacylglycerols (DAG) and monoacylglycerols (MAG). So, Hydrolysis increases the amount of FFA, DAG and MAG content in oils. Generation of FFA and their oxidized compounds lead to rancid flavor and further to unsuitability of frying oil for consumption.

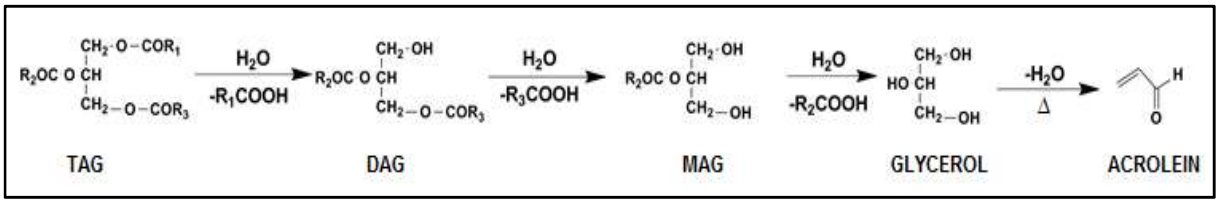


Figure 1.4 Hydrolysis pathway of TAG during deep fat frying.

The primary source for the hydrolysis is water in food material during frying. Figure1.4 explains the mechanism of hydrolysis while frying process. The decomposition of the ester linkage is feasible while heating in the presence of water. Even though evaporation of water with the increase of temperature can retard the rate of hydrolysis, the volatilization of formed glycerol above 150 °C will speed up the hydrolysis [28]. Replenishment of fresh oil during the frying process might minimize the formation of DAGs or MAGs and slow down the hydrolytic changes [29]. In the frying process all enzymes are unstable at higher frying temperature. There is no enzymatic activity during oil degradation was reported. However, hydrolytic rancidity could be possible in oils at lower temperature levels. Several factors including the position and number of carbons, length of carbon chain, steric hindrance of the aliphatic chains, moisture content, and temperature influences the breakage site of ester bond on glycerol backbone [30].

B. Thermo-oxidation:

During the frying process, oxygen reacts with oil and leads to oxidation on the fatty acid groups on TAG. It is well known that the autoxidation is a significant degradation reaction,

which is attributed to the rancidity of oil and fat. It is as well the major reaction occurred while frying along with an increase in temperature. Oxidation occurs at a higher rate than hydrolysis during frying and produces hydroperoxides and then small molecular volatile compounds such as aldehydes, ketones and short-chain alkanes [25]. The phenomenon of thermal oxidation is same as the autoxidation mechanism, except in reaction speed [31]. Figure 1.5 demonstrates the mechanism of the thermal oxidation, which involves the initiation, propagation and termination of the reaction.

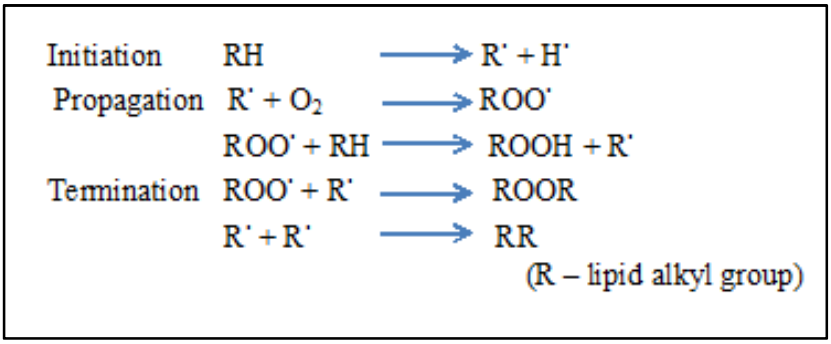


Figure1.5 Oxidation pathway of frying oil.

Alkyl radical formation through removal of hydrogen in the oil is known as the initiation step of the primary oxidation reaction of oil. The initiation step involves the formation of radical ion on TAG. Oil in non-radical state does not react with the triplet state di-radical oxygen due to the spin barrier. Radical oxygen requires radical oil for the oxidation of oil.

Rate of oxidation of different fatty acids during thermal oxidation or autoxidation is mainly influenced by the bond strengths of carbon-hydrogen (C-H) of fatty acids. The weakest C-H bond of fatty acid on TAG is removed primarily to form alkyl radical. The initiation step is catalyzed by many factors such as light, heat, and metal ions [32]. The

location of radical formation is different for saturated fatty acids and unsaturated fatty acids such as oleic or linoleic acids. The alkyl radical from the saturated fatty acids is formed at α-position of the carboxyl group, which are having electron withdrawing property. Whereas in the case of unsaturated fatty acids, it is formed at allylic position of the double bond. These alkyl radicals form peroxy radicals and propagate the oxidation process. These alkyl radicals can also react with other alkyl, alkoxy and peroxy radicals to yield dimers and polymerized products [30-32].

Propagation step of the oxidation process involves the generation of peroxy radicals (ROO•) which then abstract hydrogen from other organic substrates to form hydroperoxides (ROOH). These hydroperoxides are very unstable and easy to give rise to β-scission homolytic cleavages of the O-O, C-C and C-O groups around peroxide group to decompose into short-chain compounds [33]. The propagation step proceeds through decomposition of hydroperoxides. Termination step concludes with the formation of volatile and non-volatile compounds. This final step involves the combination of free radicals thus prevent the propagation step to further [34]. These compounds include a homologous series of small molecular alcohols, aldehydes, hydrocarbons etc. These short chain volatile compounds are odor active that cause off-flavor of fried material and frying oil. Most volatiles evaporate from frying oil by steam while deep fat frying and presence of moisture in the frying oil could reduce the concentration of those compounds in oil [35]. Other factors like nature of the oil, food material, and frying conditions also influence the concentration of volatile compounds in the oil [36].

C. Polymerization:

The major degradation products of frying oil are polar compounds that mainly include triacylglycerol dimers and polymers. These Dimers and polymers are large molecules with high molecular weight, formed by combination of -C-C-, -C-O-C, and -C-O-O-C- bonds [37]. Different dimers including dehydroxy dimer, ketone hydro dimer, dehydro dimer of linoleate, and dehydro dimer of oleate are found in soybean oil while frying at 195 ◦C [38]. Therefore, based on the presence of oxygen, two different kinds of polymeric products formed. They are non-polar polymers without oxygen and polar polymers with oxygen. Continuous production and accumulation of polymers leads to color deepening and increase in viscosity and further deterioration of frying oil [39]. Other factors including frying condition and the nature of frying oil affects the structure and quantity of polymers formed in frying oil [40].

Two major reactions including free radical chain reaction and Diels-Alder reaction are mainly used to interpret the formation mechanism of polymers. Allyl radicals are formed preferably by methylene carbons α to the double bonds and dimers are formed through the reactions of these alkyl radicals [41]. Acyclic and cyclic polymers are formed through different positional carbons reacting with allyl radicals. Allyl radical forms a dimeric radical by reacting with another radical of unsaturated molecule and the generated dimeric radical reacts with hydrogen radical to form acyclic dimer. Usually, TAGs with polyunsaturated fatty chain lost hydrogen radical and form a radical similar to the conjugated diene. This conjugated diene can combine with another unsaturated molecule to form a dimeric radical

intermediate; consequently intramolecular additions took place and form a cyclic dimer. The rest of high-molecular polymers can be formed in the same manner (Figure1.6).

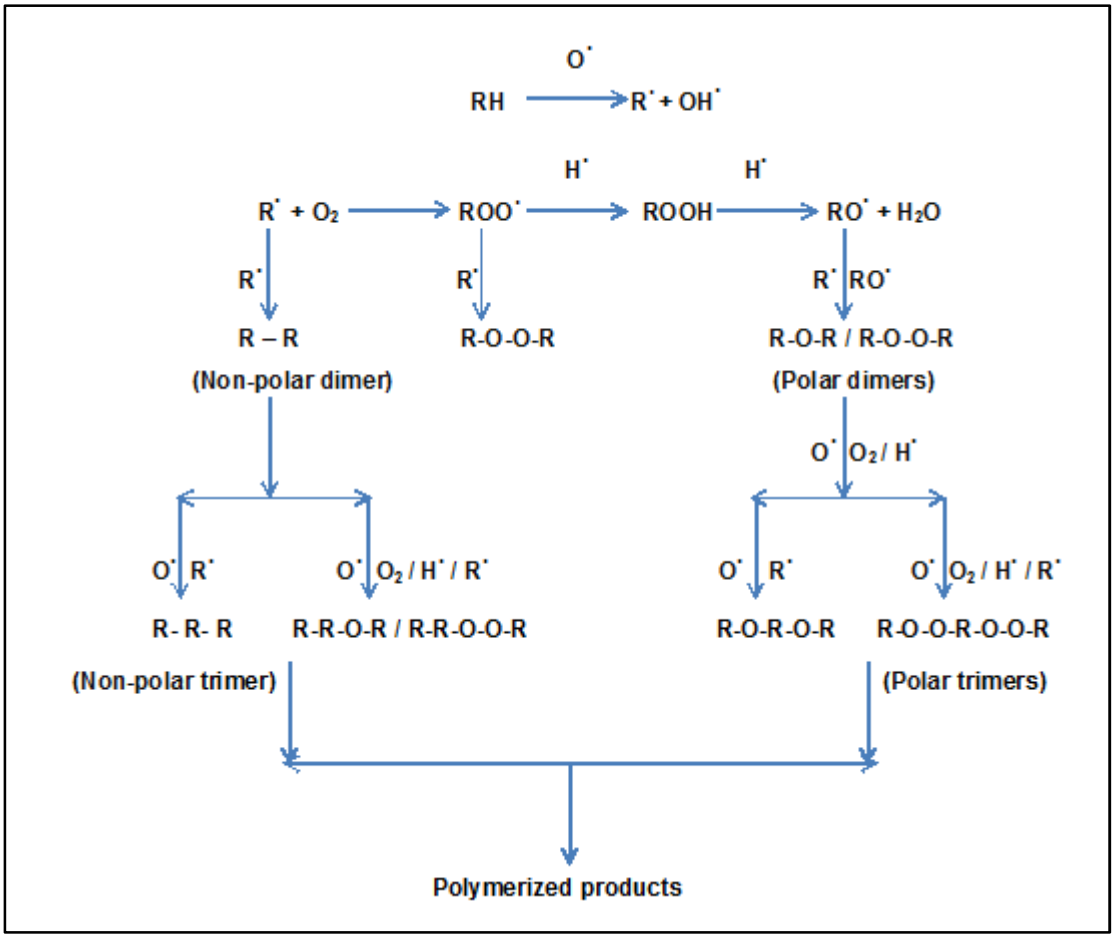


Figure 1.6 Polymerization reaction pathway of frying oils.

Oxidized TAG monomers which have one or several oxygen-contained groups are formed from extra oxygen involved in chemical reactions during deep fat frying. Under the effect of the radical catalysis, these oxygen-contained TAG monomers would be polymerized through –C–C–, –C–O–C– and –C–O–O–C– bonds [42]. Moreover, additional oxygen atoms could be formed in the dimers, trimers or oligomers when the sample was heated at frying

temperature for prolonged time [43]. Allyl radical produced by oxidation could combine with an alkoxy radical formed by breaking of hydroperoxides to produce oxydimers. Similarly, two molecules of peroxyl radicals bonded together and formed a peroxy dimer [25]. Furthermore, the simultaneous formation of these non-polar and polar polymers and their ease of formation are still needed to be investigated. The structural complexity and the lack of literature, highlights the importance to study the structure analysis and formation pathways of polymers produced during deep fat frying.

The formation of dimers and polymers depends on the nature of oil, frying temperature, and the number of frying cycles. As the number of frying cycles and temperature increase, the quantity of polymer formation increases [44]. The oils rich in linoleic acid (PUFA) such as soybean oil and sunflower oil are more easily polymerized during frying compared to the oil rich in oleic acid (MUFA) such as palmolein [45]. The formation of cyclic compounds including polymers in frying oil depends on the degree of unsaturation and also frying temperature [46-47]. Cyclic compounds are not formed until the temperature in frying oil reaches 200 ◦C to 300 ◦C. The formation of cyclic fatty acid monomers and also polymers is directly related to the amount of linolenic acid increased [48]. Several studies highlighted the possibility of formation of tricyclic dimers and bicyclic dimers as well as cyclic monomers while frying in soybean oil [49]. However, oxidized polymer compounds accelerate the oxidation of oil and degrade oil quality and increase the oil viscosity. Further, they reduce the heat transfer, produce foam during frying and also cause the high oil absorption to foods [50-51].

1.6 Factors Influencing the Quality of Oil during Deep Fat Frying

The quality of oil during the frying process is influenced by several factors namely, initial oil quality, replenishment of oil, frying time and temperature, composition of frying food, type of fryer, antioxidants, and oxygen content, etc. The effects of various factors on the degradation of frying oil can be studied by different analytical methods.

1.6.1 Initial quality of frying oil

The quality of frying oils not only depends on the oil used for frying but also it is influenced by frying condition. So, it is most important to select stable frying oil with good quality to maintain minimum degradation during the frying process. The desired quality for frying oils is lower FFA and lower unsaturated fatty acids content. Because, rate of oxidation of oil increases with the increase in unsaturated fatty acids content of frying oil. Palm oil and corn oil with less unsaturated fatty acid are better frying oils than soybean oil with higher unsaturated fatty acid content [52]. Mainly, the contents of linoleic and linolenic acid are critical to the frying performance and stability of oil. It has been reported that polar compounds formation in soybean oil which is rich in linoleic acid, was found to be increased during the frying of potato chips [53]. Hydrogenation increases the frying stability of oil by decreasing the unsaturated fatty acids of oil. However, hydrogenation produces *trans* fatty acids in the oil which is detrimental to health [54]. Therefore, oils with lower unsaturated content by genetic modification are suggested to be a useful alternative to the hydrogenated

frying oil [55]. Blending of different oils can modify the fatty acid compositions of oils and can decrease the oxidation of oils during deep fat frying [56-57].

1.6.2 Replenishment of fresh oil

Initially, oil quality is high at early stages of frying. But, while frying due to formation of FFA, peroxides and polymers the quality of oil deteriorates. In the industrial frying process, replenishment of fresh oil is performed to maintain an equal level and uniformity in the quality of oil. It has been found that higher ratio of replenished oil to the total oil provides better frying oil quality [58]. Frequent replenishment of fresh oil decreases the concentration of total polar compounds, diacylglycerols, FFA and increases the frying quality of oils [59].

1.6.3 Frying time and temperature

Prolonged frying time during continuous frying or intermittent frying process degrades the frying oil quality [60]. Progression of frying process gradually leads to increase in the formation of polar compounds rapidly. Polar compounds such as oxidized triacylglycerols, triacylglycerol dimers and polymers formed at higher concentration in the oil used for more frying cycles [61-62]. Similarly, high frying temperature accelerates thermal oxidation and polymerization of oils [63]. It has been reported that, high frying temperature decreases the formation of polymers with peroxide linkage and increases the formation of polymers with ether linkage or C-C direct linkage [64]. The intermittent frying process leads to higher degree of deterioration of oils than continuous frying process due to the high oxygen solubility when the oil cools down [65]. It has been observed that 25% of linoleic acid in the

sunflower oil was degraded during intermittent frying, whereas in continuous frying process only 5% was degraded [66].

1.6.4 Compositions of foods

Degradation of frying oils depends not only on the nature of the oil or initial quality of oil but also on the type of food material being fried. Higher moisture contents in foods exchange into oil during heat and mass transfer, and increases the hydrolysis of oil during deep-fat frying. This causes an increase in FFA of the oil and further deterioration. On the contrast, moisture in the food can form a steam blanket over the fryer and reduces surface oxidation [67-68]. Similarly, Lecithin from frying foods caused foam formation at the initial stages of deep-fat frying. Presence of starch also increases the degradation of frying oil while presence of amino acids was found to be protecting the oil from degradation during the frying process [63]. Transition metals such as iron present in the meat that could accumulate in the oil during frying which will increase the rate of oxidation and also thermal degradation of oil [18].

1.6.5 Types of fryers

Different types of fryer could affect the frying oil degradation. Polymerized fat deposited on the fryer causes the formation of gum, foam, dark color, and further deterioration of frying oil. Usually, lower surface to volume ratio of fryer is recommended for minimizing the oil - air contact is recommended for better frying practice with less degradation of oil. It has been reported that oxidation of oil was reduced by modifying a fryer with higher ratio of oil depth

to the surface area. Similarly, Copper and Iron fryers are found to be accelerating the oxidation of frying oil [69].

1.6.6 Antioxidants

Antioxidants are compounds which can inhibit the oxidation of other compounds. Many natural antioxidants, which protect oils from oxidation to a certain extent, are reported (Figure 1.7). Similarly, various synthetic antioxidants are utilized by the food industry in order to enhance oils stability or shelf life for longer duration. Antioxidants are found to have greater influence on the quality of oils during deep fat frying.

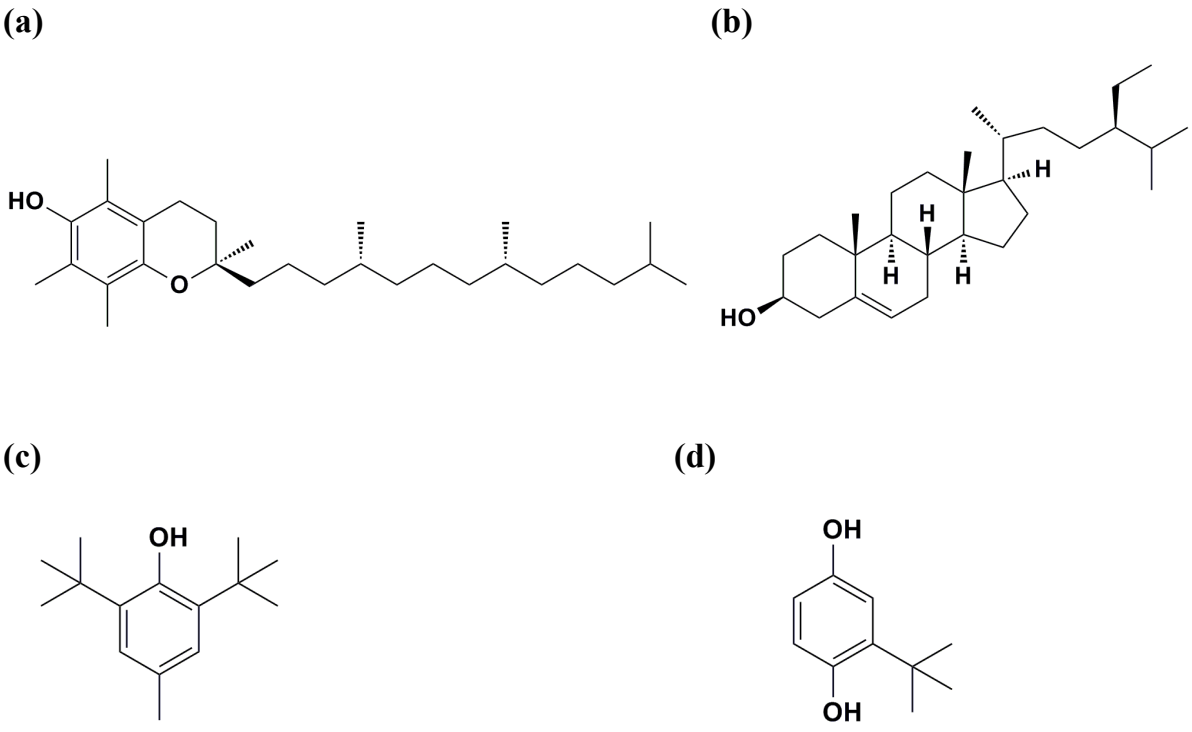


Figure 1.7 Structures of natural and synthetic antioxidants (a) *α*- tocopherol (b) *β*-sitosterol (c) BHT (d) TBHQ.

Several studies reported the efficacy of both natural and synthetic antioxidants added to oils on the improvement of oil quality during different frying models [70-72]. Shahidi *et al.* (1997) reviewed the wide variety of antioxidants in lipid research extensively. Natural antioxidants including tocopherols and sterol, synthetic antioxidants including butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT), and *ter*- butyl hydroquinone (TBHQ) were proved to retard the oxidation of oil at room temperature [73]. However, they are less effective at frying temperature due to their low thermal stability [74].

Carotenes were found to be ineffective in preventing the oil from thermal oxidation at frying temperature in the absence of other antioxidants. However, Carotenes are major antioxidants that in red palmolein they provide stability and react with radicals. Tocotrienols could regenerate carotenes from carotene radicals. Synergistic effects of tocotrienols and carotenes in decreasing the oxidation of oil during frying of potato chips were reported [75].

Sesame lignan compounds of sesame oil including sesamol, sesamin and sesamolin, were found to be more thermally stable during heating and could provide high oxidative stability to sesame oil during frying temperature [76]. Soybean oil blended with roasted sesame oil lowered the formation of conjugated dienoic acids than control soybean oil at frying temperature. Similar results were observed with the addition of sesame oil and rice bran oil to high oleic sunflower oil which showed higher oxidative stability at elevated temperatures [77].

Ascorbyl palmitate was found to be lowering the dimerization in edible oils at frying conditions [78]. Similarly, sterols and their fatty acid esters showed higher oxidative stability of oils during deep fat frying [79-80]. Rosemary and sage extracts are potent antioxidants which showed reduction of oil deterioration during intermittent deep fat frying of potato chips [81-84]. Similarly, extracts of thyme and Curcuma showed considerable effect on frying oil quality [85-86].

1.6.7 Additives

Modern lipid research focused on the food additives and their efficacy in improving the oxidative stability of oils and also anti-polymerizing activity in the frying process. Earlier reports discussed the silicone and its ability in protecting the oil from oxidation during deep fat frying by the formation of a protective layer at the air-oil interface and the small convection currents of frying oil [87-89]. The combination of silicone and antioxidants are also found to have a synergistic effect on decreasing the oxidation of frying oils [90].

Dimethyl polysiloxane (DMPS) is an anti-foaming agent that is commonly used in industrial frying process. DMPS at lower concentrations showed anti-polymerizing activity during discontinuous frying process [91-92]. However, higher levels of DMPS in frying oils showed increase in acrylamide formation in potato chips in the recent research. Gertz *et al*. (2004) reviewed the activity of various oil improving agents including anti-foaming agents, emulsifiers and found that utilization of filter aids or mineral adsorbents could improve the stability of oil in repeated frying process [93].

1.6.8 Dissolved oxygen content in oils

There is little information available about the influence of dissolved oxygen content on the frying oil deterioration. At frying temperatures, reduction in partial pressure of oxygen cause rapid decomposition of hydroperoxides leads to faster degradation of oil [94]. Earlier reports discussed the flushing of nitrogen, and carbon dioxide gases to decrease the dissolved oxygen in the oil which reduced the oxidation of oil during the frying process. Moreover, carbon dioxide is found to have higher protection from oxidation because of its higher solubility than nitrogen [95] suggested that a minimum of 15 min of nitrogen or 5 min of carbon dioxide flushing prior to heating decreases the oxidation of oil during deep-fat frying.

1.7 Biological effects on consumption of frying oils and fried foods

In the recent past, rising consumption of deep fried products causing increase in total energy intake in both developing and developed countries. The rise in consumption is mainly due to the desirable flavor, color, crispy texture and ready to eat nature of fried foods. During the frying process, a complex series of reactions leads to formation of both volatile and nonvolatile compounds which may have important physiologic effects. Nonvolatile products of degradation including polymers and the polar compounds gain more importance than other compounds because they remain in the frying oil and migrate into food material [96]. Similarly, oxidation products of unsaturated fatty acids (FA) including 13-hydroperoxyoctadecadienoic acid (13-HPODE), 9-hydroxy- and 13-hydroxyoctadecadienoic acid (9-HODE, 13-HODE) as well as cyclic FA monomers (CFAM) are found to be potent atherogenic compounds in frying oils. Inevitably, these substances are absorbed by the fried

food during the frying process and eventually ingested during their consumption. Fat content of potato crisps (35-40%), donuts (20-25%), and French fries (10-15%) signifies the necessity of the studies on their detrimental effects [97-98].

1.7.1 Biological effects of oxidized lipids formed during frying

Feeding experiments with rats, revealed that ingestion of frying oils compared with regular fats, lead to many biological effects in mammals including oxidative stress [99], impaired glucose tolerance [100], thyroid dysfunction [101] and lipid metabolism alterations [102]. Moreover, recent studies clearly showed 9-HODE, 13-HODE, and 13-HPODE in frying oils are potent ligands and activators of peroxisome proliferator-activated receptors (PPAR) [103-104]. PPARs are nuclear receptors, which include *α, β/δ,* and *γ* transcription factors which could be activated by FA and their metabolites such as eicosanoids etc. Similarly, hydroxylated FAs are stronger activators of PPAR than non-hydroxylated FAs. This point explains the weak activation of PPAR by consumption of fresh oils. But, consumption of frying oils with high concentration of hydroxylated FAs lead to strong activation of PPAR. Usually, PPAR stimulates the expression of target genes by binding to specific DNA sequence elements. Therefore, components of frying oil are capable of exhibiting both stimulatory and inhibitory effects on gene expression through the activation of PPAR [105]. Consumption of thermally degraded animal fats is causing a higher risk compared to that of heated vegetable oils as animal fats are rich sources of cholesterol and phospholipids oxidation products. Recent studies have highlighted the adverse effect of cholesterol oxides on cholesterol metabolism, cell membranes and triggering several health risk factors including cytotoxicity, carcinogenicity, and atherosclerosis [106].

1.7.2 Physiological effects of *trans* fatty acids formed during frying

Usually, TFA does not develop to a significant extent during the frying process. However, recent studies demonstrated the formation of TFA during prolonged frying and heating processes [107]. Mechanism of formation of TFA mainly proceeds through generation of free radicals [108]. Earlier studies of biological effects demonstrated the impact of the consumption of TFA on cardiovascular risk and lipoprotein metabolism. High plasma concentrations of total cholesterol, LDL cholesterol and low levels of HDL cholesterol were also observed with TFA intake through food. Consumption of TFA can promote inflammation, vascular endothelial dysfunction and shows negative effects on the eicosanoid metabolism [109]. The eicosanoids are cyclic bioactive compounds which primarily originate from arachidonic acid by the action of cyclooxygenases and lipoxygenases. They have diversified functions in tissues at low levels, especially related to inflammation. Fatty acids in the diet with *trans* double bonds could potentially inhibit eicosanoid metabolism by reducing the availability of substrates or by inhibiting specific enzymes [110]. However, both mono and poly *trans* unsaturated fatty acid can show many harmful biological effects upon their regular intake through fried food materials.

1.7.3 Physiological effects of cyclic fatty acids formed during frying

Cyclic fatty acids are a group of fatty acids having ring system in their structure. Sebedio *et al*. (1989) elucidated the structures of the major cyclic fatty acid isomers (CFAM) formed from linoleic and α-linolenic acids while frying, by using mass spectrometry [111]. Prior research works found that the formation of CFAM is very less and highly depends on

frying temperature (> 240 ^{o}C) and fatty acid composition of oil. However, α-linolenic acid is highly prone to the formation of CFAM, that can be accumulated into all types of tissues during metabolism and show various biological effects [112]. CFAM present in thermally stressed oils have shown very high level of intestinal absorption depending on the structure and position in the triacylglycerol. They mainly metabolized, selectively incorporated into tissues and further oxidized in a similar manner as the precursor essential fatty acids. CFAM can influence the activities of enzymes responsible for synthesis and oxidation of lipids in the liver [113-114]. However, these cyclic fatty acids are not a primary concern or toxic, when the oils are utilized under normal frying conditions.

1.8 Characterization of frying oil degradation through mass spectrometry

Lipidomics is a branch of science that deals with the study of pathways and networks of cellular lipids in biological systems and it involve the characterization of lipid molecular species and of their biological roles [115-116]. The word "lipidome" describes the complete lipid profile of a cell and lipidomics is an advanced research field that has been driven by rapid advances in technologies such as mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy with the recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis and diabetes [117-118].

Usually, lipids are analyzed through chromatographic techniques because of their low volatility, especially by gas chromatography. However, it includes derivatization such as hydrolysis and esterification that makes those techniques complicated and time consuming. Moreover, chromatographic methods only quantify the compounds rather than providing

information about compounds [119]. The development of mass spectrometric methods for Matrix-Assisted Laser Desorption Ionization (MALDI) and electrospray ionization (ESI) have advantage to perform structural elucidation of lipid molecules based on their molecular weight [120-121]. To enhance the accuracy and resolution in the MALDI, it is generally coupled with a fast scanning time-of-flight (TOF) mass analyzers [122]. The methods developed for the screening of thermo-oxidation products of triacylglycerols (TAG) through liquid chromatography coupled with atmospheric pressure chemical ionization-mass spectrometry (APCI/MS) and electrospray chemical ionization-mass spectrometry (ESI/MS), is much complicated and laborious [123-124]. The recent trends in analytical methods show an increasing usage of TOF-MS for structural elucidation, which allows precise empirical formula assignments for unknown compound confirmation. In addition to this, assessing the oxidative stability of edible oils through MALDI-TOF/MS is a growing interest in lipid science with its rapid profiling of complex mixtures and easy sample preparation [125-127].

The working principle of MALDI-TOF/MS is based on the utilization of a matrix that absorbs the energy from the laser source followed by the generation of ionized molecules [128]. Usually, small organic molecules with acid moiety such as 2, 4- dihydroxybenzoic acid (DHB) or α- cyanohydroxycinnamic acid (CHCA) are utilized in the preparation of matrix solution [129-130].However, several molecules with azo and nitro groups were synthesized for enhancing the resolution of MALDI spectra in recent years.

On the other hand, the suitability of a particular compound as matrix is determined by the type of the laser and its emission wavelength [131]. UV lasers have emission wavelength of 337 nm and majority of MALDI instruments are primarily with this laser type [132]. An

efficient or suitable matrix exhibits a significant absorption at the laser wavelength (337 nm) and shows high stability at higher vacuum conditions. It also enables sufficient stability for ions until they are detected at the mass analyzer [133]. When the pulsed laser beam strikes the sample mixed with matrix, primarily the energy is absorbed by the matrix and transfer that energy to analyte, upon which it evaporates into gaseous phase along with the analyte [134-135]. A solid sample between the analyte and the matrix is commonly analyzed by MALDI-TOF/MS. The matrix solution absorbs the energy emitted from the laser and protects the analyte from severe fragmentation. During this process, protonated (H^+) and alkylated (Na^+) ions are exchanged between the matrix, and the sample that eventually leads to the formation of charged analyte molecules. These generated ions are called quasimolecular ions [128, 136]. These ions could be both cations and also anions and detecting the cation mode spectra is commonly followed in MALDI. On the other hand, there is a difference between general radical ions which are often produced in the electron impact (EI) mass spectra and quasimolecular ions that are produced by MALDI [137]. Molecular radical ions are formed by the abstraction of an electron from the analyte, which has the same mass (*m/z*) compared to the analyte. But, quasimolecular cations are formed by the addition of a proton or cation to the analyte. So, the mass of the quasimolecular ion is higher than analyte molecule based on the ion formed.

In the recent past, few studies discussed the application of MALDI-TOF/MS in the characterization of frying or thermal degradation of edible oils. Schiller *et al.,* (2002) studied the degradation pathway of saturated fatty acids in thermally stressed coconut oil and also oxidation products in heated olive and linseed oil through MALDI-TOF/MS [138]. Picariello

et al., (2007) demonstrated the characterization of TAG from animal fat by MALDI-TOF/MS. They also studied the polar and non-polar fractions in heated sunflower, olive oils and highlighted the significance of the enrichment of polar compounds in screening the thermal degradation of edible oils through MALDI-TOF/MS [139-141]. Stutts *et al.,* (2013) characterized phosphatidylcholine oxidation products by MALDI/MS and illustrated the enhanced selectivity of MS for the identification of lipid oxidation products [142]. From all these studies, it can be clearly understood that MALDI-TOF/MS could be utilized effectively to study frying oil degradation.

1.9 Fate of natural and synthetic antioxidants during the frying process

One of the most important aspects of frying which needs extensive study is the fate of the minor constituents present in oils such as tocopherols, tocotrienols, sterols, carotenoids and other phytochemicals. In addition, it is also necessary to understand the new compounds formed from these constituents and their biological impact. Among these minor constitutes, tocols are the most potent natural antioxidants because of their lipophilic character [143-144]. They usually scavenge lipid peroxyl radicals, which propagate lipid peroxidation. Tocopherolquinones were found to be major compounds to be originated from frying process [145]. Tocopheroxyl radical formed during lipid oxidation yields 8α-substituted tocopherones that further hydrolyzed to 8α-hydroxytocopherones that rearrange spontaneously to form α-tocopherolquinones. Other minor degradation products including epoxy quinones, tocopherol dimers and trimers were reported [146]. These quinones also possess antioxidant nature and their biological effects are not widely studied [147].

Cholesterol and other phytosterols including sigmasterol, stigmasterol, campesterol, brassicasterol and avanosterol are highly susceptible to oxidation at frying temperature and produce several oxidation products which are commonly referred as oxysterols [148-149]. 7-keto oxi derivatives were found to be the most abundant form of oxysterols at higher temperature. 7α-hydroxy, 7β-hydroxy, 5, 6 α-epoxy, 5, 6 β -epoxy and 5, 6, 7-triol derivatives were reported as other minor degraded products of sterols [150-151]. Recently, dimers and oligomers of sitosterol and also stigmasterol were identified during thermo-oxidation [152-153]. These oxysterols are potential health hazards and they are cytotoxic and also associated with atherosclerosis [154].

Carotenoids represent a class of hydrocarbons produced by an extensive variety of plants, and they possess important physiological properties. β - carotene is the most important carotenoid in oils such as palm oil or red palmolein. Nutritionally, β - carotene is a precursor of vitamin A dimer structure, and it imparts redness to the oils. Lutein is another carotenoid compound that is commonly present in corn oil. Due to their highly unsaturated composition, carotenoids are highly susceptible for thermal degradation during processing and storage [155-156]. Earlier studies have found that natural carotenoids are in *cis* form but during the degradation process, they change into *trans* forms. Both β -carotene and lutein found to form *trans*-β-carotene and *trans*-lutein respectively [157-159]. Moreover, other degradation products including 13-*cis*- and 9-*cis*-β-carotene, and 13-cis-, and 9-cis-lutein, epoxy carotenoids were also detected in heated palmolein [160-161].

Sesame lignans including sesamin, sesamol, sesamolin are commonly found in sesame oil. They possess higher thermal stability compared to other natural antioxidants. However,

during prolonged thermal treatment they decompose into minor fragments. Earlier findings reported that sesamolin hydrolyze and forms sesamol, sesamin and sesaminol [162-163]. Among these, sesamol is a stable compound which could constitute the dimer during peroxidation of oil, and both were found to possess similar antioxidant capacity [164-165]. Moreover, flax lignans and pinoresinol from olive oil were proven to have higher thermal stability than sesamolin. On the other hand, aglycones and glycosides of lignans degrade at higher roasting temperature [166] and additionally there is not much information about degraded products during frying. Synthetic antioxidants are widely used in commercial oils and the elucidation of new compounds arise from their degradation is one of the critical studies. Previous studies found that BHT and TBHQ have high volatility at frying temperature compared to BHA and PG [167-168]. BHA and BHT could form dimerized compounds during heating. Similarly, tertiary butyl benzoquinone (TBBQ) was found as the primary degradation product of TBHQ with minor degradation products with residual antioxidant activity [169-170]. In the recent past, many studies are focusing on the efficacy of plant extracts as substitutes to the several synthetic antioxidants used in commercial oils. These additives targeted to exert both antioxidant and anti-polymerizing activities in frying oil stabilization. Studying the efficacy of different plant based antioxidants such as sesame lignans, rosemary and sage extracts in the frying process is of growing interest in the edible oil industry to develop highly thermally stable frying oils. This in turns leads to the formulation of antioxidant emulsions, blending with different oils and screening of unconventional antioxidants for increasing the stability of frying oils. The limit of the addition of synthetic antioxidants to refined edible oils is 0.02 % by different food laws.

However, most of the studies discussed the efficacy of antioxidants in higher limits (up to 0.1 %) and tested the effect of those additives in the refined oil which already contains synthetic antioxidant that causes synergestic or antagonistic effect on the antioxidant activity and further to underestimation or overestimation of targeted antioxidant effect. This necessitates to study on the various antioxidants or additives at same concentration and their impact on the frying oil degradation. In this context, the aim of the present study is to understand the thermal degradation of palmolein and soybean oils in different models of frying operations and to characterize the new compounds formed in frying through MALDI-TOF/MS. This study also investigates efficacy of various antioxidants on the degradation of frying oils and to evaluate the thermally stable antioxidants to maximize the stability of frying oils. As this thesis "Thermal degradation studies on edible oils during deep-fat frying process" completely concentrates on the degradation studies on edible oils, there was no attention has given to the fried food products.

CHAPTER 2
MATERIALS AND METHODS

2.1 Chemicals and solvents

Standards of fatty acids methyl esters (FAME) (C4:0 Methyl butyrate, C6:0 Methyl caproate, C8:0 Methyl capryllate, C10:0 Methyl caprate, C12:0 Methyl laurate, C14:0 Methyl myristate, C14:1-9c Methyl myristoleate, C14:1-9t Methyl myristelaidate, C16:0 Methyl palmitate, C16:1-11c Methyl palmitoleate, C16:1-11t Methyl palmitelaidate, C17:0 Methyl heptadecanoate, C18:0 Methyl stearate, C18:1-9c Methyl oleate, C18:1-9t Methyl elaidate, C18:1-11t Methyl vaccenate, C18:2-9, 12 Methyl linoleate (cc, ct, tc&tt isomers), C18:3-9, 12, 15 Methyl linolenate (isomeric mixture), C20:0 Methyl arachidate, C20:5 n-3 Methyl eicosapentaenoate, C22:6 n-3 Methyl docosahexanoate) were purchased from Sigma Chemicals (Steinheim, Germany).

Standard TAG including Glyceryl tripalmitate (PPP), Glyceryl trioleate (OOO), Glyceryl trilinoleate (LLL), Glyceryl trilinolenate (LLL), Glyceryl tristearin (SSS) and 1, 3-dipalmitoyl-2-oleoglycerol (POP), were purchased from Sigma (Milan, Italy). All solvents and chemicals of the highest purity grade were purchased from Spectrochem (Bangalore, India). Silica gel 60 and thin-layer chromatography (TLC) Aluminum plates (20×20 cm) with silica gel 60 F254 were purchased from Merck (Darmstadt, Germany). High-Performance Liquid Chromatography (HPLC) grade solvents were purchased from Merck (Darmstadt, Germany). All other chemicals were of laboratory grade from Spectrochem (India).

Tocopherols mixture (α, β, γ and δ), α -tocopherol, γ-tocopherol, β-sitosterol, β-sitostanol, sesamol, gallic acid, caffeic acid, ferulic acid and quercetin were purchased from Sigma (Milan, Italy). γ- oryzanol was purchased from Tokyo chemical industries (TCI, Japan). Butylated hydroxytoluene (BHT) and *ter*- butyl hydroxyquinone (TBHQ) and Curcumin were purchased from Alfa aesar (Hyderabad, India). Rosemary extract (Herbalox, type O) was gifted from Kalesc Inc. (Miami, U.S), Sucrose acetate isobutyrate (SAIB 100) was provided by Eastman Chemical Company (Tennessee, U.S) and pure soy lecithin was provided by Haneil Soyatech Pvt. Ltd. (Nagpur, India).

2.2 Oil samples and food materials

Commercial samples of refined palmolein and soybean oil along with potatoes, chicken nuggets, and all other food samples were purchased from the local market (Trivandrum, India). Refined soybean oil without any added synthetic antioxidants or additives was sourced from Sakthi soya group (Pollachi, India). Refined palmolein, rice bran oil, sesame oil, groundnut oil and sunflower oils, with and without addition of synthetic antioxidants or additives were obtained from Kamani oils Industries Pvt Ltd (Mumbai, India).

2.3 Frying operation methods

2.3.1 Intermittent frying operation

Prior to frying experiment, potatoes were washed and peeled out. Potatoes were cut into French fries in a length of 5-10 cm. Potato strips were rinsed twice with 400 mL of water and

put in a cup of water (400 mL) for 15 min to get rid of surface starch. Then all strips were blanched for 10min in hot water and finally dried with a paper towel [171]. 4 liter of refined palmolein and refined soybean oils were subjected to discontinuous frying of French fries for 18hrs (6hrs/day) at 180^{o}C. Everyday oil was preheated for 1 hr and after stabilizing the frying temperature first frying cycle started. This operation was continued for 3 days simultaneously with 36 frying cycles. During this process, every 2 hrs, oil was collected and physico-chemical parameters of oils were studied. A blank heating operation was performed in the absence of food material at similar conditions mentioned above.

2.3.2 Continuous frying operation

2.3.2.1 Uni-pot frying process

4 L of refined palmolein and soybean oils were subjected to continuous frying of French fries and chicken nuggets separately for 10 hrs (20 frying cycles) at 180^{o}C. For each 2 hrs (4th frying cycle), oil was collected from fryer and physico-chemical studies were carried out. Simultaneously, a blank heating of the two oils were carried out in the absence of food material for comparative studies.

2.3.2.2 Multi-pot frying process

4 L of refined palmolein and soybean oils were subjected to continuous frying of French fries and chicken nuggets in combination for 10 hrs (20 frying cycles) at 180^{o}C. First 5 hrs (10 frying cycles) potato frying was carried out followed by frying of chicken nuggets. At the end of each 2 hrs (4th frying cycle), oil was collected from fryer and physico-chemical

studies were carried out. Simultaneously, a blank heating of the two oils were carried out in the absence of food material for comparative studies.

2.4 Physico-chemical quality studies

2.4.1 Free fatty acid value (FFA)

Free fatty acid (FFA) value is defined as the number of milligrams of alkali (KOH) required for neutralizing 1 g of fat. FFA contents of all samples were determined using AOCS official method Ca 5a-40, and results were reported as the percentage of oleic acid [172].

2.4.2 Peroxide value (PV)

The peroxide value is defined as the amount of peroxide oxygen per 1 kg of fat or oil. Traditionally this was expressed in units of mill equivalents per kilogram. Peroxide values of all the samples were determined by standard AOCS official method Cd 8-53 [172].

2.4.3 *p*-Ansidine value (pAV)

It can be defined as 100 times the O.D measured at 350 nm in 1 cm cuvette of a solution containing 1 g of the oil in 100 ml of the solvent mixture. *p*AV of all samples were determined by standard AOCS official method Cd (18) – 90 [172].

2.4.4 Conjugated diene value (CD)

Diene value is a measurement of conjugated double bonds formed prior to peroxide formation. CD values of the all heated oil samples were determined according to the standard IUPAC method [173].

2.4.5 Total polar compounds

Polar and non-polar fractions were obtained by chromatography on silica gel as per the the official IUPAC procedure [174]. Briefly, 500 mg of heated/fried oil dissolved in 2 ml of hexane/diethyl ether 90:10 (v/v) and loaded on a silica gel column. The non-polar fraction containing the TAG was eluted with hexane/diethyl ether 90:10 (v/v) and the polar fraction was then eluted by diethyl ether (100%) and finally dried. The separation of two fractions was monitored by TLC by using pre-coated silica gel plates eluted with hexane/diethyl ether 80:20 (v/v) and visualized by iodine vapors. The clear separation between the two fractions was achieved. Weight of the polar fraction was quantified and total polar compounds level was expressed in percentage.

2.4.6 Color analysis

Color measurements of the oil samples were carried out using a spectrophotometer (model CM-3500d, Konica Minolta Sensing, Inc., Osaka, Japan). The color values were expressed as L* (brightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). Total color difference was calculated using the equation: where, L_0, a_0 and b_0 were the L*, a* and b* values of fresh oil and L_t, a_t and b_t refer to the color values of oil at various frying cycles [172].

2.5 Fatty acid composition by gas chromatography (GC)

Fatty acid methyl esters (FAME) from oil samples were prepared according to the International Union of Pure and Applied Chemistry (IUPAC) procedure [173]. The FAME was analyzed by using Shimadzu GC 2010 fitted with a split injector (250^{o}C) and a Flame Ionization Detector (FID 300 ^{o}C). An SP-2560 Supelco column (75m, 0.18mm id and 0.14μm film thickness) was utilized and operated at 200^{o}C. Nitrogen was used as a carrier gas at a velocity of 13.0cm/s. Methyl heptadecanoate was utilized as the internal standard. This method was developed and standardized in our laboratory [175]. FAME was identified by comparison of their retention time with authentic standards, and the peaks were quantified by using digital integration according to AOCS official method Ce 1-62 [172]. Fatty acid levels were reported as relative proportions of the total composition.

2.6 MALDI-TOF/MS analysis of frying oils

MALDI-TOF/MS analysis was performed with a Shimadzu Biotech Axima CFR Plus instrument equipped with an N_2 laser (337 nm, 3 ns pulse width, 20 Hz repetition rate). Mass spectra were acquired in both the linear and reflector ion and a range of m/z 300-4000 ranges was explored. The instrument was operated with an accelerating voltage of 20 kV. The matrix solution was prepared by dissolving 10 mg of crystalline α-cyano-4-hydroxycinnamic acid (CHCA) in 1 mL of methanol containing 0.1% TFA. Heated and fried oils, as well as the fractionated samples, were dissolved in Chloform at a concentration of 10 μL/ mL. An aliquot of the $CHCl_3$ layer (10 μL) was mixed with the matrix (1:1, v/v), and 1 μL of the resulting solution was deposited directly onto the sample plate and air-dried. Typically, 196 laser pulses were acquired for each mass spectrum. External mass calibration was performed

with a separate acquisition using a mixture of standard TAG and standard peptides. Lipid characterization was performed by comparing with standard TAG wherever possible and also mass measurements for other compounds were performed with the LIPID MAPS prediction tool (http://www.lipidmaps.org/tools/index.html). To check the repeatability, samples were analyzed in triplicate.

2.7 Addition of antioxidants to oils and heating procedure

A standard amount of 0.02% of each antioxidant added to 20 g of additive free refined soybean oil samples (recovered directly from Sakthi soya industries refinery, Pollachi) in different steel vessels (30 ml capacity) and heated on a hot plate assembled with a temperature sensor (IKA, India) at 180^{o}C for 2 hrs by maintaining exact frying temperature throughout the study. In addition, the temperature at different parts of the oil was monitored through the temperature reader and also occasionally stirred to maintain almost uniform temperature with little variance. A control sample without any added antioxidant was heated at same conditions mentioned above. All oil samples were cooled down after heating and subjected to further characterization immediately.

2.8 Preparation of oil blends

Refined palmolein (PO) and refined soybean oils (SO) free from additives were blended separately with each of refined rice bran oil (RBO), sesame oil (SeO), sunflower oil (SNO), groundnut oil (GNO) and coconut oils (CO) free from additives in 80/20 ratio and 200 ppm of BHT was added. Blends were heated in steel vessels at frying temperature as discussed earlier.

2.9 Statistical analysis

Results were expressed as the mean and standard deviations of the control from three independent experiments. Data were analyzed by one-way ANOVA and the significance of differences between the mean was calculated by Duncan's multiple range tests using SPSS for Windows standard version 7.5.1 (SPSS, Inc.). Note that $p \leq 0.05$ was considered to be significant.

CHAPTER-3.0
RESULTS AND DISCUSSIION

3.1 Thermal Degradation of palmolein and soybean oils during deep fat frying processes

3.1.1 Degradation of palmolein during intermittent frying process

The intermittent frying process is a discontinuous frying process, which is one of the most common domestic or household cooking practices. In this study, we did not replenish the fresh oil to the frying oil, which will influence the quality of frying oil. During 36 frying cycles (18 hrs frying at 180^{o}C) of French fries, soybean oil was degraded more than palmolein. Similarly, both oils showed greater degradation during frying compared to the heating process.

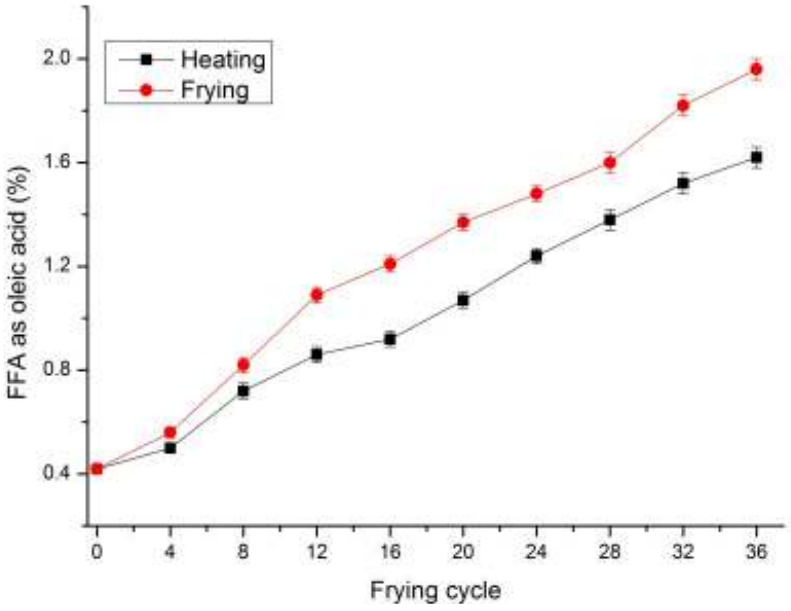
Figure 3.1 (a), (b), (c) and (d) showed the formation of FFA, PV, *p*AV and TPC in the palmolein during heating and frying processes (Values are showed in supplementary information; Table 3.1S). Fresh palmolein with 0.42 ± 0.02% of FFA reached 1.96 ± 0.04% at the end of the 36th frying cycle, whereas after heating it showed 1.62 ± 0.04% of FFA. In the heating process, FFA forms by limited moisture and thermal break down of TAG. Whereas in the frying process, moisture of food material also causes hydrolysis of the triglycerides in addition and generates FFA. This result in the formation of higher FFA values during the frying process compared to heating.

An interesting phenomenon with respect to peroxide values was observed during heating and frying of palmolein. During heating higher peroxide value (from 3.63 ± 0.40 mEq/Kg to 28.21 ± 1.00 mEq/Kg) was obtained compared to frying (from 3.93 ± 0.50 mEq/Kg to 17.69 ± 0.90 mEq/Kg). During the oxidation process, abstraction of oxygen by triglyceride radicals

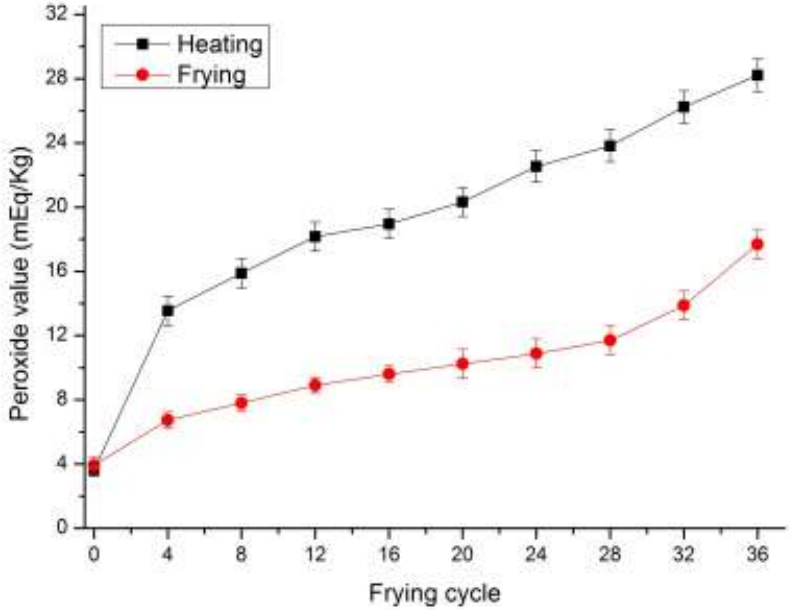
leads to the formation of hydroperoxides. These hydroperoxides are highly unstable at high frying temperature and immediately proceeds to secondary oxidation and several breakdown products [25, 176]. This explains the lower peroxide value during frying compared to heating. Our results clearly explain that peroxide value is not a reliable indicator for monitoring frying oil quality which is in agreement with previous reports [177]. However, the higher peroxide value leads to rancidity and faster degradation of the oil. *p*AV represents secondary oxidation of the oil and palmolein showed higher secondary oxidation during the frying process compared to heating. In this study, fresh palmolein showed a *p*AV of 5.64 ± 1.20 which was increased to 90.03 ± 2.20 during the frying process while during the heating process it was recorded at 82.03 ± 2.30.

Quantification of polar compounds was proposed as the most reliable method for the evaluation of quality of oils during frying [178]. Initial polar compound content in palmolein is 6.00 ± 0.25%, which is higher than other edible oils because of the presence of large amounts of DAG and MAG. The polar compound content was quantified as 17.00 ± 0.50% and 22.00 ± 0.50% at the end of heating and frying process respectively. This is mainly due to higher dimerization and polymerization along with oxidation in palmolein during the frying process. It is known that 24% of polar compounds are considered as a limit of complete degradation of any frying oil. Palmolein reaching this limit, within 18 hrs of the frying process (36 frying cycles) showing that palmolein is completely degraded. These results also suggest that repeated frying cycles lead to the oil unsuitable for consumption.

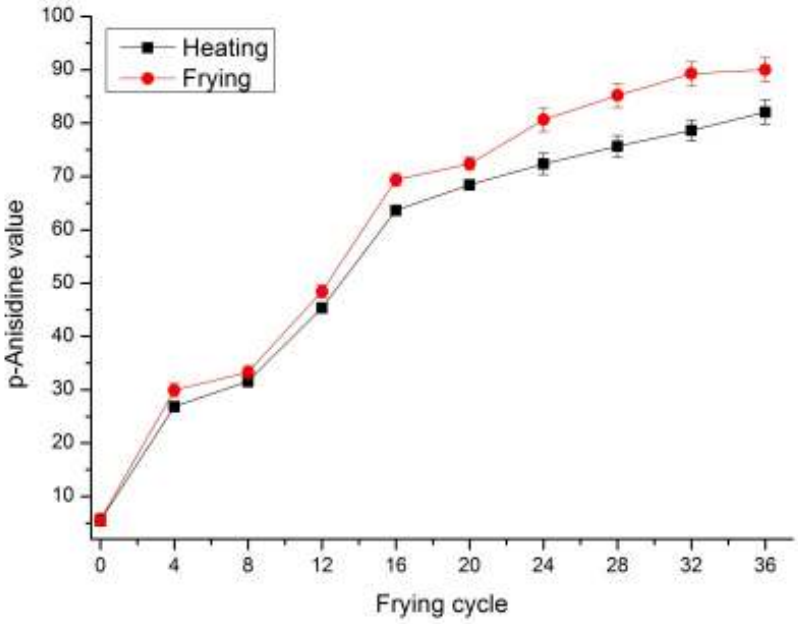
(a)


Heating
Frying
FFA as oleic acid (%)
2.0
1.6
1.2
0.8
0.4
0
4
8
12
16
20
24
28
32
36
Frying cycle

(b)


Heating
Frying
Peroxide value (mEq/Kg)
32
28
24
20
16
12
8
4
0
0
4
8
12
16
20
24
28
32
36
Frying cycle

(c)



(d)

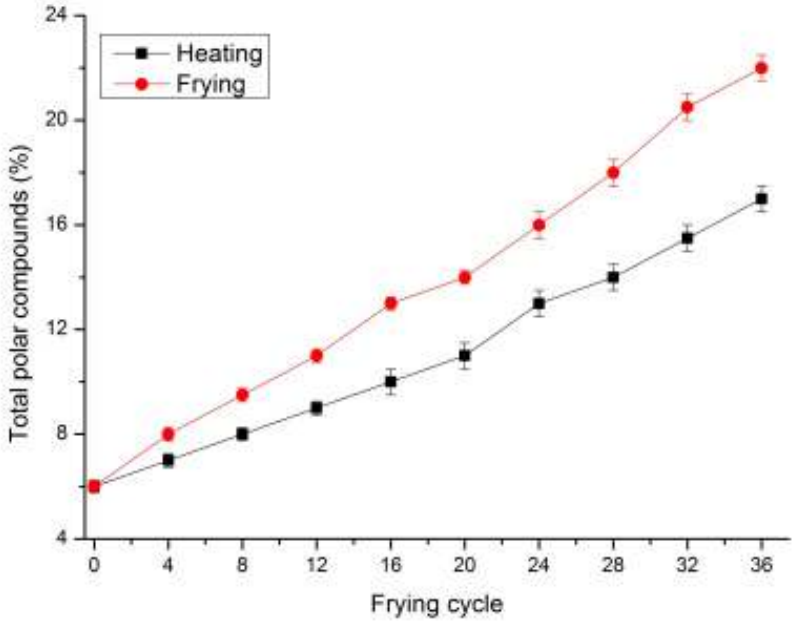


Figure 3.1 Quality parameters of palmolein during intermittent frying process (a) FFA (b) PV (c) *p*AV (d) TPC.

Fatty acid degradation is one of the most common phenomena of heating and frying processes. Figure 3.2 showed the fatty acid degradation in palmolein during frying process (Values are showed in supplementary information; Table 3.2S). In our study, GC results showed that the fresh palmolein is mainly composed of palmitic acid (50%), oleic acid (37%), and linoleic acid (9%). Higher level saturated fat gives more oxidative and frying stability to the oil. Interestingly, with respect to the MUFA and PUFA, fresh palmolein (53.71± 0.3% of SFA, 37.33± 0.2% of MUFA, 8.96± 0.3% of PUFA) showed lesser degradation after 36 frying cycles (61.27± 0.5% of SFA, 31.21± 0.4% of MUFA, 7.52± 0.5% of PUFA). But secondary oxidation results (*p*AV and CD) showed higher degree of degradation during frying which leads to the complete degradation of palmolein after 36 frying cycles. This is mainly due to overlapping of oxidized fatty acids with unoxidized fatty acids in GC analysis [179]. These results clearly demonstrate that studying fatty acid degradation is not an effective method of determining frying oil quality. On the other hand, there was no TFA formation was observed during heating and frying processes of palmolein.

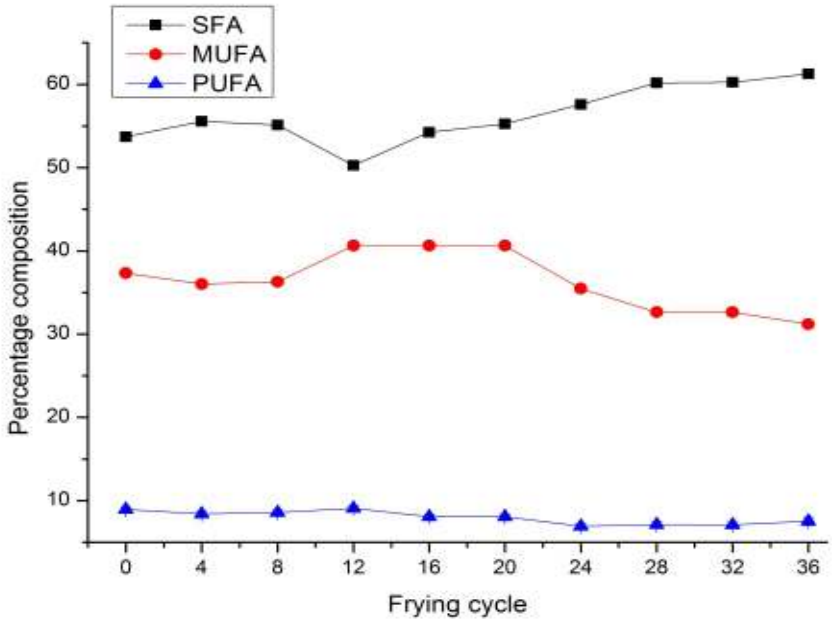
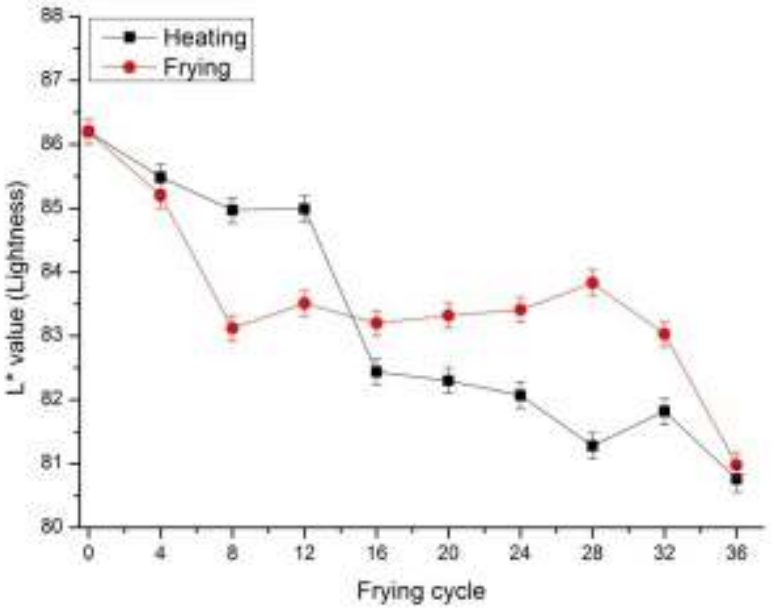


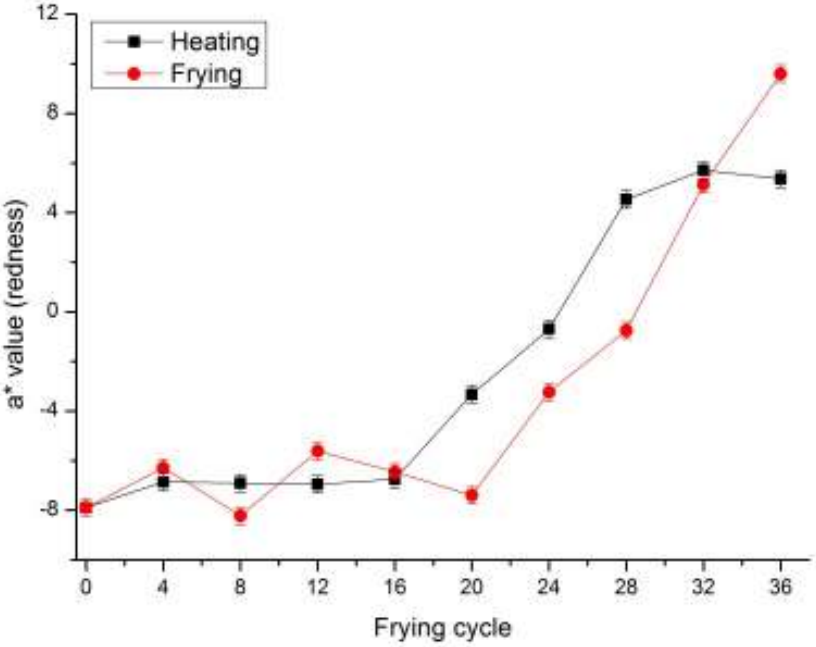
Figure 3.2 Fatty acid degradation in palmolein during intermittent frying process.

Degradation of color parameters of palmolein during heating and frying process were shown in Figure 3.3 (a), (b), (c), (d). Increase in redness and decrease in lightness and yellowness is one of the indicators for frying oil degradation (Values are showed in supplementary information; Table 3.3S). Compared to heating, during frying conditions, redness of the palmolein was found higher. Redness of any frying oil mainly depends on polymers formation and food material being fried [180]. Similarly, there was a significant reduction the lightness and yellowness during frying. Total color difference demonstrates the change of color during each frying cycle. In our study, both heating operation and frying operation including 36 frying cycles showed a lower color difference in comparison between heating and frying process (Fig 3.3 d). So, this showed that French fries do not have significant effect on the total color difference.

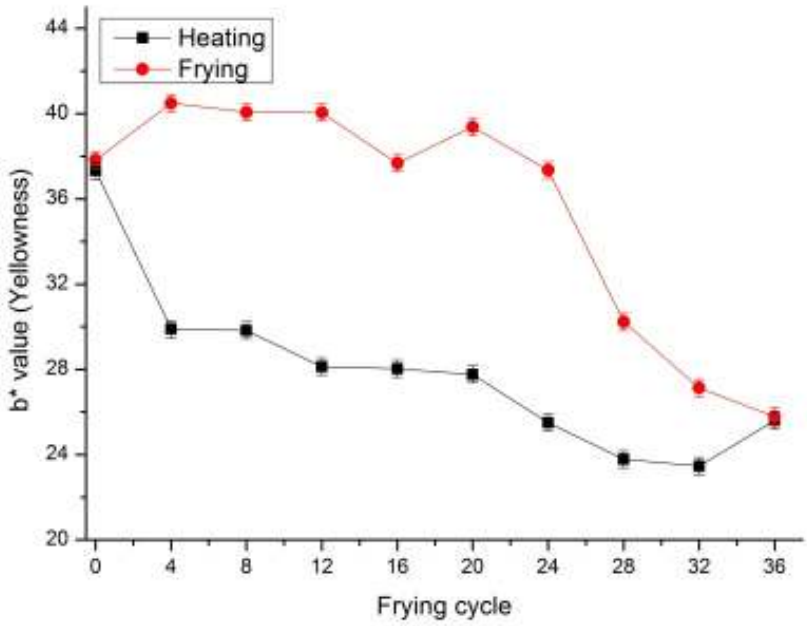
(a)


Heating
Frying
L* value (Lightness)
Frying cycle
88
87
86
85
84
83
82
81
80
0
4
8
12
16
20
24
28
32
36

(b)


Heating
Frying
a* value (redness)
Frying cycle
12
8
4
0
-4
-8
0
4
8
12
16
20
24
28
32
36

(c)



(d)

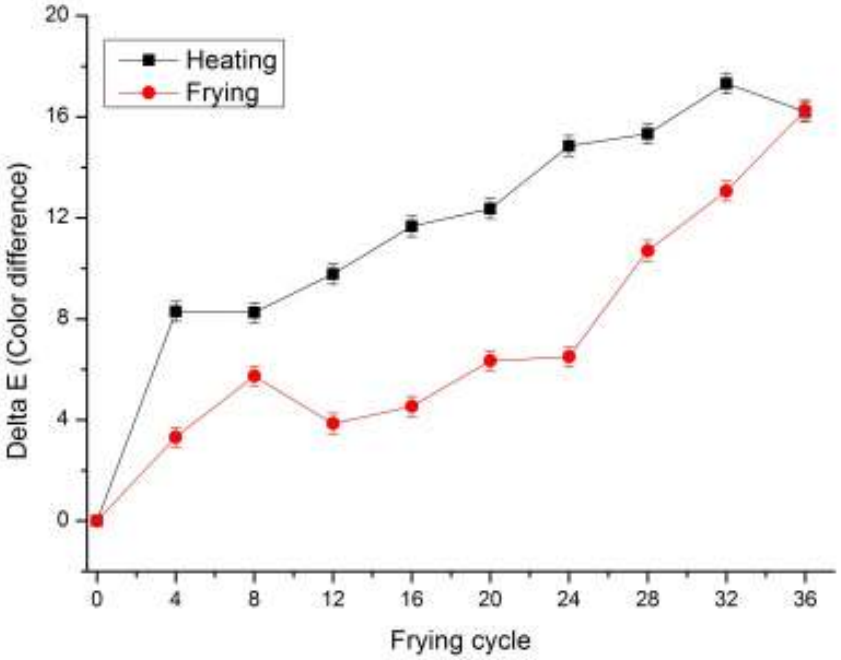


Figure 3.3 Degradation of palmolein color during intermittent frying process (a) lightness (b) redness (c) yellowness (d) color difference.

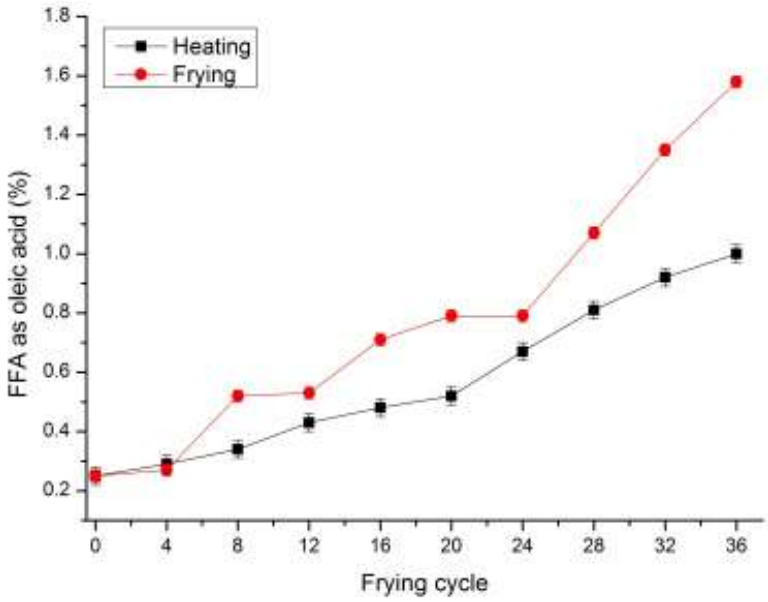
3.1.2 Degradation of soybean oil during intermittent frying process

Soybean oil is the largest consumed cooking oil across the world. Fresh soybean oil had a lower FFA (0.25 ± 0.03%) compared to fresh palmolein. Figure 3.4 (a), (b), (c), (d) showed the formation of FFA, PV, *p*AV and TPC in the soybean oil during heating and frying processes (Values are showed in supplementary information; Table 3.4S). During the heating, soybean oil showed lesser formation of FFA (1.00 ± 0.03%) compared to frying process (1.58 ± 0.02%). Soybean oil showed higher peroxide values during heating (from 3.30 ± 0.42 mEq/Kg to 14.84 ± 0.53 mEq/Kg) than frying process (from 3.34 ± 0.42 mEq/Kg to 24.60 ± 0.51 mEq/Kg). Primary oxidation of soybean oil is lower compared to that of palmolein. But, *p*AV of soybean oil (73.27 ± 1.5 after heating, 94.67 ± 1.6 after frying) clearly demonstrated that the secondary oxidation in soybean oil is much higher than palmolein. Higher unsaturation in soybean oil influences more secondary oxidation. This further leads to the development of rancidity and this undesirable rancid flavor makes soybean oil less acceptable to the food industry compared to vegetable oils [100]. Soybean oil with low linolenic acid content has been developed as an alternative for improved thermal stability towards the industrial frying purpose [67].

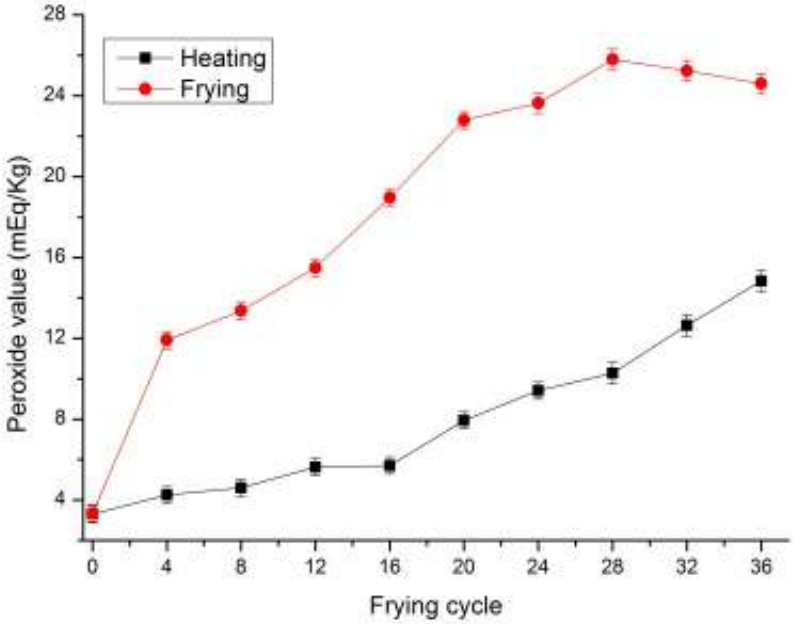
Total polar compound formation in soybean oil increased gradually throughout the frying process. Fresh soybean oil contains only 4.00 ± 0.25% TPC, which is lower than that of fresh palmolein. But, after 36 frying cycles, the formation of TPC in soybean oil (20.00 ±

0.50% by heating and 23.50 ± 0.50% by frying) is much higher than palmolein. Soybean oil with lower oxidative stability tends to form a higher percentage of polar compounds. Earlier reports suggested greater degradation of soybean oil during the intermittent process is influenced by the secondary oxidation reactions occur between two consecutive heating periods (in the form of peroxides and dissolved oxygen) and also higher polymerization that directly affect the rate of formation of TPC [181-182]. However, during the real time frying process, oxygen supply is rather limited by steam blanketing from the food, the polymerization reaction dominates oxidation [183].

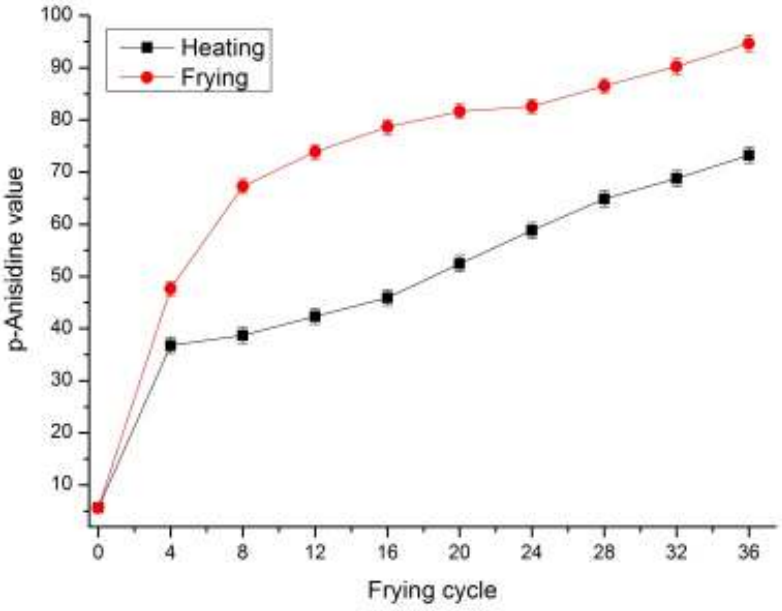
(a)



(b)


Heating
Frying
Peroxide value (mEq/Kg)
Frying cycle
0
4
8
12
16
20
24
28
32
36

(c)


Heating
Frying
p-Anisidine value
Frying cycle
10
20
30
40
50
60
70
80
90
100
0
4
8
12
16
20
24
28
32
36

(d)

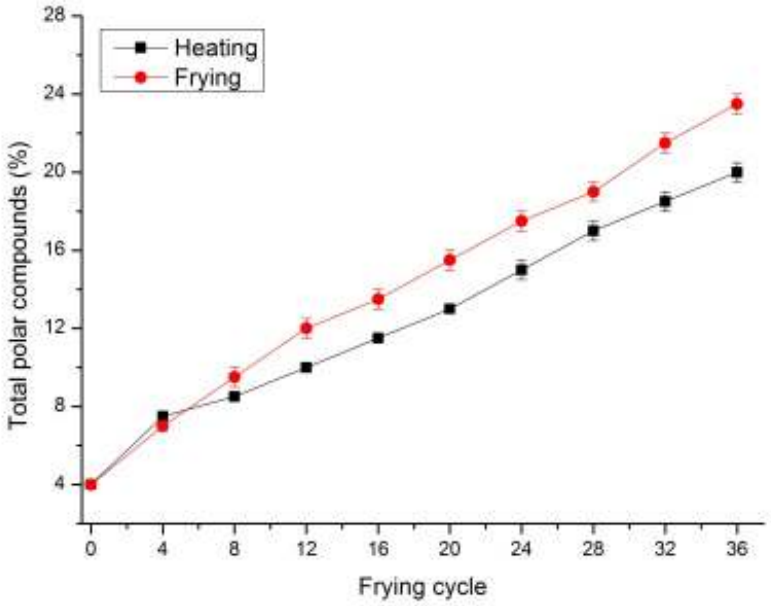


Figure 3.4 Quality parameters of soybean oil during intermittent frying process (a) FFA (b) PV (c) *p*AV (d) TPC.

Fatty acid degradation in soybean oil during intermittent frying was shown in Figure 3.5 (Values are showed in supplementary information; Table 3.5S). GC analysis showed that major fatty acids in the soybean oil are palmitic acid (16%), oleic acid (23%), linoleic acid (50%) and linolenic acid (4%). Higher content of MUFA (23.00 ± 0.3%) and PUFA (54.00 ± 0.4%) than SFA (21.69± 0.2%) have resulted in soybean oil more susceptible for degradation at frying temperature. But, GC results showed that there is no considerable change in the fatty acid content of soybean oil after frying process (26.92± 0.4% of SFA, 24.04± 0.2% of MUFA, 49.04± 0.5% of PUFA). These results once again explain that GC analysis of fatty acids is not a suitable method for studying the frying oil degradation as explained in the case of palmolein. Recent research suggests that deep fat frying promotes production of TFA

[184]. But, in our study it was observed that there was no formation of TFA during the heating as well as frying process of soybean oil. Recent research focused towards the hydrogenation of soybean oil to reduce the level of oleic acid and linolenic acids to make it less prone to oxidation during frying [185-187].

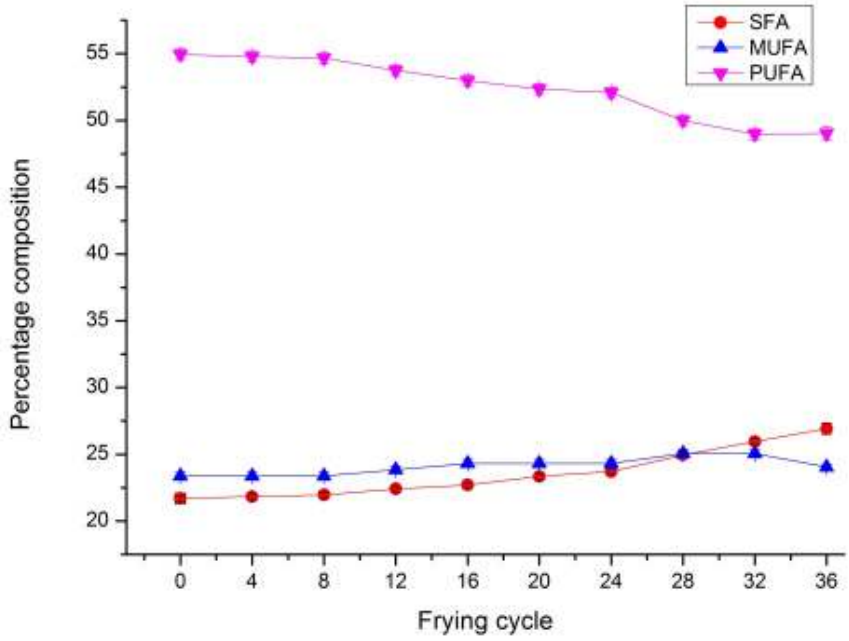
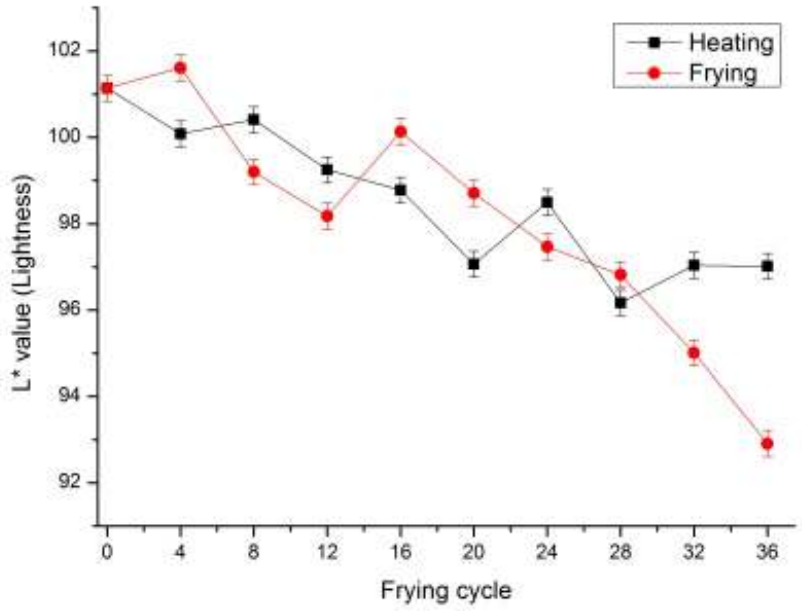


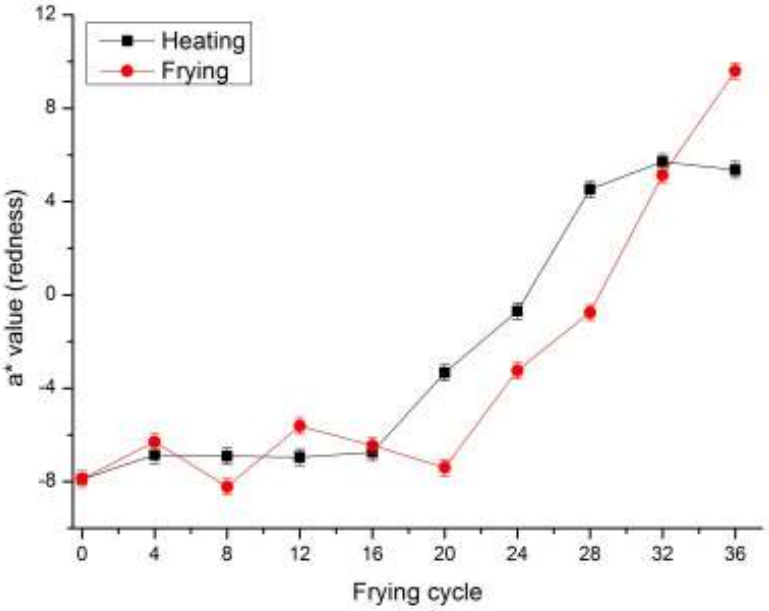
Figure 3.5 Fatty acid degradation in soybean oil during intermittent frying process.

Comparison of color degradation of soybean oil during the heating and frying process was shown in Figure 3.6 (a), (b), (c), (d) (Values are showed in supplementary information; Table 3.6S). Compared to palmolein, soybean oil showed lesser degradation with respect to color. This could be due to the higher initial color values of palmolein than that of soybean oil. Minimal change in lightness, yellowness and redness were observed even after last frying cycle.

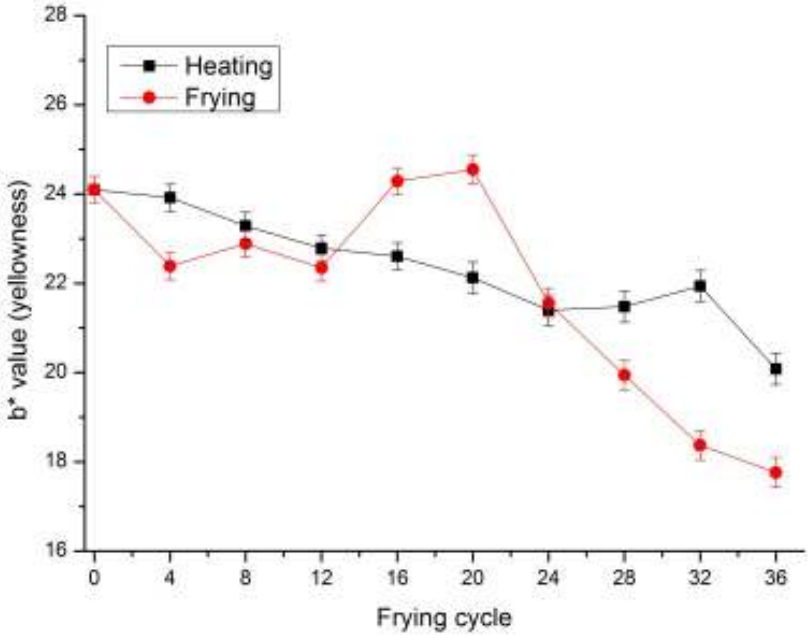
(a)


Heating
Frying
L* value (Lightness)
102
100
98
96
94
92
0
4
8
12
16
20
24
28
32
36
Frying cycle

(b)


Heating
Frying
a* value (redness)
12
8
4
0
-4
-8
0
4
8
12
16
20
24
28
32
36
Frying cycle

(c)



(d)

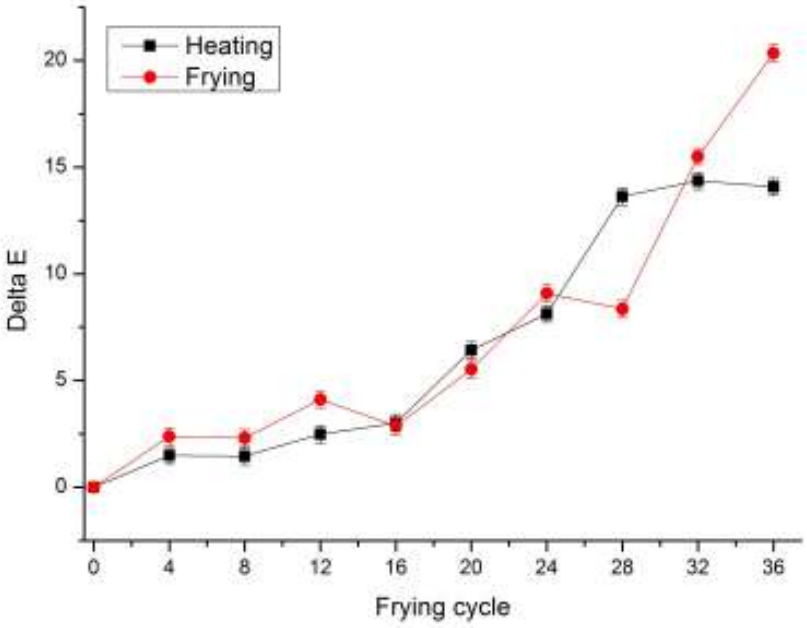


Figure 3.6 Degradation of soybean oil color during intermittent frying process (a) lightness (b) redness (c) yellowness (d) color difference.

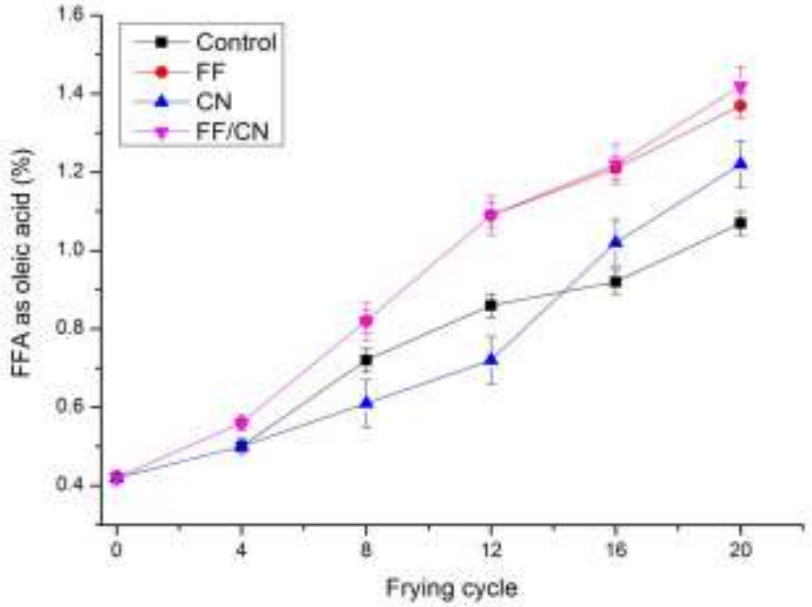
3.1.3 Degradation of palmolein during continuous frying process

Continuous frying process is a type of industrial frying process and mainly followed in commercial outlets where one or more food samples are subject to frying continuously. To mimic these conditions in this study, we performed multiple frying operations by using different food materials. French fries and chicken nuggets were fried separately and also in combination to explore the effect of food material on the frying oil deterioration. Blank heating oil without any food material was performed simultaneously as a control. Figure 3.7 (a), (b), (c), (d) showed the formation of FFA, PV, *p*AV and TPC in the palmolein during continuous frying processes (Values are showed in supplementary information; Table 3.7S). Fresh palmolein showed 0.42 ± 0.02% of FFA. After frying of French fries, it has reached to 1.37 ± 0.03% while after frying of chicken nuggets it was 1.22 ± 0.06%. After frying both foods in combination, it was recorded at 1.42 ± 0.05%. Moreover, the difference between FFA after frying and heating processes was very less. These results have shown that influence of food material on the frying oil hydrolysis was not significant. Higher FFA value of oil after potato strips frying than chicken nuggets was caused by higher moisture content of potato strips than chicken nuggets.

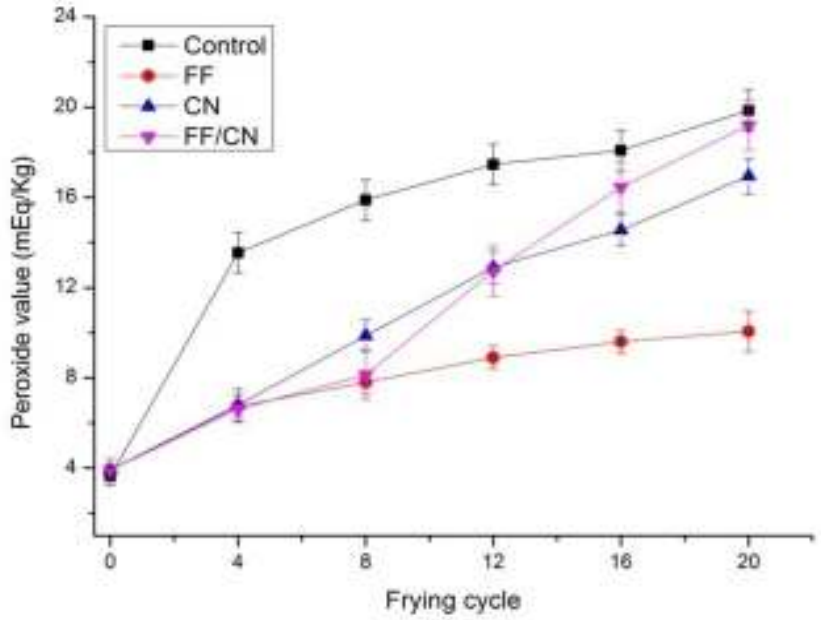
Peroxide values of palmolein after French fries (10.06 ± 0.9 mEq/Kg), chicken nuggets (16.94 ± 0.8 mEq/Kg) and in combination (19.2 ± 1.1 mEq/Kg) has varied with the control (19.8 ± 0.9 mEq/Kg). Control sample had higher peroxide value than that of the sample fried with combination of food materials due to higher secondary oxidation in the later. These results explain higher primary oxidation of palmolein during frying of chicken nuggets. On the other hand, *p*AV during both frying conditions (70.46 ± 1.40 for FF and 72.45 ± 1.34 for

CN) were similar. This could be caused by the presence of phospholipids in the chicken fat, which led to higher thermo-oxidation in the initial stages of frying. However, peroxide values of frying oil are not reliable as they degrade rapidly into secondary oxidation products as discussed earlier.

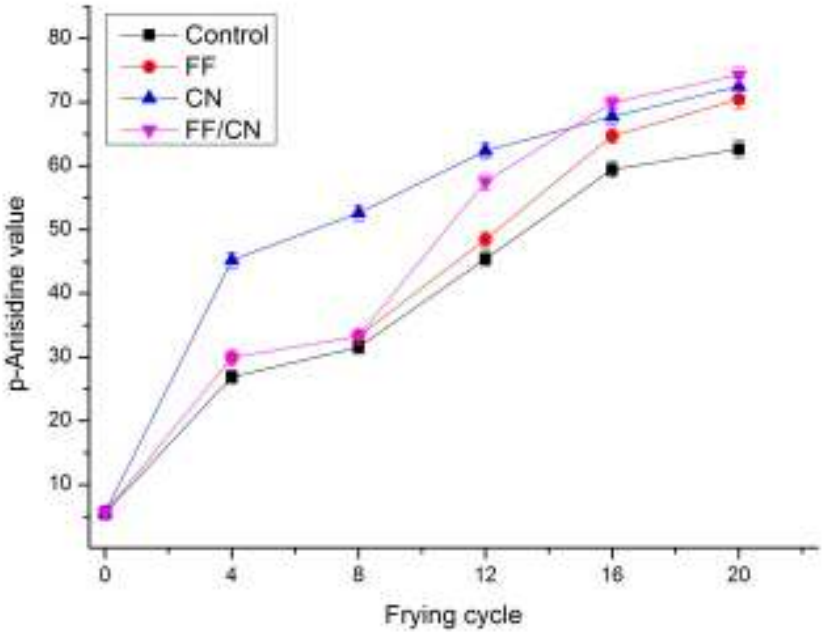
(a)



(b)



(c)



(d)

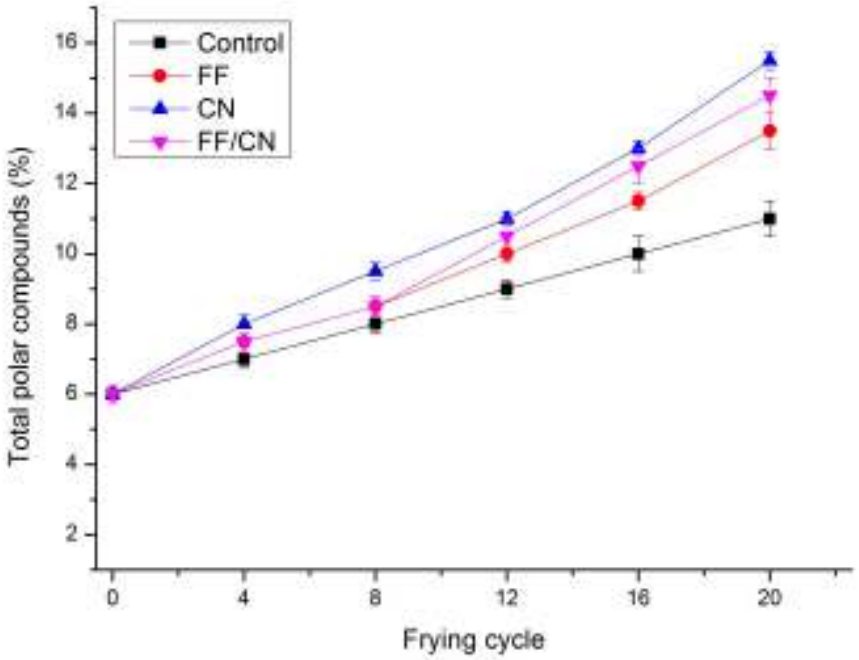


Figure 3.7 Quality parameters of palmolein during continuous frying process (a) FFA (b) PV (c) *p*AV (d) TPC.

Total polar compound formation in all models of frying using palmolein was similar. Frying of French fries resulted in 13.50 ± 0.5% while chicken nuggets resulted in 15.25± 0.25%. When French fries and chicken nuggets were cooked in combination, it was recorded at 14.50 ± 0.25%. Few earlier reports showed that chicken nuggets results in higher degree of degradation of palmolein compared to French fries [188-189]. But, on contrary to them, our results have shown that frying of single food item and different foods in combination exhibit only similar effect on the degradation of oil.

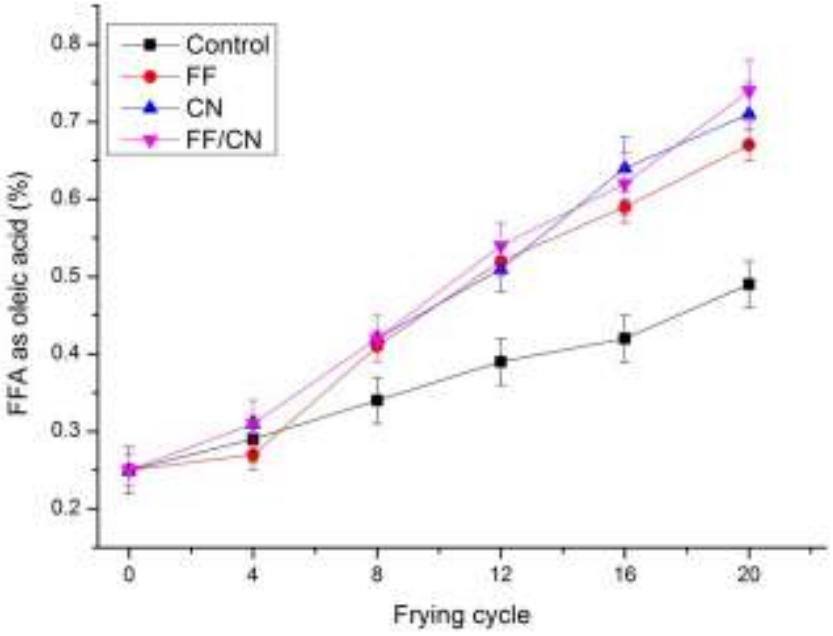
3.1.4 Degradation of soybean oil during continuous frying process

Degradation of soybean oil during continuous frying process was found to be similar to palmolein. Figure 3.8 (a), (b), (c), (d) showed the formation of FFA, PV, *p*AV and TPC in the soybean oil during continuous frying process (Values are showed in supplementary information; Table 3.8S). FFA content at the end of 20th frying cycle of French fries and chicken nuggets was determined as 0.67 ± 0.02% and 0.71 ± 0.04% respectively. After cooking both food materials in combination, FFA level reached to 0.74 ± 0.03%. These results once again showed the insignificant effect of different food materials on the frying oil hydrolysis. When moisture from food material is released into the oil, it is quickly evaporated out of the frying medium and there is too little time for moisture to react with the oil. This could be the reason for the insignificant effect of food material in different frying models on the formation of FFA [190].

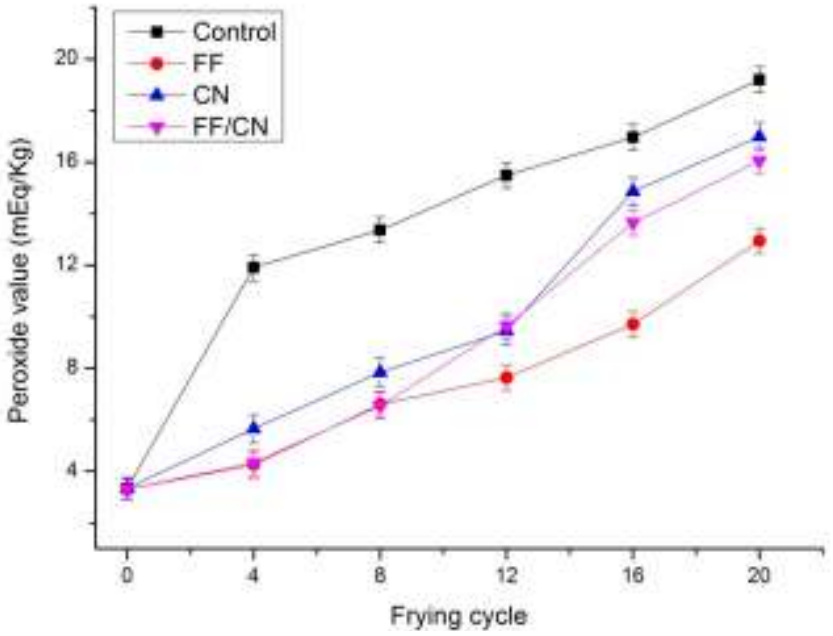
Peroxide values of soybean oil were once again found to be lower than the control group. Primary oxidation during frying of the French fries (12.94 ± 0.65 mEq/Kg) was

recorded lower than that of chicken nuggets (16.99 ± 0.55 mEq/Kg) and combinational frying (16.06 ± 0.50 mEq/Kg). However, the secondary oxidation of soybean oil was found to be similar in all the cases of frying (78.62 ± 1.40 of *p*AV after French fries, 81.30 ± 2.0 after chicken nuggets and 84.69 ± 1.80 after both foods in combination). Overall results convey that both hydrolysis and thermo-oxidation of frying oil is unaffected by the type of food material. Moreover, these results also showed that the possible influence of the initial quality of oils on their degradation during the frying process as discussed in the recent reports [191]. Similar results were obtained in the case of total polar compounds. After 20 frying cycles heated oil showed 12.00 ± 0.50% of polar compounds. After frying of French fries it reached to 14.00 ± 0.50%, after chicken nuggets it was 15.5 ± 0.50% and both items in combination it was reached 15.0 ± 0.50%. The natural presence of fat and phospholipids in the chicken nuggets compared to the French fries might have resulted in the difference in the polar compound formation [192]. It can be observed that polar compound formation in the palmolein and soybean oils at the end of 20th frying cycle is similar to that of same frying period in the intermittent frying process. Moreover, other quality parameters including fatty acid degradation and color degradation of both palmolein and soybean oil during continuous frying process are same as intermittent frying process.

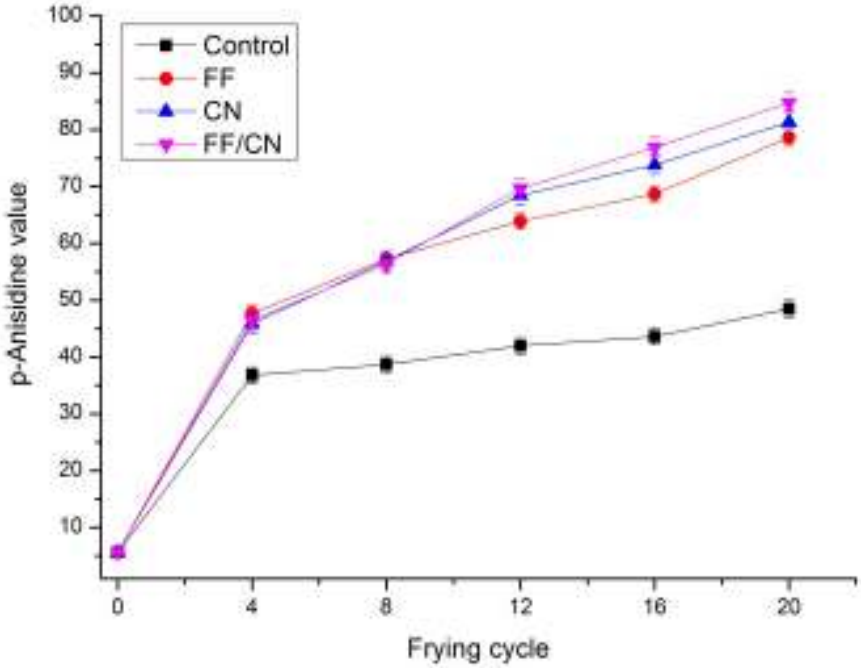
(a)


Control
FF
CN
FF/CN
FFA as oleic acid (%)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
4
8
12
16
20
Frying cycle

(b)


Control
FF
CN
FF/CN
Peroxide value (mEq/Kg)
4
8
12
16
20
0
4
8
12
16
20
Frying cycle

(c)



(d)

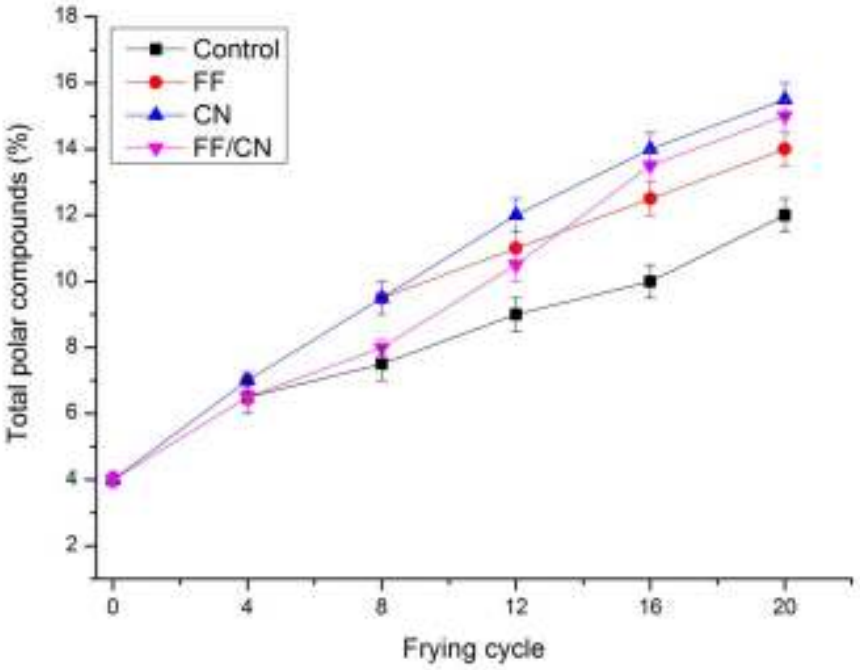


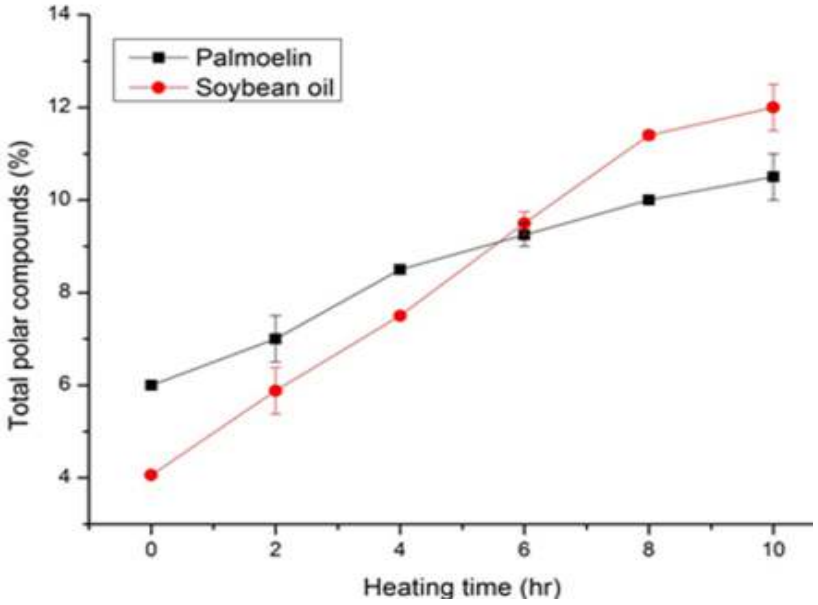
Figure 3.8 Quality parameters of soybean oil during continuous frying process (a) FFA (b) PV (c) *p*AV (d) TPC.

Interpretation and analysis of all these results lead to three significant observations. Firstly, there is no large difference in the degradation of oil during heating or frying process. Secondly, deterioration of oil is positively correlated with the number of frying cycles. Finally, the deterioration of any frying oil is significantly influenced by its nature but not by the type of food material subjected to frying. These observations are in agreement with earlier reports suggesting that oil type, oil quality, and the frying procedure have a significant impact on the fried potatoes [193].

3.2 MALDI-TOF/MS Analysis of Thermal Degradation products of Edible oils during the Deep Fat Frying Process

Primarily, in this study, we observed that the quantity of the polar fraction was increased throughout the heating/frying process. After 10 hrs of heating process, palmolein (from 6% to 10.5%) and soybean oils (from 4% to 12%) showed a gradual increase in the polar compound formation (Figure3.9a). After 10 hrs of frying process, 20 frying cycles of potato fries, palmolein (from 6 % to 14 %) and soybean oils (from 4 % to 15.5 %) showed increase in polar compounds in large extent which is higher than that of heating (Figure 3.9b). From these results, it can be observed that polar compounds are degraded products of non-polar products. Several research papers discussed 2, 5 di-hydroxy benzoic acid (DHB) matrix solution for analysis of lipids through MALDI. In this study, we performed an analysis on both CHCA and DHB and found better results with CHCA in the detection of polymeric triacylglycerols (PTAG) over DHB. Earlier reports [145-146] discussed the difficulty in detection of PTAG by direct MALDI-TOF/MS and we continued further studies by using CHCA matrix in linear mode of detection. Ionization of crude oils, polar and non-polar fractions by MALDI produces both protonated species, $[M + H]^+$ and alkali metal adducts such as $[M + Na]^+$. Further, characterization of non-polar compounds, including TAG, polar compounds, including diacylglycerols (DAG), oxidized triacylglycerols (OTAG), dimeric triacylglycerols (DTAG) and PTAG were structurally elucidated based on their *m/z* values.

(a)



(b)

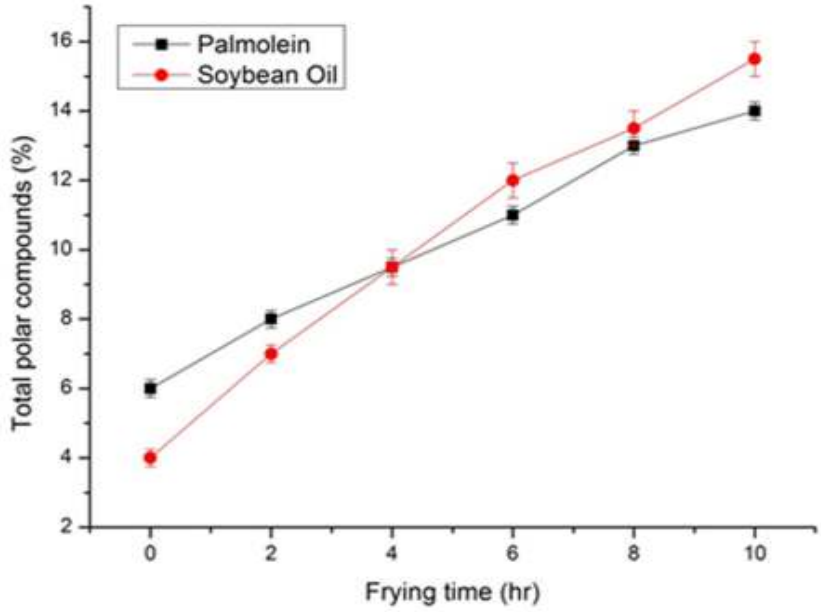


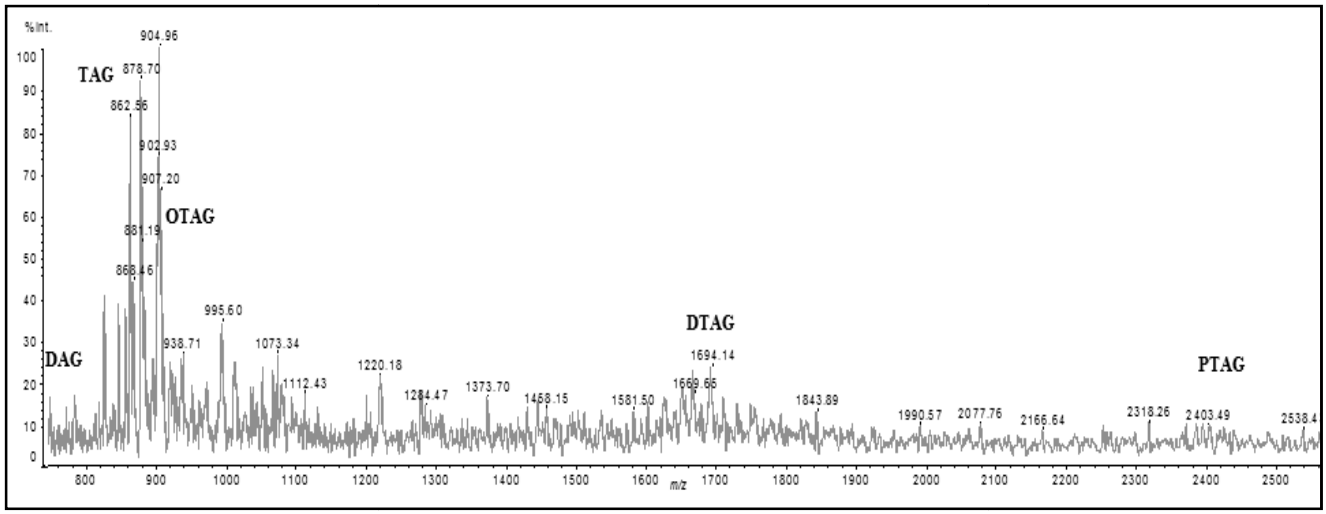
Figure 3.9 Total polar compounds formation in palmolein and soybean oils (a) during heating (b) during frying.

3.2.1 Thermal degradation of palmolein

Table 3.1 (a) and (b) showed the different TAG, DAG, OTAG, DTAG and PTAG detected in palmoelin during frying process. Earlier reports suggested that the major TAG in palmolein composed of POP (42.8%), POO (30.4%), PLO (12.6%), PLP (10.7%) and OOO (8%) [194]. In the present study the significant TAG identified was POP (855.18 *m/z*), POO (857.01 *m/z*) and PLO (856.73 *m/z*) which is in agreement with earlier studies. Palmolein with 50% palmitic acid, 37% oleic acid and 9% linoleic acid (analyzed by gas chromatography) mostly composed of PLO and POP. During earlier stages of frying/heating some TAG were identified with an *m/z* difference of one less to parent TAG, and these were TAG radicals, which were originated before peroxidation. However, non-polar fractions of unused oil showed accurate *m/z* of TAG corresponding to their standards.

Figure 3.10 (a) showed the formation of different compounds including DAG, OTAG, PTAG and *β*-scission compounds during frying. Figure 3.10 (b) showed the spectra of the polar fraction isolated from fried oil (illustrated figures are that of palmolein). In the case of frying of palmolein, no formation of OTAG was found till 2 hrs of frying, but at 4th hr (8th frying cycle) sodium adducts of mono-oxygenated fragments of PLO (895.76 *m/z*) and POP (910.32 *m/z*) were found. By the 6th hour, (12th frying cycle), di-oxygenated fragment of PLO (911.64 *m/z*) was found. Similarly, peroxides of OLL, OOL and LLL were found in the frying process. After few frying cycles, percentage abundance of OTAG tended to have decreased due to formation of *β*-scission fragments of hydroperoxides.

(a)



(b)

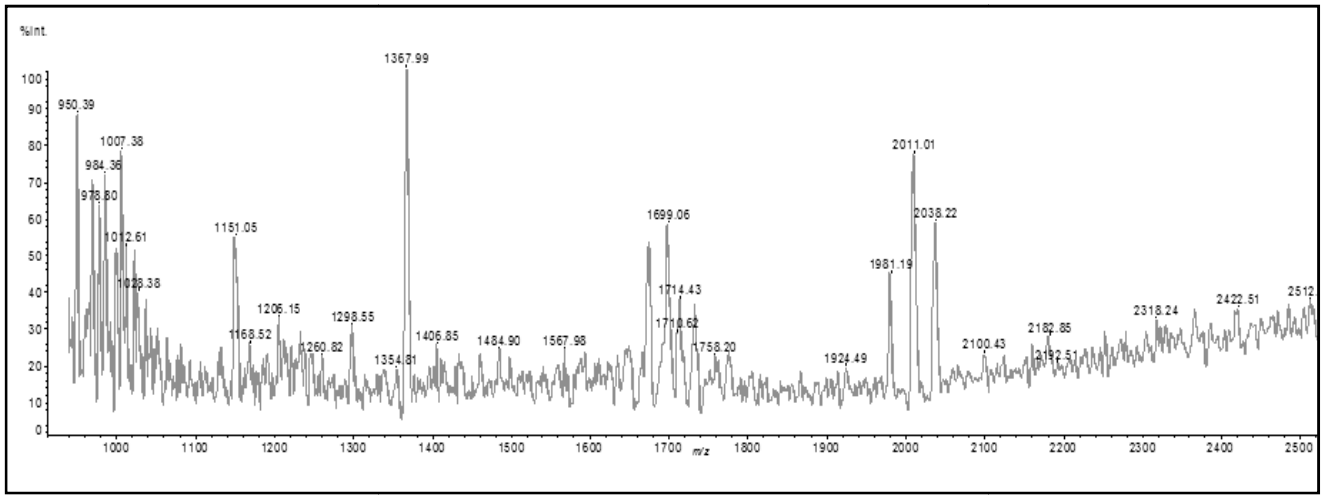


Figure 3.10 MALDI-TOF/MS spectra (a) thermally stressed palmolein (b) polar fraction of fried palmolein.

Table 3.1 (a) Major compounds identified in the non-polar fraction of fried palmolein.

m/z	TAG
807.76	$C_{48:0}$ (PPP)
852.04	$C_{52:5}$ (PLLn)
855.18	$C_{50:1}$ (POP)Na^+
856.73	$C_{52:3}$ (PLO)
857.01	$C_{52:2}$ (POO)
872.71	$C_{54:9}$ (LnLnLn)
878.53	$C_{54:6}$ (LLL)
880.87	$C_{54:5}$ (LLO)
881.88	$C_{52:2}$ (POO)Na^+
883.73	$C_{54:4}$ (LOO)
892.56	$C_{54:0}$ (SSS)
908.88	$C_{54:3}$ (OOO)Na^+
941.43	$C_{54:2}$ (OSO)Na^+

(b) Major compounds identified in the polar fraction of fried palmolein.

m/z	DAG
568.96	$C_{32:0}$ (PP)
594.90	$C_{34:1}$(PO)
613.33	$C_{34:3}$(PLn) Na^+
615.63	$C_{34:2}$(PL)Na^+
618.68	$C_{34:1}$(PO)Na^+ / $C_{36:3}$(LO)
644.23	$C_{36:2}$(OO)Na^+
664.40	$C_{36:2}$(LL)2Na^+
667.22	$C_{36:2}$(OO)2Na^+

m/z	OxTAG
872.75	$C_{52:3}$ (PLO)+^{16}O
863.02	$C_{50:2}$(PLP)+2 ^{16}O
894.72	$C_{52:4}$ ((PLL)+^{16}O) Na^+
895.64	$C_{54:6}$ (LLL)+^{16}O
913.59	$C_{52:2}$ ((POO)+2 ^{16}O) Na^+
918.57	$C_{54:6}$ ((LLL) +^{16}O) Na^+
924.57	$C_{54:3}$ (OOO) +^{16}O
930.09	$C_{52:2}$ ((POO)+ 3^{16}O) Na^+

m/z	TAG dimers
1713.47	C_{50} - C_{52}(PLP-POO)
1714.91	C_{52} - C_{52}(POO-PLO)
1715.85	C_{50} - C_{52}(PLP-POO) +^{16}O
1732.83	C_{50} - C_{54}(POP-OOO) +^{16}O
1735.00	C_{54} - C_{52}(OOL-PLO)
1739.44	C_{52} - C_{52}(POO-POO)Na^+
1745.45	C_{52} - C_{52}(PLO-PLO) +2 ^{16}O
1748.38	C_{52} - C_{52}(POO-POO) +2 ^{16}O
1754.53	C_{52} - C_{52}(POO-POO) +3 ^{16}O
1766.88	C_{54} - C_{54} (OOO-OOO)
1788.75	C_{52} - C_{54}(POO-OOO) +3 ^{16}O
1814.50	C_{54} - C_{54}(OOO-OOO) +3 ^{16}O

m/z	TAG Trimers/Polymers
2485.96	C_{50} - C_{50} - C_{50}(PLP-PLP-PLP)
2564.84	C_{52} - C_{52} - C_{52}(PLO-PLO-PLO)
2668.44	C_{54} - C_{54}- C_{54} (OOO-OOO-OOO)+^{16}O
2788.22	C_{54} - C_{54}- C_{54} (OOO-OOO-OOO)+9 ^{16}O
3549.68	C_{54} - C_{54}- C_{54} - C_{54}(OOO-OOO-OOO-OOO)+ ^{16}O
3630.39	C_{54} - C_{54}- C_{54} - C_{54}(OOO-OOO-OOO-OOO)+6 ^{16}O

These results demonstrate that the rate of degradation of oil during first few frying cycles is lower. In the case of the heating process, break down products of TAG including DAG and MAG were identified. For instance, 2 hrs heated palmolein was abundant in sodium adducts of POP (856.86 *m/z*) and OOO (908.84 *m/z*), while, 8 hrs heated palmolein was rich in PO fragment (618.42 *m/z*) and OO fragment (644.56 *m/z*), which arose from hydrolysis of POP and OOO respectively. Other DAG found in the heated/fried palmolein includes PP (568.96 *m/z*), PL (615.63 *m/z*) and PLn (613.33 *m/z*) fragments. Percentage abundance of intensity of oxidation products was higher during heating compared to the frying process. Usually, in frying process oxidation was not higher because of the steam blanket on the surface of frying oil which retards oxidation to a greater extent [25]. However, most of the peaks are common in heated and fried oils with difference in relative abundance. Overall results demonstrate that

mechanism of degradation during heating and frying processes is similar except variation in the percentage abundance of the fragments.

To understand the formation of OTAG, it is needed to discuss the mechanism of lipid oxidation in detail. Frankel (1980) and Choe *et al*. (2006) profoundly discussed the pathway of fatty acid degradation in edible oils and highlighted that fatty acid composition is a major factor that affects lipid oxidation [195-196]. Basically, the rate of oxidation depends on the percentage of unsaturation in oil. So, soybean oil with 50% linoleic acid and 23% oleic acid is prone to faster degradation compared to palmolein with 37% oleic acid and 9% linoleic acid. Fatty acid oxidation on TAG proceeds with hydroperoxides formation which further undergoes β-scission fragmentation and eventually leads to the formation of different volatile and non-volatile compounds. These hydroperoxides and glycerol bound aldehydes are biologically active, which play an important role in the atherogenesis [197-198]. The formation of these hydroperoxides during lipid oxidation varies with the position of the double bond. It was reported that the relative amounts of 26-28 % each of C8 and C11 hydroperoxides can be seen in oleic acid (C18:1) oxidation [196]. In this study, glycerol bound fatty aldehydes from PLnO (729.59 *m/z*), PLO (731.55 *m/z*), POO (733.01 *m/z*) and OOO (803.11 *m/z*) triglycerides (through breakdown of 8-hydroperoxide and also through 10-hydroperoxide on oleic acid group) were detected with higher intensity in the range of 720 - 840 *m/z*. In addition to glycerol bound aldehydes, glycerol bound hydrocarbons were also found in frying process in the range of 770 - 780 *m/z*. These are all minor degradation products that impart odor and flavor to frying oil and fried food products.

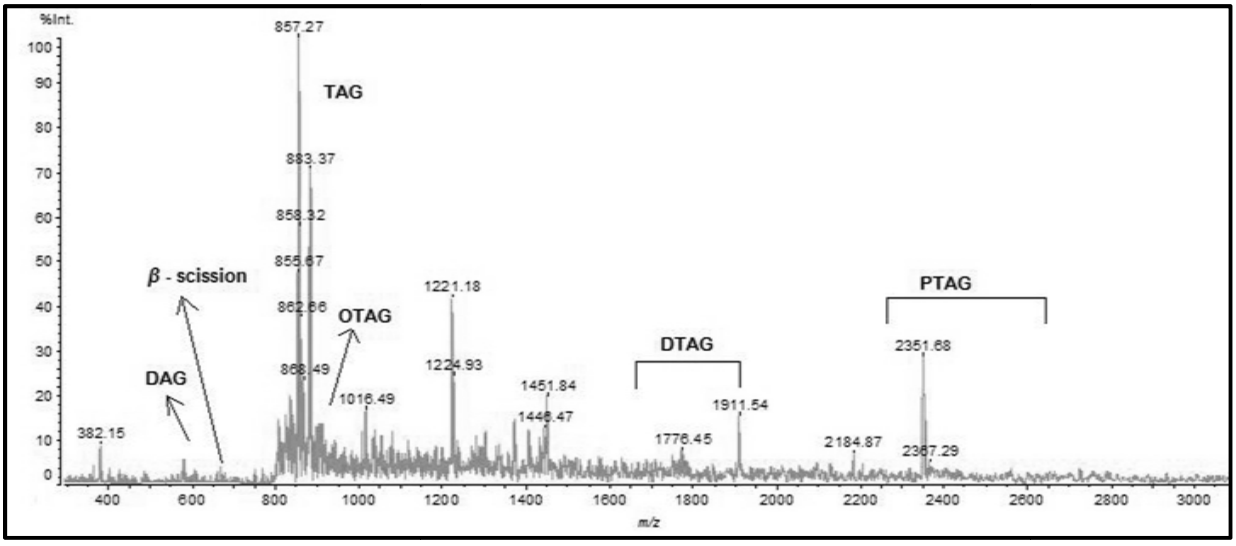
DTAG can be mainly differentiated into polar and non-polar DTAG based on the presence of the oxygen group (C-C or C-O-C/C-O-O-C). Non-polar dimers of OOO (1766.88 *m/z*) and POO (1739.44 *m/z*), and polar dimers of tri-oxygenated OOO (1814.50 *m/z*) and POO (1754.53 *m/z*) were identified as major dimers in the dimeric region of heated palmolein spectra. The primary PTAG identified in heated/fried palmolein are trimers of PLP, PLO, OOO and also tetramer of oxygenated triolein. We observed that the majority of polar and non-polar polymers were retained in the non-polar fraction due to their high molecular weight and non-polar nature, which is in agreement with previous findings,. These results clearly suggest that studying direct oil by MALDI without fractionation gives more information about identification of PTAG formed during thermal processing. Most of the PTAG are oxygenated and they are in abundance in heated palmolein compared to that of fried palmolein. Heating differs from the frying process due to the absence of food material with moisture and also agitation. This may result in more polymerization during heating compared to the frying process. These PTAG accelerates degradation of the oil by increasing the oil viscosity, foaming and reduce the heat transfer, which eventually leads to higher oil absorption of foods [25]. Patrikios *et al.* (2014) discussed the formation of oligomers of oleic acid during thermo-oxidation of virgin olive oil through free radical polymerization, and those oligomers were found to be the potential hemagglutinins [199]. These results clearly demonstrate that the formation of these oligomers/polymers not only degrades the quality of frying oil but also the nutritional quality of food products.

3.2.2 Thermal degradation of soybean oil

Table 3.2 (a) and (b) showed the different TAG, DAG, OTAG, DTAG and PTAG detected in soybean oil during heating/frying. Earlier reports [194] suggested that major TAGs in soybean oil are OLL (23.4%), LLL (22.6%), PLL (17.0%), OOL (12.7%), OLLn (8.3%) and OOO (4.9%). In our study, we observed that soybean oil with 50% linoleic acid, 23% oleic acid, 16% palmitic acid, 4% linolenic acid (analyzed by gas chromatography) mainly composed of OLL (880.40 *m/z*), OOL (905.71 *m/z*), OLLn (879.45 *m/z*) and PLO (856.71 *m/z*). Peaks correspond to LLL (878.29 *m/z*) and LnLnLn (895.39 *m/z*) were also found with lower percentage abundance compared to other TAGs. Similarly, Major DAG identified in this study are LnLn (612.54 *m/z*), LL (616.74 *m/z*), LO (618.22 *m/z*) and OO (621.97 *m/z*) (Figure 3.10 c, d).

Figure 3.11 showed the MALDI-TOF/MS spectra of soybean oil at different frying intervals. After 2 hr frying, hydroperoxides of OLL (935.11 *m/z*) were detected. By the end of 4 hr frying period mono-oxygenated OLL (920.31 *m/z*) was identified, that were formed by the breakdown of the peroxide. Similarly, hydroperoxides of OOL (937.49 *m/z*) and OOO (939.22 *m/z*) were identified from spectral data of 2 hr heated soybean oil and their degradation products were identified in further frying cycles. In this study, we observed that the intensity of OTAG fragments was higher during heating/frying of soybean oil compared to palmolein due to its high unsaturation (mainly through linoleic acid). Dissolved oxygen is another significant factor that influences the rate of oxidation in frying. Rate of diffusion of atmospheric oxygen into oil at frying temperature is lower than that at room temperature. So, at early stages of frying process OTAG formation completely depends on dissolved oxygen,

(c)



(d)

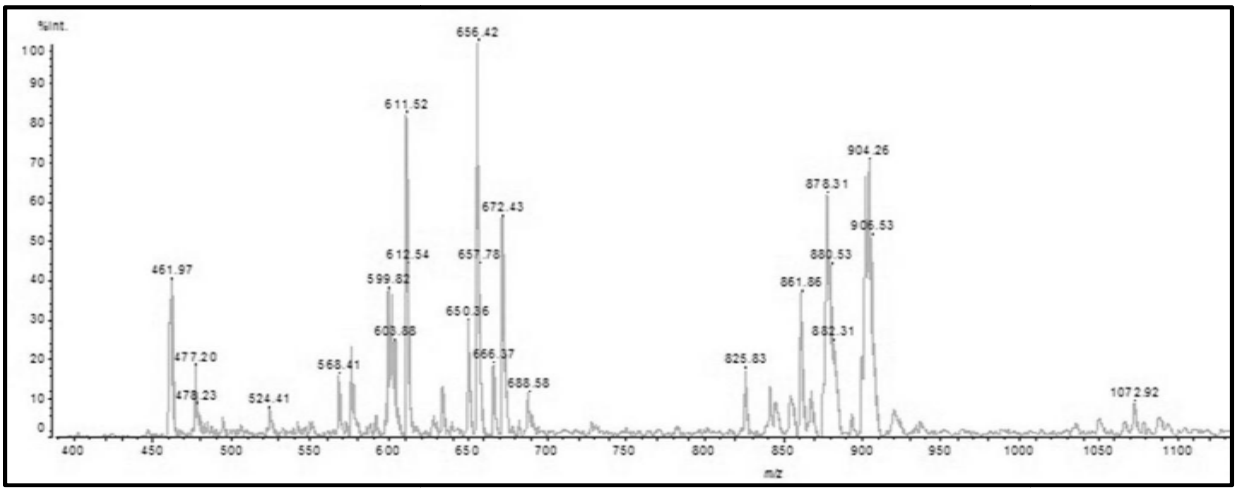


Figure 3.10 MALDI-TOF/MS spectra (c) thermally stressed soybean oil (d) polar fraction of fried soybean oil.

We analyzed many samples and taken appropriate spectra. But, in the thesis we mentioned the spectra for clear understanding and the ranges of DTAG and PTAG can be seen the enlarged versions of different spectra and the data provided in table 3.2 was based on the repetitions of respective compounds those are analyzed from various spectra.

but in the later stages it is influenced by DTAG and PTAG formation. These OTAG are more polar and easily absorbed in the surface of frying food and consumption of these OTAG through frying oils or fried food products show undesirable biological effects, including influencing of peroxisome proliferator-activated receptors (PPAR) further to lipid metabolism to a larger extent [200-201].

Table 3.2 (a) Major compounds identified in the non-polar fraction of fried soybean oil.

m/z	**TAG**
829.92	$C_{48:0}$(PPP)Na^+
834.62	$C_{50:0}$ (PPS)
852.13	$C_{52:5}$ (PLLn)
855.18	$C_{50:1}$ (POP)Na^+
857.01	$C_{52:2}$ (POO)
856.71	$C_{50:3}$ (PLO)
878.29	$C_{54:6}$ (LLL)
879.45	$C_{54:6}$ (OLLn)
880.40	$C_{54:5}$ (OLL)
882.31	$C_{54:5}$ (OOL)
895.39	$C_{54:9}$ (LnLnLn)Na^+
904.26	$C_{54:4}$ (OLL)Na^+

(b) Major compounds identified in the polar fraction of fried soybean oil.

m/z	**DAG**
568.41	$C_{32:0}$ (PP)
592.33	$C_{34:2}$(PL)
594.90	$C_{34:1}$(PO)
612.54	$C_{36:6}$(LnLn)
616.74	$C_{36:4}$(LL)
618.22	$C_{34:1}$(PO)Na^+ / $C_{36:3}$(LO)
621.97	$C_{36:2}$(OO)
623.02	$C_{36:1}$(OS)

m/z	**OxTAG**
870.72	$C_{54:3}$ [(PLP) + ^{16}O] Na^+
897.89	$C_{54:3}$ [(POO) + ^{16}O] Na^+
923.37	$C_{54:3}$ [(OOO) + ^{16}O] Na^+
937.49	$C_{54:4}$ [(OOL) + 2 ^{16}O] Na^+
940.73	$C_{54:3}$ [(OOO) + 2 ^{16}O] Na^+
955.25	$C_{54:3}$ [(OOO) + 3 ^{16}O] Na^+
966.79	$C_{54:6}$ [(LLL) + 4 ^{16}O] Na^+
970.27	$C_{54:4}$ [(OOL) + 5 ^{16}O] Na^+

m/z	**TAG dimers**
1733.65	C_{52} - C_{52}(POO-POO)+ ^{16}O
1736.96	C_{52} - C_{52}(PLO-POO)Na^+
1760.26	C_{50} - C_{52}[(PLL-PLL) +2 ^{16}O] Na^+
1766.75	C_{54} - C_{54}(OOO-OLL)
1783.49	C_{54} - C_{54}(OOO-OOO)+ ^{16}O
1795.91	C_{54} - C_{54}(OOL-OOL) + 2 ^{16}O
1798.07	C_{54} - C_{54}[(OLL-OLL) +^{16}O] Na^+
1808.64	C_{54} - C_{54}[(LLL-LLL) +2 ^{16}O] Na^+
1814.28	C_{54} - C_{54}[(OLL-OLL) +2 ^{16}O] Na^+
1820.41	C_{54} - C_{54}[(OOO-OOO) +2^{16}O] Na^+
1821.29	C_{54} - C_{54}(LLLn-LLLn) +3 ^{16}O
1830.35	C_{54} - C_{54}[(OLL-OLL) + 3 ^{16}O] Na^+

m/z	**TAG Trimers/Polymers**
2576.55	C_{52} - C_{52}- C_{52} (PLO-PLO-PLO) ^{16}O
2632.22	C_{54} - C_{54}- C_{54} (LLL-LLL-LLL)
2641.09	C_{54} - C_{54}- C_{54} (OOL-OOL-OOL)
2652.34	C_{54} - C_{54}- C_{54} (OOO-OOO-OOO)
2668.44	C_{54} - C_{54}- C_{54} (OOO-OOO-OOO)+^{16}O
2683.65	C_{54} - C_{54}- C_{54} (OOO-OOO-OOO)+2 ^{16}O
2700.09	C_{54} - C_{54}- C_{54} (OOO-OOO-OOO)+3 ^{16}O
3464.14	C_{52} - C_{52}- C_{52}- C_{52} (PLO-PLO-PLO-PLO)+2 ^{16}O
3518.35	C_{54} - C_{54}- C_{54} - C_{54} (OLL-OLL-OLL-OLL)
3581.68	C_{54} - C_{54}- C_{54} - C_{54} (OOO-OOO-OOO-OOO)+3 ^{16}O

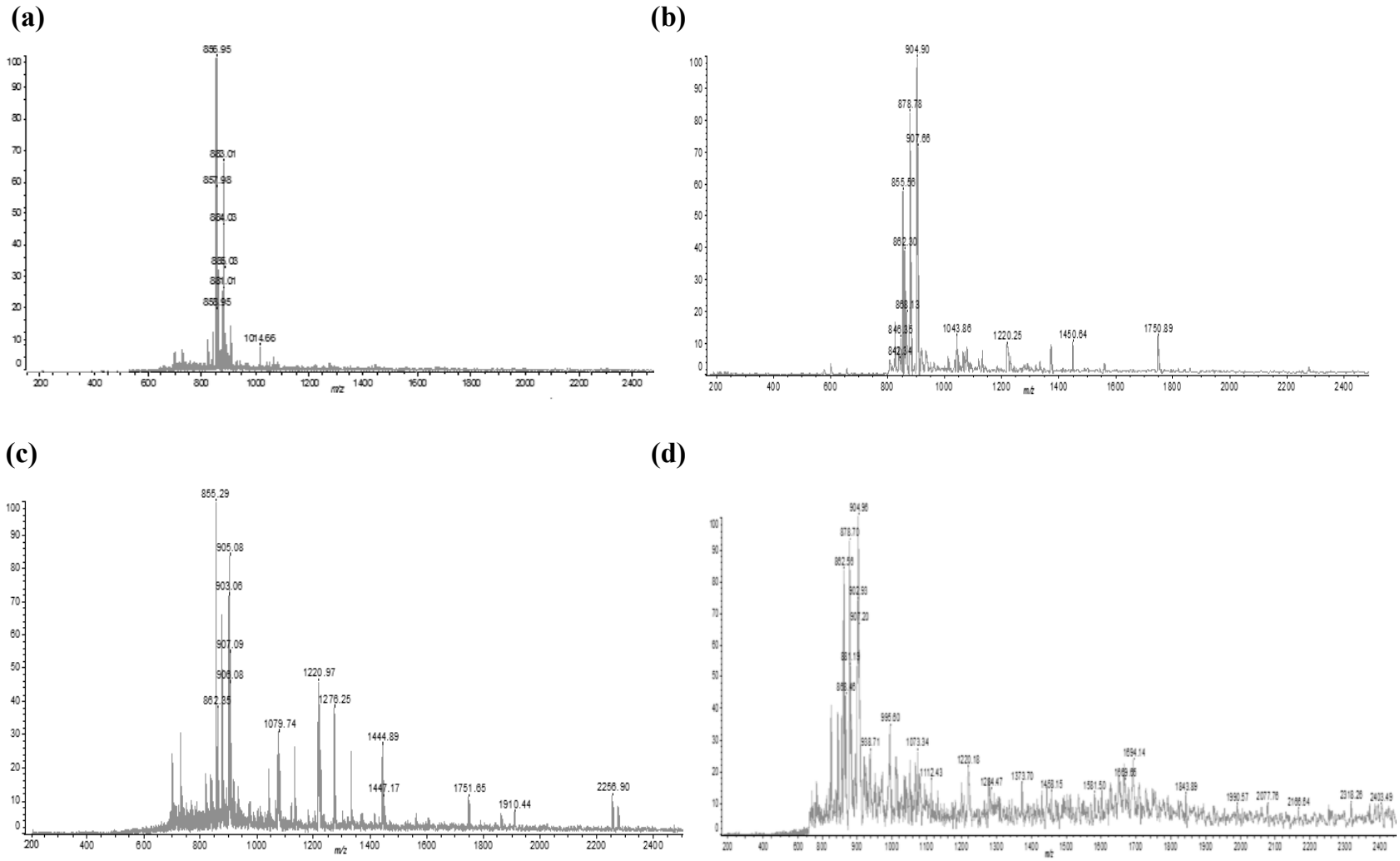


Figure 3.11 MALDI–TOF/MS spectra of soybean oil at different frying intervals (a) 0hr (b) 4hr (c) 8hr (d) 10hr.

In soybean oil frying, minor tri-unsaturated TAG such as LLL, LnLnLn, and OLLn were affected by oxidation than other TAG, through epoxidation and hydroperoxidation. Linoleic acid (C18:2) tends to form C9 and C13 hydroperoxides to the extent of 48-53% while, linolenic acid (C18:3) tends to form C9 and C13 hydroperoxides to the extent of 28-35% [202].So, the probability of forming *β*-scission fragmentation fragments in soybean oil is higher than palmolein. In line with that, several *β*-scission products were identified in the thermally stressed soybean oil in the range of 700 – 850 *m/z* which originated from the breakdown of oleic, linoleic and linolenic acids. Earlier reports explained the presence of 13-hydroxy octadecadienoic acid atherosclerotic lesions and the importance of both 9- and 13-hydroperoxides in cellular lipid peroxidation [203-204]. Prior works [145] discussed about the formation of covalent adducts of the TAG with fatty acids and also with *β*-scission products. Scanning the spectra between 1000 – 1200 *m/z* corresponding to the mass range of those newly formed products. In our study, covalent adduct of OOO with oleic acid (1167.27 *m/z*) and adduct of OOO with undecenal (1054.02 *m/z*) are detected with significant intensity and scarcity of much information and analytical standards are hurdles to identify this kind of newly formed compounds fully.

In the frying process, soybean oil tends toward higher dimerization and polymerization compared to palmolein. Both homo dimers and heterodimers of DTAG were elucidated in thermally stressed soybean oil. Among these, oxidized dimers of OLL (1798.07 *m/z*), OOL (1795.91 *m/z*) and OOO (1783.49 *m/z*) were identified as major compounds during the progression of frying time. There is not much information available regarding dimers of

DAG. But, a prominent peak of 1222.15 *m/z* with 25% intensity was identified in 4 hrs heated soybean oil. By considering the DAG of di-linolenin (LnLn at 612.54), this peak was assigned to be as dimer of di-linolenin. Unlike palmolein, soybean oil showed higher fragments of PTAG during heating and frying. Basically, OTAG and DTAG are intermediates in the formation of PTAG. So, higher unsaturation of soybean oil caused more polymerization than palmolein. Bastida *et al*. (2002) found that the polymerization was greater in more unsaturated oils and showed that higher oligomeric content in olive oil than sunflower oil during frying [205]. In this study, several trimers and tetramers of triolein (OOO) with and without additional oxygen containing functional groups were identified in thermally stressed soybean oil. Few reports published on identification of ammonium adduct of trimers and tetramers of triolein through Liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS) [43] and through electrospray ionization Fourier transform cyclotron resonance mass spectrometry (ESI-FTICR-MS) [206]. Other major PTAG includes trimers of PLO, OLL, and OOL. Interestingly, in this study, we observed that OOO tends more to polymerization than oxidation and breakdown. Most probably, this is because of lower content of OOO compared to other triglycerides and effect of anti-oxidants from unsaponifable matter.

3.3 Effect of antioxidant enrichment, phenolic compounds and blending on the thermal stability of frying oils

3.3.1 Effect of antioxidants on frying stability of edible oils

The effect of several natural and synthetic antioxidants, to retard the formation of polar compounds and thermo-oxidation during prolonged frying conditions, was studied. All the antioxidants were tested at the concentration of 0.02% in refined soybean oil and refined palmolein without addition of any other additives including antioxidants. Majority of the antioxidants utilized in this study were food grade, and they were listed out in the table 3.3.

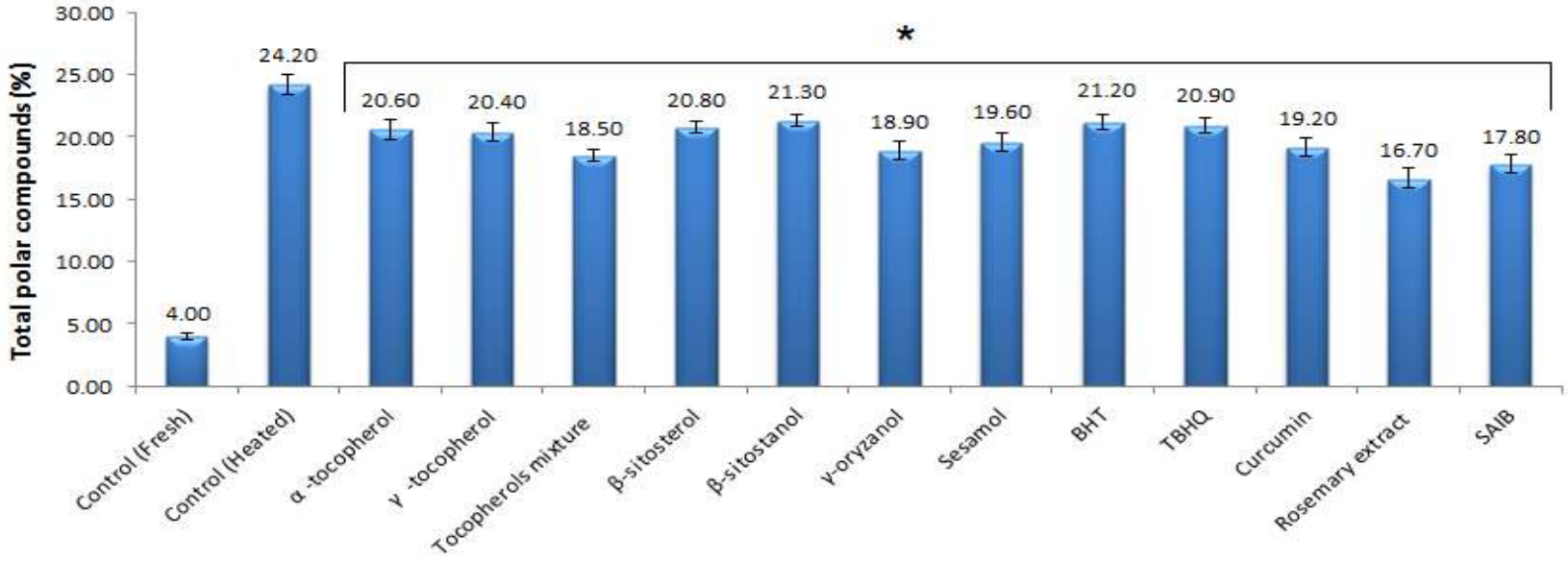
Table 3.3 List of antioxidants tested for the frying stability.

S. No	Test sample (Conc. = 200 ppm)	E number*
1	α -tocopherol	E307
2	γ -tocopherol	E308
3	Tocopherols mixture (α, β, γ and δ)	E306
4	β-sitosterol	E499
5	β-sitostanol	NA
6	γ-oryzanol	NA
7	Sesamol	NA
8	Butylated hydroxy toluene (BHT)	E321
9	*ter*-Butyl hydroxyquinone (TBHQ)	E319
10	Curcumin	E100
11	Rosemary extract	E392
12	Sucrose acetate isobutyrate (SAIB)	E444

*E numbers are codes for substances which can be used as food additives for use within the European Union

3.3.1.1 Effect of antioxidants on polar compound formation in the soybean oil

Figure 3.12 showed the effect of various antioxidants on the formation of polar compounds in the refined soybean oil at frying temperature after 2 hr. In this study, steel vessels (30 ml capacity) and heated on a hot plate assembled with a temperature sensor (IKA, India) at 180^{o}C for 2 hrs by maintaining exact frying temperature throughout the study to mimic the frying pan composition. In addition, the temperature at different parts of the oil was monitored through the temperature reader and also occasionally stirred to maintain almost uniform temperature with little variance. Fresh soybean oil showed 4.00 ± 0.25% polar compounds while heated oil showed 24.20 ± 0.75%, which is closer to the limit (25%) of deterioration for frying oils. Usually, this limit acquires in oils after 20-25 frying cycles in the real time frying operations [191, 207]. This higher value explains the influence of steel vessel on the extensive degradation of oils utilized in this study. Tocopherols, sitosterol, BHA and BHT showed only minor effect at frying temperature and they could retard the polar compound formation levels in the range of 20.40% - 21.20% (figure 3.12). Moreover, sitostanol which is a saturated form of sitosterol also found to be ineffective similar to those explained earlier. These results clearly explain the ineffectiveness of conventional antioxidants during the prolonged frying process which is in agreement with earlier reports [208-209].



*significant difference from the control ($p \leq 0.05$)

Figure 3.12 Effect of various antioxidants on polar compound formation in the refined soybean oil.

Tocopherols mixture showed a higher reduction in the polar compound formation compared to individual tocopherols. A mixture of tocopherols limited the polar compound level to 18.50 ± 0.50%, which is mainly caused by the synergistic effect of combination of tocopherols. On the other hand, *γ*-oryzanol, a principle bioactive compound of rice bran oil, sesamol from sesame oil and curcumin, a major compound from turmeric have shown a significant effect in limiting the polar compound formation. These results suggest that these compounds could be utilized in the oil industries to increase the thermal stability of oils.

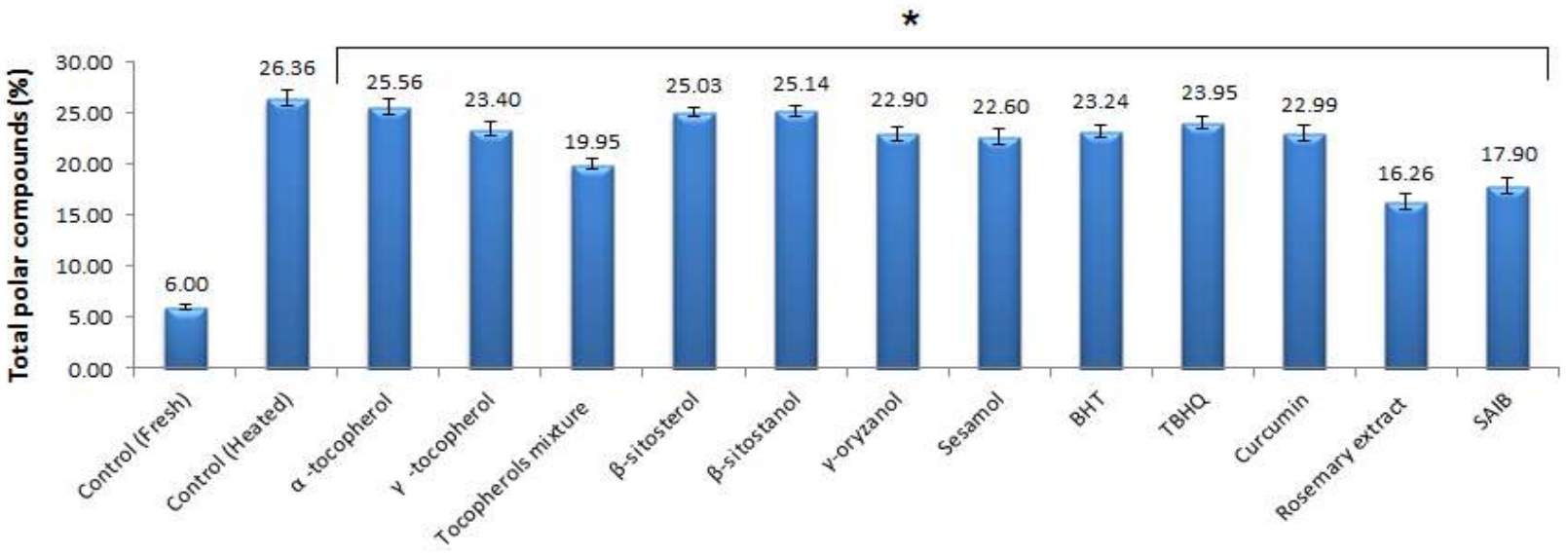
In this study, we identified rosemary extract and SAIB as the most thermally stable antioxidants for frying process. Soybean oil added with the same concentration (0.02%) of rosemary and SAIB showed that they limited the polar compound formation to 16.70 ± 0.75% and 17.80 ± 0.70% respectively as against 24.20 ± 0.75% of polar compound formation in the case of the control sample. Overall the results showed that the difference between the control (heated) and all other samples are significantly different at $p < 0.05$. Further, rosemary, SAIB, tocopherol mixture added samples are more stable compared to BHT, TBHQ, and sitosterol added samples. The rosemary and SAIB imparts about 30% more stability in comparison with a control sample with respect to the total polar compounds formation. Antioxidant properties of rosemary extracts are well known due to the contents of diterpenes (10-15%) including carnosic acid and carnasol [210]. On the other hand, SAIB is a food additive that is generally used in the beverage industry. Toxicological studies showed SAIB is one of the safest food additives with maximum acceptability up to 2000ppm [211-212]. However, SAIB is not a conventional antioxidant for oil processing industries instead several other natural and synthetic antioxidants are being used. But, in this study, we utilized

SAIB because of its high thermal stability and found that it is the best replacement to many other synthetic antioxidants.

3.3.1.2 Effect of antioxidants on polar compound formation in the palmolein

Figure 3.13 showed the effect of various antioxidants on the formation of polar compounds in the refined palmolein at frying temperature after 2 hr. Fresh palmolein showed 6.00 ± 0.20% polar compounds while heated palmolein showed 26.36 ± 0.60%. These results showed palmolein was degraded extensively similar to the soybean oil ($p < 0.05$). Individual tocopherols (α and γ), BHT and TBHQ, showed little effect on the reduction of polar compounds formation (25.56± 0.80% in the case of α- tocopherol and 23.95 ± 0.70% by TBHQ).

Sesamol (22.60 ± 0.75%), curcumin (22.99 ± 0.59%), γ-oryzanol (22.90 ± 0.80%) and tocopherol mixture (19.95 ± 0.50%) showed positive effect on retarding the polar compound formation similar to the case of soybean oil. Rosemary (16.26 ± 0.60%) and SAIB (17.90 ± 0.80%) showed the significant effect on the stability of palmolein. Rosemary extract was found to have potent antioxidant nature in real time frying process of palmolein [213]. These results once again demonstrate the potential of rosemary as well as SAIB in improving the stability of frying oils. In addition, these results also showed that those antioxidants can be used in all other oils, as they showed higher effect in both highly unsaturated (soybean oil) and medium unsaturated (palmolein) oils.



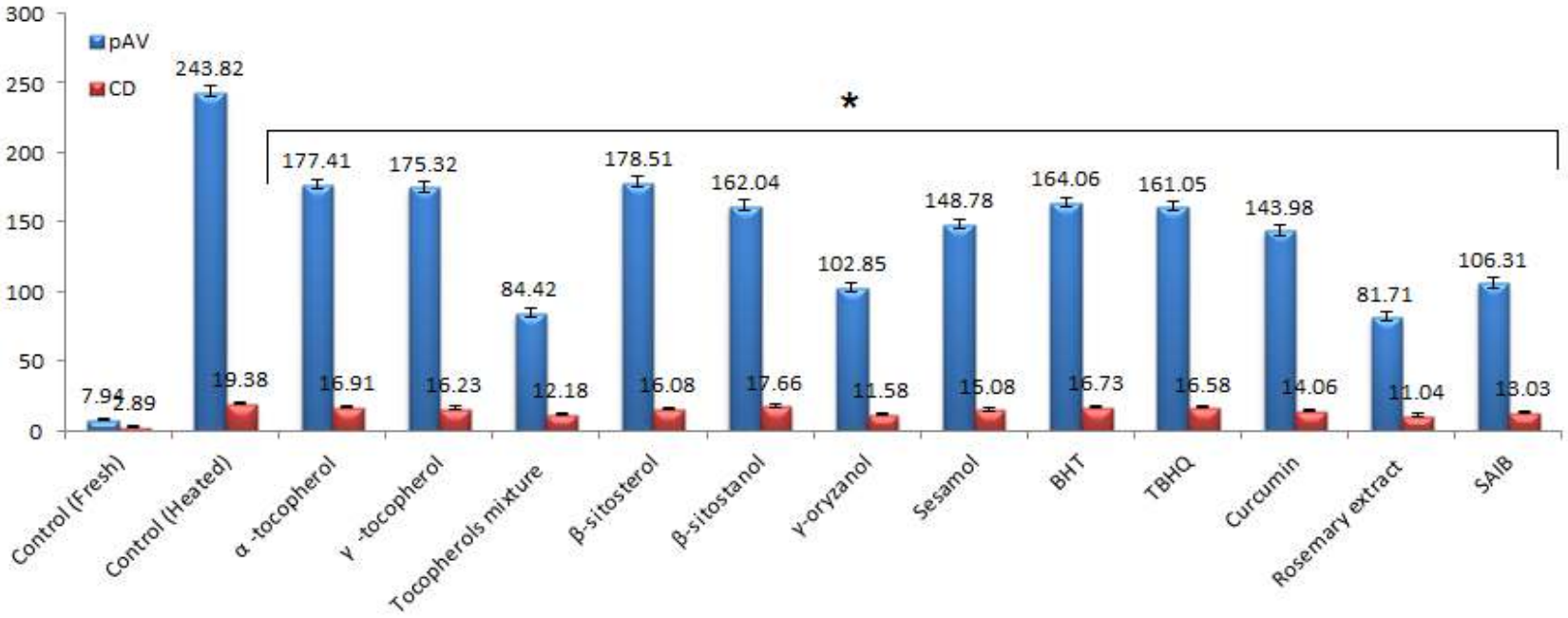
*significant difference from the control ($p \leq 0.05$)

Figure 3.13 Effect of various antioxidants on polar compound formation in the refined palmolein.

3.3.1.3 Effect of antioxidants on thermo-oxidation of the soybean oil

The oxidative stability of frying oil is an important factor in determining the shelf-life of fried products during storage [214]. Thermo-oxidation of frying oils involves both primary and secondary oxidation. But, secondary oxidation continues because of the least stability of peroxides at frying temperature. Oxidation further proceeds to the formation of minor compounds, including aldehydes, ketones, and dienes. This is one of the main reasons for not considering the peroxide value or fatty acid profiling as indicators in determining the frying oil degradation. Moreover, *p*AV and diene values provide useful information about the degradation of oils at frying temperature. *p*AV and diene values of oils treated with different antioxidants are shown in Fig. 3.14. *p*AV and diene values showed extensive degradation of the control sample within 2 hrs (7.94 ± 1.0 to 243.82 ± 3.5 of *p*AV and 2.89 ± 0.5 to 19.38 ± 0.8 of CD) without the addition of any antioxidants. But oils added with the tocopherol mixture, *γ*-oryzanol, SAIB and rosemary extract showed significant resistance towards thermo-oxidation. All these values are significantly different at $p < 0.05$ as inferred from statistical analysis. Rosemary extracts showed potent antioxidant capacity towards the thermo-oxidation (Figure 3.14) which is in agreement with earlier studies [88]. It is also found that tocopherol mixture and *γ*-oryzanol which showed relatively lesser effect in retarding the polar compound formation compared to SAIB, could retard oxidation more effectively compared to SAIB treated oils. These results clearly explain that SAIB could be useful to reduce polar compounds formation whereas tocopherol mixture and *γ*-oryzanol could be useful in retarding oxidation. Similarly, even though Rosemary showed higher activity than SAIB in terms of reducing the polar compound formation, the color retention of

the oil added with SAIB was better than rosemary even after heating the oils for 6 hr (color measurements were not taken as oil was 100% polymerized). Moreover, oils added with BHT, TBHQ, sterol and tocopherols completely lost their color (Figure 3.15). These results clearly explain the anti-polymerizing activity of SAIB over many other antioxidants.



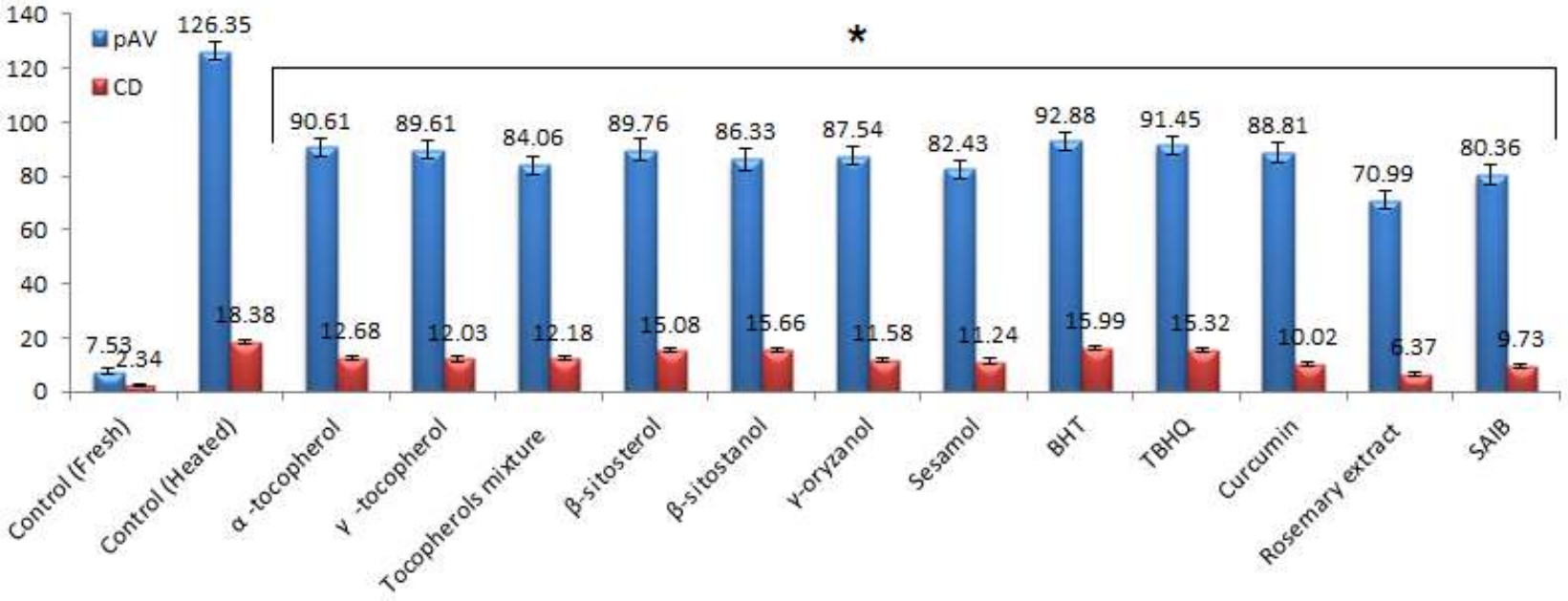
*significant difference from the control ($p \leq 0.05$)

Figure 3.14 Effect of various antioxidants on thermo-oxidation in the refined soybean oil.

Figure 3.15 Physical appearance of soybean oil with different antioxidants after 6 hrs heating at frying temperature.

3.3.1.4 Effect of antioxidants on thermo-oxidation of the palmolein

Figure 3.16 showed the *p*AV and CD values of the palmolein added with different antioxidants. Palmolein without any added antioxidant (control) showed (126.35 ± 3.8 of *p*AV and 18.38 ± 1.0 of CD) lesser oxidation at the end compared to soybean oil due to high saturated fat content. However, even though it has shown lower oxidation, the polar compound formation was similar to that of soybean oil. These observations demonstrate that the formation of polar compounds and thermo-oxidation are not positively correlated. Palmolein added with rosemary extract (81.71± 3.20 of *p*AV and 11.04± 0.70 of CD) and SAIB (106.31± 3.8 of *p*AV and 13.03± 0.55 of CD) showed phenomenal effect compared to that of BHT, TBHQ, sitosterol, and tocopherols. Tocopherol mixture, *γ*-oryzanol sesamol, curcumin also showed significant effect (Figure 3.16). These results demonstrate that natural antioxidants like rosemary and oryzanol possesses higher activity than synthetic antioxidants such as BHT and TBHQ. Moreover, greater efficacy of oryzanol shows that rice bran oil can be a real alternative to soybean oil in the industrial frying process.



*significant difference from the control ($p \leq 0.05$)

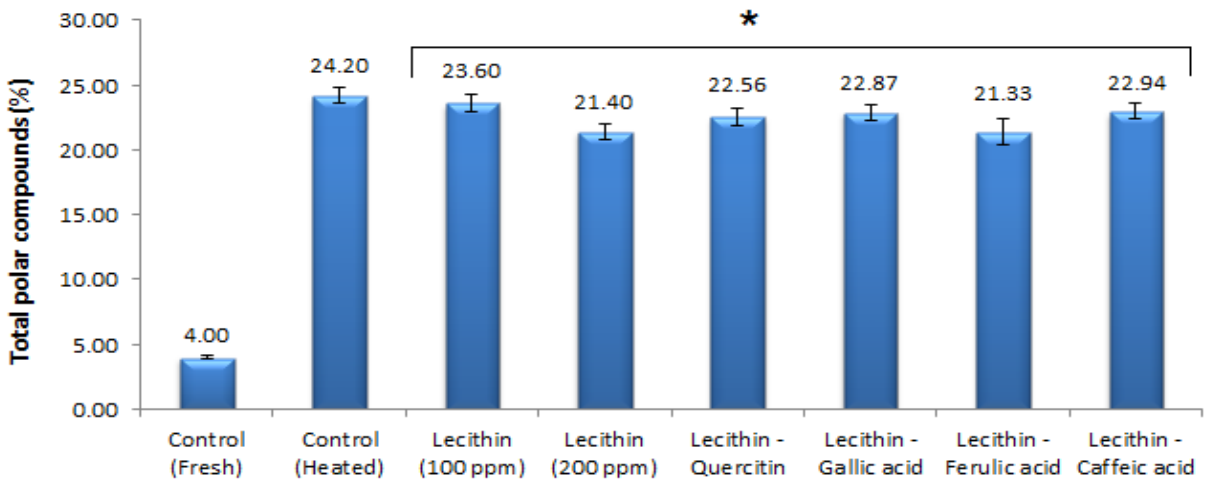
Figure 3.16 Effect of various antioxidants on thermo-oxidation in the refined palmolein.

3.3.2 Effect of phenolic compounds on the frying stability of oils

Phenolic antioxidants such as flavonoids and phenolic acids were found to be the most effective natural antioxidants compared to the conventional synthetic antioxidants, but they lack lipophilicity [215]. Addition of plant extracts containing phenolic compounds to the oil was proved to increase the stability in prolonged frying process [26]. Similarly, soy lecithin was found to exert antioxidant effect in the frying oils, and that could significantly increase the oil stability [200, 216]. Lecithin is a commonly used emulsifier (E 322) in the food processing industry. It contains fatty acids, glycolipids and phospholipids (mainly phosphatidylcholine and phosphatidylethanolamine). It has been reported that quercetin showed improved antioxidant activity in combination with soy lecithin in a triolein model system [217-218]. So, the lipophilicity of the phenolic compounds and their solubility in the oil could be increased through lecithin. In our study, Lecithin and different phenolic compounds mixed in equal proportions (0.01% each) to the oil and studied the polar compound formation along with thermal oxidation. In addition, two control samples added with 0.02% of lecithin and 0.01% of lecithin were considered.

3.3.2.1 Effect of lecithin/phenolic compounds on polar compound formation in the soybean oil

Figure 3.17 showed the polar compound levels in the heated soybean oil with different phenolic acids mixed with lecithin. Soybean oil added with 0.01% of lecithin (23.60 ± 0.60%) showed no difference between the control samples (24.20 ± 0.65%) upon thermal treatment. Upon increasing the lecithin concentration to 0.02%, little effect has been observed in terms of inhibiting polar compound level (21.40 ± 0.57%). 0.01% each of quercitin, gallic acid, and caffeic acid mixed with 0.01% of lecithin could not show more effect compared to 0.02% of lecithin (Figure 3.17). Same composition of ferulic acid and lecithin showed slightly better effect (21.33 ± 1.00%) than other phenolic compounds. Previous reports demonstrated that the antioxidant synergy between α- tocopherol and phospholipids could increase oil stability at room temperature [219-220]. But, our results suggested that phenolic compounds mediated with lecithin are not stable at frying temperature. This could be due to the de-emulsification of lecithin/phenolic system at frying temperature and eventually led to its degradation. Moreover, 0.02% of lecithin is also ineffective compared to the same concentration of BHT. So, based on these results we may conclude that emulsifier mediated phenolic compound incorporation in oil may not be an effective method for increasing the frying oil stability.

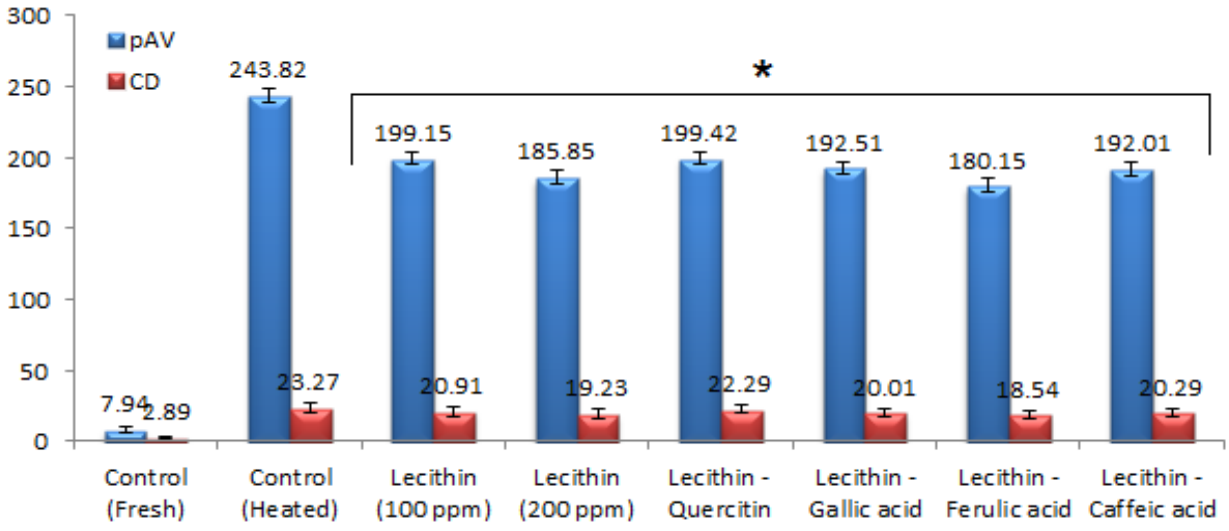


*significant difference from the control ($p \leq 0.05$)

Figure 3.17 Effect of various lecithin/phenolic compounds on polar compound formation in the refined soybean oil.

3.3.2.2 Effect of lecithin/phenolic compounds on thermo-oxidation of the soybean oil

Figure 3.18 showed the oxidation pattern of soybean oil added with different phenolic compounds through lecithin. 0.01% lecithin (199.15 ± 4.8 of *p*AV and 20.91 ± 3.3 of CD) and 0.02% (185.85 ± 3.9 of *p*AV and 19.23 ± 2.7 of CD) showed similar oxidative stability. Moreover, except ferulic acid (180.15 ± 5.1of *p*AV and 18.54 ± 2.3 of CD) all other phenolic compounds such as quercitin, gallic acid and caffeic acids showed no difference in their efficiency in inhibiting thermo-oxidation. These results suggest that phenolic compounds and lecithin degraded at early stages of frying conditions.



*significant difference from the control ($p \leq 0.05$)

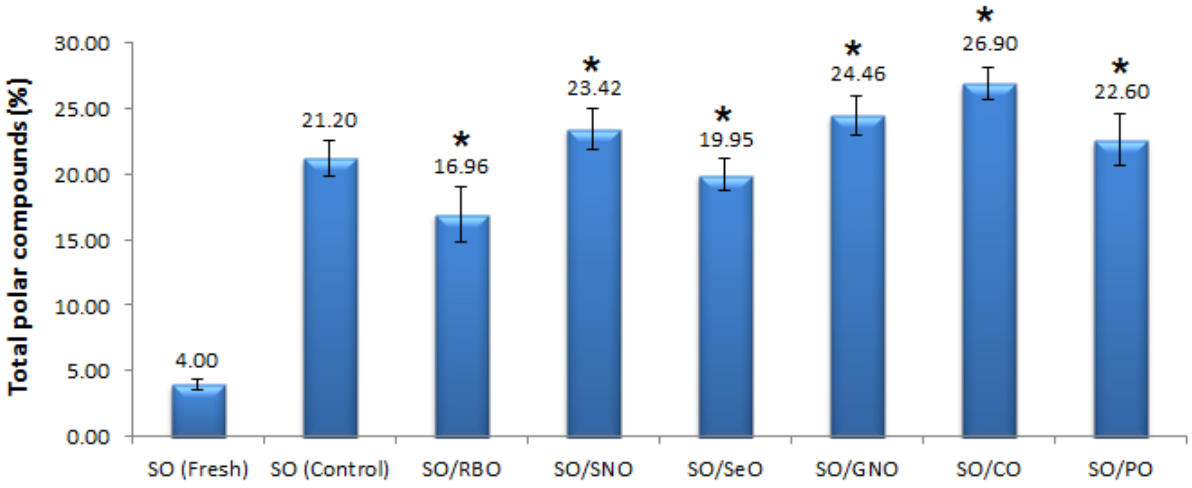
Figure 3.18 Effect of various lecithin/phenolic compounds on thermo oxidation in the refined soybean oil.

3.3.3 Effect of blending on the frying stability of oils

Blending refers to an admixture of two or more edible vegetable oils [221]. The blending process improves the fatty acid profile and increases the antioxidant activity which results in oxidative stability of the oils. Moreover, blending of vegetable oils is an economical way of modifying physicochemical characteristics of oils besides enhancement in oxidative stability [222-223]. In this study, refined palmolein (PO) or refined soybean oils (SO) were blended with either refined rice bran oil (RBO), sesame oil (SeO), sunflower oil (SNO), groundnut oil (GNO) or coconut oil (CO) in 80/20 ratio. Degradation of blends was studied in terms of polar compounds formation and thermo-oxidation.

3.3.3.1 Effect of blending on the polar compound formation in the soybean oil

Polar compounds of soybean oil blended with different oils were shown in figure 3.19. As explained earlier, fresh soybean oil had 4.00 ± 0.25% of total polar compounds. After heating for 2 hrs at frying temperature, it had reached 21.20 ± 0.40%. Soybean oil blended with palmolein (22.60 ± 1.60%), sunflower oil (23.42 ± 1.48%) and groundnut oil (24.46 ± 1.52%) showed higher polar compound percentage than the control. This is caused by the increase of unsaturation of oil as all PO, SNO and GNO are rich in monounsaturated fat (triolein). On the other hand, soybean oil blended with rice bran oil (16.96 ± 2.10%) and sesame oil (19.95 ± 1.24%) showed lower quantity of polar compounds compared to that of control. Even though these oils are rich in MUFA, due to the contents of antioxidant such as oryzanol (rice bran oil) and sesame lignans (sesame oil) along with the other natural antioxidants made those oils more stable at frying temperature compared to other oils. Recently published reports also demonstrated the stability of sesame oil towards industrial frying process [224-225]. Coconut oil blend showed the highest percentage of polar compounds (26.90 ± 1.20%) compared to other oils that were blended with soybean oil. From these results, it is evident that rice bran and sesame oils are suitable to make commercial blends with soybean oil while coconut oil is the least appropriate.



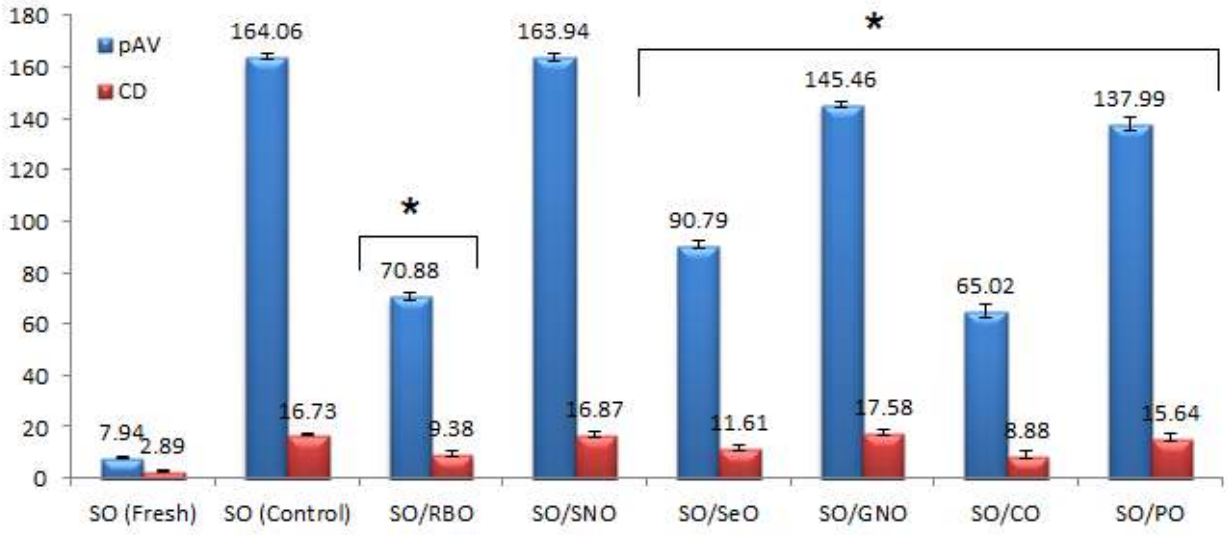
*significant difference from the control ($p \leq 0.05$)

Figure 3.19 Effect of blending on polar compound formation in the refined soybean oil.

3.3.3.2 Effect of blending on thermo-oxidation in the soybean oil

*p*AV and CD values of different blends of palmolein along with a fresh and control samples were shown in figure 3.20. Oxidative stability of any frying oil depends on how well it retards the thermo-oxidation process. Soybean oil is well known for its lesser oxidative stability due to the higher unsaturated fatty acid composition. Control sample showed (164.06 ± 1.26 of *p*AV and 16.73 ± 0.64 of CD) twenty-fold increase in comparison to the fresh soybean oil (7.94 ± 0.49 of *p*AV and 2.89 ± 0.90of CD). Rice bran oil and sesame oil blends could reduce oxidation efficiently compared to other blends such as groundnut oil, sunflower oil and palmolein. Interestingly, soybean oil blended with coconut oil showed lowest levels of oxidative deterioration (65.02 ± 2.57 of *p*AV and 8.88 ± 1.47 of CD). From these observations, it can be concluded that higher contents of short chain and medium chain fatty

acids of coconut oil lead to more polar compound formation due to breakdown products (DAG and MAG) while its highest saturation showed the lowest thermo-oxidation. However, coconut oil is not recommended to blend due to its faster degradation compared to all other oils.

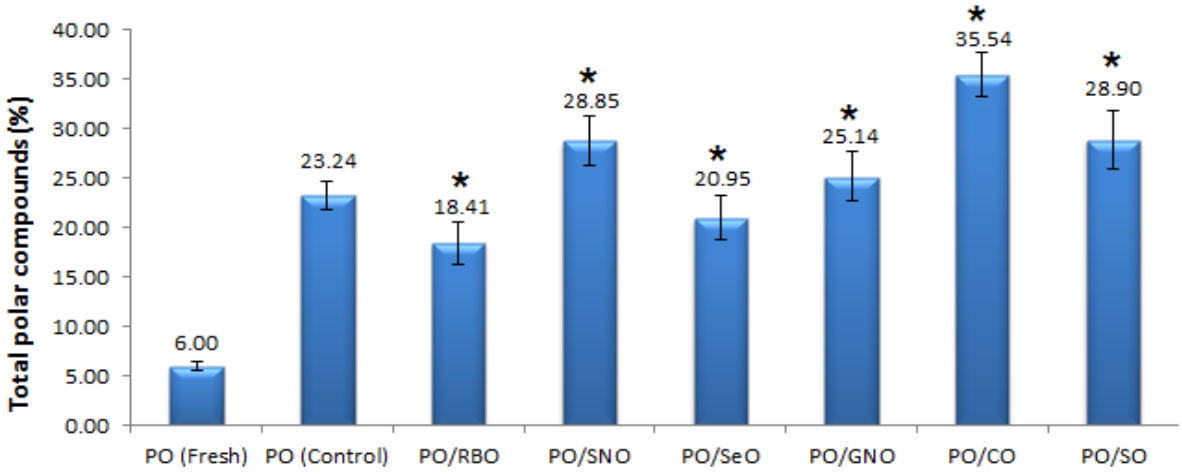


*significant difference from the control ($p \leq 0.05$)

Figure 3.20 Effect of blending on thermo-oxidation in the refined soybean oil.

3.3.3.3 Effect of blending on polar compound formation in the palmolein

Figure 3.21 showed the polar compounds content in the heated palmolein as well as palmolein blended with different oils. Heated palmolein showed 23.24 ± 1.36% of polar compounds against the 6.00 ± 0.25% of fresh palmolein. Similar to the soybean blends discussed earlier, palmolein blended with sunflower oil (28.85 ± 2.50%), groundnut oil (25.14 ± 2.54%) and soybean oil (28.90 ± 3.00%) in same ratios showed higher percentage of polar compounds than control sample. Earlier reports suggested that blending of the highly unsaturated oils like soybean oil and sunflower oil with palmolein leads to formation of higher polar compounds during frying, even though they have optimum tocopherol content [226-227]. Palmolein blended with rice bran oil (18.41 ± 2.10%) and sesame oil (20.95 ± 2.24%) showed lower polar compound content due to their higher oxidative stability. These results suggested that two oils of the same nature are not a good choice for blending unless either of the one possesses high antioxidant capacity. However, palmolein blended with coconut oil showed 35.54 ± 2.24% of polar compounds, which proved its unsuitability towards industrial frying process as discussed earlier.

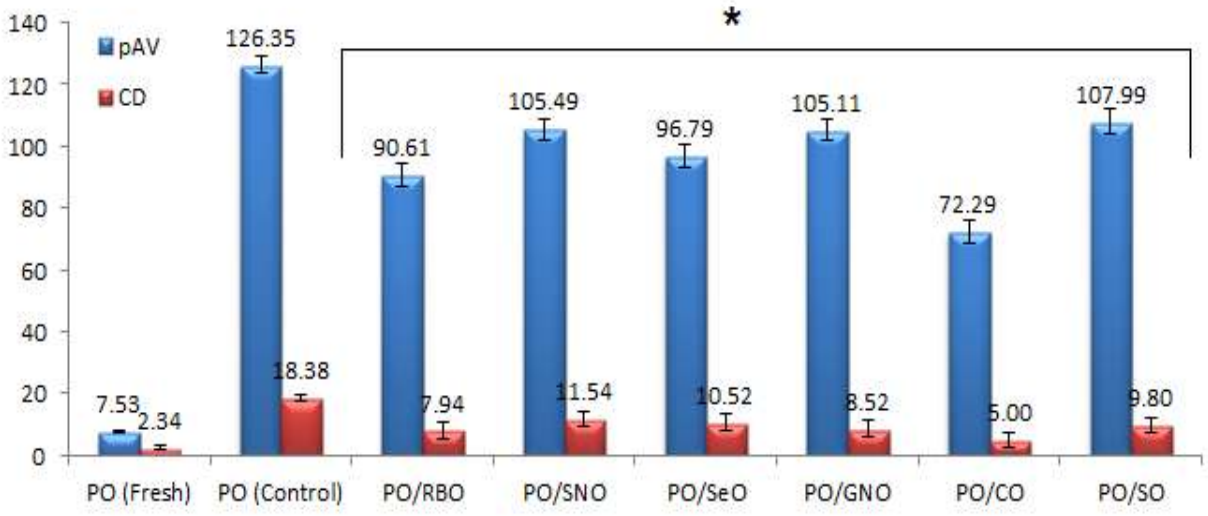


*significant difference from the control ($p \leq 0.05$)

Figure 3.21 Effect of blending on polar compound formation in the refined palmolein.

3.3.3.4 Effect of blending on thermo-oxidation in the palmolein

Thermo-oxidation of palmolein and its blends in terms of *p*AV and CD values were shown in figure 3.22. Heated palmolein (126.35 ± 2.50 of *p*AV and 18.38 ± 0.80of CD) showed lesser oxidation compared to soybean oil. Coconut oil blend (72.29 ± 4.00 of *p*AV and 5.00 ± 1.50 of CD) showed the lowest oxidation followed by rice bran oil (90.61± 3.50 of *p*AV and 7.94 ± 2.50 of CD) and sesame oils (96.79 ± 3.37 of *p*AV and 10.52 ± 2.70of CD). Sunflower oil, groundnut oil and soybean oil also showed similar results and found to be sensitive towards prolonged frying temperature. Overall the whole study showed that rice bran and sesame oils are most thermally stable frying oils to prepare commercial blends of different edible oils and also the best alternative to other oils for industrial frying process.



*significant difference from the control ($p \leq 0.05$)

Figure 3.22 Effect of blending on thermo-oxidation in the refined palmolein.

CHAPTER 4
SUMMARY AND CONCLUSION

4.0 Summary and conclusion

Deep fat frying is one of the most widely used culinary art, and it makes fried food more palatable. Heat and mass transfer between oil and food during the frying process produces the desirable and unique quality of fried foods. But, frying leads to thermal degradation of the oil that involves thermo-oxidation, hydrolysis, and polymerization. This phenomenon leads to the formation of polar compounds and degrades frying oil further. These degradation products, transport into fried food and reduce the nutritional quality of frying oil as well as fried food. Addition of potent antioxidants can decrease the formation of polar compounds and thermo-oxidation in frying oils during extensive thermal treatment. So, it is crucial to study the thermal degradation products formed during the frying process and to find ways to inhibit their formation. The present study focused on scientific understanding of the above with the following objectives:

1. To study the influence of frying time and food material on the formation of polar compounds and thermo-oxidation in the frying oil.
2. To characterize the type of polar compounds formed during frying process through mass spectrometry for the better understanding of degradation of frying oil.
3. To study the effect of various natural and synthetic antioxidants including phenolic compounds on the formation of polar compounds and thermo-oxidation in frying oil.

The results obtained and observations made during this study are discussed, and a summary of the findings and the conclusions are presented below.

In this study, we carried out a comprehensive analysis of the degradation of palmolein and soybean oils during both intermittent and continuous frying process. In the intermittent frying process, French fries were being fried for 18 hrs, including 36 frying cycles. Both oils were found to be degraded completely in the end, with respect to oil quality and total polar compounds. From these observations, 25-35 frying cycles or polar compound content of 25% were set to be the limit of usability of these frying oils. Moreover, this study also established that fatty acid degradation and peroxide value determination are not reliable parameters to arrive at the frying quality of the oil. Instead, the total polar compound content determination is a dependable choice in determining the frying oil quality.

In the continuous frying process, French fries and chicken nuggets, and also both the food materials in combination were subjected to frying for 10 hrs including 20 frying cycles. During this process, it was observed that the type of food products have no significant effect on the degradation of oil. It was also found that there was not much difference in the degradation levels when single or multiple food products in combination were fried. Moreover, the moisture content of the food product had not substantially influenced the hydrolysis process, which could be observed from the FFA values. Similarly, the degradation of oil during intermittent and continuous frying process was similar for the same period of frying. These observations conclude that the deterioration of the oil mainly depends on the nature of the oil.

After understanding the role of polar compounds in the oil degradation, the structural elucidation of polar compounds was performed by MALDI-TOF/MS. In this study, we identified several oxidized, dimerized and polymerized TAG along with DAG fragments

formed during heating/frying, which provided the information about the mechanism of degradation and simultaneous changes occurred during prolonged frying conditions. From these findings, it could be inferred that the oxidation predominates over hydrolysis, and continuous oxidation leads to dimerization and polymerization. Dimers and polymers were identified as polar and non-polar ones. Oxidation continued even after polymerization and turned non-polar polymer TAG into polar polymers. However, few non-polar polymers were also identified in the non-polar fraction of fried oil. In this process, we found larger content of trimers and tetramers of OOO, PLO, POO and OLL triglycerides. It has been also found that more unsaturated oil such as soybean oil undergone higher degree of polymerization than palmolein and oxidized rapidly. These results can serve as a valuable database for the mass spectrometrists in lipid science. This study also highlighted the importance of MALDI-TOF/MS as a tool for rapid and sensitive detection of fried product quality and edible oil adulteration with used oils.

From the previous phases of work, we conclude that oxidation causes continuous degradation of oil, and if we can slow down or inhibit the oxidation, we can increase the stability of oil towards the prolonged frying process. In this scenario, we tested the efficacy of several conventional and potential antioxidants to inhibit the polar compound formation as well as thermo-oxidation in the palmolein and soybean oils. Commercial oils possess added synthetic antioxidants that may exert a synergistic effect with the antioxidants that were to be added for this study. To avoid this, we tested all the antioxidants in the refined oils recovered directly from the refinery which are free from any synthetic antioxidant. All oils were heated in the steel vessels at frying temperature to create necessary frying atmosphere. Among the all antioxidants studied, we found rosemary extract and SAIB could efficiently reduce the

total polar compound formation and also secondary oxidation in the oils compared to other antioxidants such as BHT, TBHQ, sitosterol and individual tocopherol isomers. Similarly, γ-oryzanol, sesamol, and curcumin showed a considerable effect on the frying oil quality. The results of this study concluded that rosemary extract and SAIB are the most commercially viable antioxidants to maximize the stability of oils at the frying temperature.

Phenolic compounds reportedly had potent antioxidant effect, but they lack lipophilicity to dissolve in the oil. To overcome this problem, we utilized lecithin, a food grade emulsifier to lipophilize the phenolic compounds, including quercetin, gallic acid, ferulic acid and caffeic acid. However, lecithin mediated phenolic compounds could not show a better effect on the oil stability compared to other synthetic antioxidants. These results could be caused by the de-emulsification effect of the lecithin and phenolic compounds. Results from this study suggested the advantage of rosemary and SAIB than lecithin mediated phenolic compounds.

Blending refers to a mixture of two or more oils that can improve the nutritional profile of oil. Previous findings suggested that blended oils have higher stability over pure oils in the storage stability studies at the room temperature. In our study, we tested frying stability of blending different oils with palmolein and soybean oil separately. Major oils utilized for this study are rice bran, groundnut, sesame, sunflower and coconut oils. One of these oils were blended with palmolein as well as soybean oil in 20/80 ratios. Findings of this study showed the higher stability of blends having rice bran and sesame oils compared to other oils. Acrylamide forms due to the condensation of sugar and protein in the food material and it does not form in oil as it is a polar compound. Since the main objective of this thesis is to study the degradation of oil we did not study the acrylamide content in the food material.

In conclusion, number of frying cycles influences the degradation of oil in the repeated frying operations. So, it is necessary to limit the number of frying cycles for the production of healthy fried foods. It was also concluded that, the oxidation process leads to continuous degradation of frying oil. So, the utilization of potential antioxidants is necessary for the frying oil stabilization. Through this study, it has been established that rosemary extracts and SAIB were found to have higher effect on improving the frying oil stability compared to conventional synthetic antioxidants. Future work may be on the natural antioxidants and the mechanistic studies of the degraded compounds formed during the frying process. The characterization of degraded compounds may lead to selection or development of the type of antioxidants for attaining the highest thermal stability towards prolonged industrial frying process. Considering the volume of fried food market and health issues, thermal degradation of oils during deep fat frying requires greater attention.

SUPPLEMENTARY INFORMATION

Table 3.1S Quality parameters of palmolein during intermittent frying process (a) FFA (%) (b) PV (mEq/kg) (c) *p*AV (d) TPC (%).

(a)

Frying cycle	Heating	Frying
0	0.42	0.42
4	0.50	0.56
8	0.72	0.82
12	0.86	1.09
16	0.92	1.21
20	1.07	1.37
24	1.24	1.48
28	1.38	1.60
32	1.52	1.82
36	1.62	1.96

(b)

Frying cycle	Heating	Frying
0	3.63	3.93
4	13.55	6.74
8	15.88	7.80
12	18.17	8.90
16	18.96	9.60
20	20.32	10.24
24	22.54	10.88
28	23.82	11.70
32	26.24	13.88
36	28.21	17.96

(c)

Frying cycle	Heating	Frying
0	5.56	5.64
4	26.86	29.92
8	31.56	33.29
12	45.39	48.46
16	63.68	69.37
20	68.47	72.39
24	72.37	80.64
28	75.68	85.20
32	78.65	89.29
36	82.08	90.03

(d)

Frying cycle	Heating	Frying
0	6.00	6.00
4	7.00	8.00
8	8.00	9.50
12	9.00	11.00
16	10.00	13.00
20	11.00	14.00
24	13.00	16.00
28	14.00	18.00
32	15.50	20.50
36	17.00	22.00

Table 3.2S Fatty acid degradation in palmolein during intermittent frying process.

Frying cycle	SFA (%)	MUFA (%)	PUFA (%)
0	53.71	37.33	8.96
4	55.55	36.04	8.41
8	55.12	36.31	8.58
12	50.26	40.65	9.09
16	54.26	40.65	8.09
20	55.26	40.65	8.09
24	57.58	35.48	6.94
28	60.20	32.65	7.15
32	60.26	32.65	7.09
36	61.27	31.21	7.52

Table 3.3S Degradation of palmolein color during intermittent frying process (a) lightness (b) redness (c) yellowness (d) color difference.

(a)

Frying cycle	Heating	Frying
0	86.20	86.20
4	85.49	85.20
8	84.97	84.12
12	84.99	83.51
16	82.44	86.20
20	82.30	83.32
24	82.07	83.41
28	81.28	85.83
32	81.82	83.03
36	80.76	80.98

(b)

Frying cycle	Heating	Frying
0	-10.02	-10.02
4	-6.41	-10.09
8	-6.80	-10.21
12	-6.94	-10.49
16	-4.06	-5.50
20	-3.23	-4.76
24	-2.05	-4.15
28	-4.82	-2.02
32	-0.62	-2.50
36	-0.25	80.98

(c)

Frying cycle	Heating	Frying
0	37.33	37.33
4	29.89	40.49
8	29.83	42.67
12	28.13	40.05
16	28.03	37.67
20	27.76	39.38
24	25.50	37.35
28	23.78	30.23
32	23.46	27.13
36	25.60	25.78

(d)

Frying cycle	Heating	Frying
0	-	-
4	8.30	3.32
8	8.25	5.73
12	9.78	3.85
16	11.67	4.53
20	12.37	6.34
24	14.85	6.50
28	15.32	10.70
32	17.32	13.06
36	16.21	16.26

Table 3.4S Quality parameters of soybean oil during intermittent frying process (a) FFA (%) (b) PV (mEq/kg) (c) *p*AV (d) TPC (%).

(a)

Frying cycle	Heating	Frying
0	0.25	0.25
4	0.29	0.27
8	0.34	0.52
12	0.43	0.53
16	0.48	0.71
20	0.52	0.79
24	0.67	0.79
28	0.81	1.07
32	0.92	1.35
36	1.00	1.58

(b)

Frying cycle	Heating	Frying
0	3.34	3.30
4	11.91	4.25
8	13.37	4.60
12	15.49	5.63
16	18.96	5.70
20	22.80	7.94
24	23.64	9.41
28	25.80	10.28
32	25.24	12.64
36	24.60	14.84

(c)

Frying cycle	Heating	Frying
0	5.65	5.63
4	36.78	47.68
8	38.69	67.27
12	42.35	73.89
16	45.96	78.67
20	52.45	81.62
24	58.82	82.60
28	64.89	86.52
32	68.78	90.24
36	73.27	94.67

(d)

Frying cycle	Heating	Frying
0	4.00	4.00
4	7.50	7.00
8	8.50	9.50
12	10.00	12.00
16	11.50	13.50
20	13.00	15.50
24	15.00	17.50
28	17.00	19.00
32	18.50	21.50
36	20.00	23.50

Table 3.5S Fatty acid degradation in soybean oil during intermittent frying process.

Frying cycle	SFA (%)	MUFA (%)	PUFA (%)
0	21.69	23.36	54.95
4	21.84	23.36	54.79
8	21.96	23.36	54.67
12	22.41	23.84	53.75
16	22.71	24.31	52.98
20	23.34	24.31	52.35
24	23.71	24.31	52.09
28	24.96	25.05	49.99
32	25.96	25.05	48.99
36	26.92	24.04	49.04

Table 3.6S Degradation of soybean oil during intermittent frying process (a) lightness (b) redness (c) yellowness (d) color difference.

(a)

Frying cycle	Heating	Frying
0	101.14	101.14
4	100.08	101.61
8	100.41	99.2
12	99.25	98.18
16	98.78	100.13
20	97.07	98.71
24	98.50	97.47
28	96.17	99.84
32	97.04	95.01
36	98.62	92.91

(b)

Frying cycle	Heating	Frying
0	-7.89	-7.89
4	-6.86	-6.29
8	-6.91	-8.22
12	-6.95	-5.62
16	-6.75	-6.45
20	-3.33	-7.40
24	-0.69	-3.24
28	4.53	-0.75
32	5.71	5.14
36	5.37	9.60

(c)

Frying cycle	Heating	Frying
0	24.10	24.1
4	23.92	22.39
8	23.29	22.89
12	22.79	22.35
16	22.61	24.29
20	22.13	29.55
24	21.40	21.56
28	21.48	19.94
32	21.94	18.37
36	20.08	17.76

(d)

Frying cycle	Heating	Frying
0	-	-
4	1.49	2.39
8	1.47	2.31
12	2.48	4.12
16	3.01	1.77
20	6.42	5.99
24	8.13	6.45
28	13.63	8.37
32	14.37	15.50
36	14.08	20.34

Table 3.7S Quality parameters of palmolein during continuous frying process (a) FFA (%) (b) PV (mEq/kg) (c) *p*AV (d) TPC (%).

(a)

Frying cycle	Control	FF	CN	FF/CN
0	0.42	0.42	0.42	0.42
4	0.50	0.56	0.50	0.56
8	0.72	0.82	0.61	0.82
12	0.86	1.09	0.72	1.09
16	0.92	1.21	1.02	1.22
20	1.07	1.37	1.22	1.42

(b)

Frying cycle	Control	FF	CN	FF/CN
0	3.63	3.93	3.93	3.93
4	13.55	6.74	6.80	6.62
8	15.88	7.80	9.87	8.12
12	17.46	8.90	12.90	12.74
16	18.08	9.60	14.56	16.45
20	19.85	10.06	16.94	19.2

(c)

Frying cycle	Control	FF	CN	FF/CN
0	5.56	5.64	5.64	5.64
4	26.86	29.92	45.21	29.92
8	31.56	33.29	52.64	33.29
12	45.39	48.46	62.35	57.52
16	59.45	64.69	67.75	69.92
20	62.63	70.46	72.45	74.26

(d)

Frying cycle	Control	FF	CN	FF/CN
0	6.00	6.00	6.00	6.00
4	7.00	7.50	8.00	7.50
8	8.00	8.50	9.50	8.50
12	9.00	10.00	11.00	10.50
16	10.00	11.50	13.00	12.50
20	11.00	13.50	15.50	14.50

Table 3.8S Quality parameters of soybean oil during continuous frying process (a) FFA (%) (b) PV (mEq/kg) (c) *p*AV (d) TPC (%).

(a)

Frying cycle	Control	FF	CN	FF/CN
0	0.25	0.25	0.25	0.25
4	0.29	0.27	0.31	0.31
8	0.34	0.41	0.42	0.42
12	0.39	0.52	0.51	0.54
16	0.42	0.59	0.64	0.62
20	0.49	0.67	0.71	0.74

(b)

Frying cycle	Control	FF	CN	FF/CN
0	3.34	3.30	3.34	3.30
4	11.91	4.25	5.65	4.33
8	13.37	6.60	7.84	6.54
12	15.49	7.63	9.45	9.64
16	16.96	9.70	14.86	13.65
20	19.20	12.94	16.99	16.06

(c)

Frying cycle	Control	FF	CN	FF/CN
0	5.65	5.63	5.65	5.63
4	36.78	47.68	45.87	46.52
8	38.69	57.27	56.96	56.40
12	41.95	63.89	68.46	69.63
16	43.62	68.67	73.83	76.89
20	48.52	78.62	81.30	84.69

(d)

Frying cycle	Control	FF	CN	FF/CN
0	4.00	4.00	4.00	4.00
4	6.50	7.00	7.00	6.50
8	7.50	9.50	9.50	8.00
12	9.00	11.00	12.00	10.50
16	10.00	12.50	14.00	13.50
20	12.00	14.00	15.50	15.00

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2. GurpurRakesh D. Prabhu, **Challa Ravi Kiran**, A. Sundaresan, Rajesh S. Mony, V.V. Venugopalan (2015). Process Development studies for recovery of bio active isolates from Spent Black Pepper generated from Ayurvedic Industry. **Industrial crops and products**, 66: 144–149.

3. **Challa Ravi Kiran**, Reshma M V, A.Sundaresan (2013). Separation of *cis/trans* isomers of fatty acids in gas chromatography and comparison to Ag-TLC method. **Grasas*y*Aceties-International Journal of fats and oils**, 64 (1): 95-102.

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