

Thermal Desorption Studies of Corn Oil at Frying Temperatures:

Thermal Scission vs Autoxidation

by

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and approved by

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## **ABSTRACT OF THE THESIS**

Thermal Desorption Studies of Corn Oil at Frying Temperatures:

Thermal Scission vs Autoxidation

By XIAOTIAN QIN

Thesis Director:  
Karen M. Schaich

Thermal degradation occurs in all oils during frying, limiting useful fry life as well as quality and shelf life of fried foods. To elucidate the reactions involved, thermal degradation processes in corn oil heated to elevated temperatures were studied using thermal desorption techniques to detect released volatiles. Corn oil was loaded into glass Purge & Trap tubes containing a Celite support to increase oil surface area, and heated to 100, 120, 150, 180, and 235 °C under nitrogen or air for up to 2 hours. Volatiles were flushed from the tubes and collected on Tenax-Carboxen thermal desorption traps at short intervals to limit the number of products present and provide a map of product changes over time, then desorbed into a Gas Chromatography-Mass Spectrometry system. Under air at 100-120 °C, oxidation was slow; major shifts in mechanism occurred between 120 and 150 °C and again at 235 °C, with exponential increase in both rates of degradation and numbers of different products at each temperature. Above 150 °C, complex product mixtures containing primarily C-4 to C-12 alkanes and alkenes, with low levels of oxidation products formed within minutes under nitrogen. Major scission points were carbons adjacent to the last double bond, yielding pentane from linoleic acid and octane and 1-decene from oleic acid. Under air, aldehydes, and alcohols of the same chain length plus 2-pentyl furan and ketones were released in much higher

quantities. Simple oxidation products formed early in heating; as heating time increased, product mixtures became quite complex and included many cyclization and rearrangement products.

Results support radicals from thermal scissions as major initiators of thermal degradation processes in oils. In air, these radicals form terminal peroxy radicals and hydroperoxides which then decompose to oxidation products of the same chain length, dimerize, or initiate autoxidation chains by abstracting radicals at C13 of linoleic acid; decomposition of the C<sub>13</sub>-OOH releases pentane and hexanal. Thermal scissions are especially important for industrial frying conducted under limited oxygen while autoxidation is the dominant degradation affecting quality in food service operations, where oils are heated in air and may be used for many days.

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## 1. INTRODUCTION

For centuries, lipid oxidation has been a serious problem for food stability and shelf life issue. This process, known as rancidity by consumers, generates characteristic painty, oily, grassy off-flavors and odors in foods, along with browning and other changes that degrade food quality (Lea 1962). Food scientists have discovered that many compounds are generated from lipid oxidation when food is contact with air and stored at high temperature during food processing and food storage. Secondary oxidation products such as aldehydes, alcohols, epoxides, are not only responsible for off-flavors, but in addition, many of these products are toxic to some extent (Claxson 1994; Kamal-Eldin 1997; Moreno 1999), and they can also react with other components in food, such as amino acids and proteins, to alter the texture and other qualities of food product.

Over the past fifty years, extensive study and research on lipid oxidation have provided much information about the basic mechanism of lipid oxidation and identified methods to inhibit the oxidation process. Indeed, the food industry thought the problem was largely under control until concerns about fat and health issues such as cardiovascular diseases forced reformulation of foods with poly-unsaturated fatty acids (PUFA) (Saguy 2003). High PUFA oils oxidize much more readily, especially when heated, and former standard approaches are inadequate for stabilizing them, particularly if the synthetic antioxidants BHT and BHA must be avoided.

Using high PUFA oils is particularly a problem in frying because the heat imposes thermal degradation processes on top of rapid oxidation, and products formed in the oil are adsorbed into food where they accelerate degradation during storage. In addition, foods release water and various oxidation catalysts into the oil, and these factors alter

degradation pathways and kinetics. The complicated system that results offers great challenges for stabilization because so many reactions occur simultaneously that it is difficult to determine which processes control the degradation. Indeed, the American Oil Chemists' Society holds a session on frying almost every year at their annual meeting, reflecting the continuing problems with frying and inability to achieve full and consistent stabilization.

A major obstacle to stabilization of frying oils is incomplete information about the chemical mechanisms involved in the degradation processes. The general sequence usually described for thermal decomposition of oils is first hydrolysis, followed by oxidation, saponification, or polymerization of the released free fatty acids (Schaich 2008), (Warner and Gupta 2003), (Velasco, Andersen et al. 2004). Common thinking holds that oxidation drives the decomposition; following Arrhenius kinetics, oxidation doubles for every 10 degrees increase in temperature (Labuza 1971), so rates at frying temperatures of 180 °C are  $10^{16}$  times faster than at room temperature. However, addition of antioxidants or other approaches based on this understanding have been almost uniformly unsuccessful, except for drastic reduction of oxygen.

Research of Wassef Nawar at the University of Massachusetts (Nawar 1967; Nawar 1969), demonstrated the existence of molecular scission pathways in thermal degradation and identified key scission points in fatty acids under air and nitrogen. This body of work has been highly recognized and respected, and yet the practical application of his basic findings remains controversial and the concept of thermal scissions has not yet been incorporated into fundamental understanding of frying chemistry.

Because heat is ever-present during frying, if thermal scissions of lipid molecules occur, they must provide a constant background load of primary radicals that set the stage for secondary degradations, initiate autoxidation chains, and consume antioxidants. Generation of thermal scissions cannot be stopped, whereas initiation and propagation chains can be intercepted. Thus, strategies for stabilizing frying oils must be very different for the two mechanisms.

Both thermal scissions and autoxidation of lipids should produce small volatile molecules as secondary products, but the specific compounds generated will not be the same. Thus, to assess dominance and contributions of these two reactions to thermal degradation of frying oils, volatiles generated under controlled heating of corn oil in a purge and trap system were trapped in thermal desorption tubes, analyzed by gas chromatography, and identified by mass spectrometry and comparison to standards. Types of products, their sequence of development, and their kinetics of generation were measured as a function of temperature and atmosphere (air vs nitrogen). Products identified were compared to products expected from thermal scission reactions according to Nawar's data and products expected from lipid autoxidation (hydrogen abstraction reactions and alkoxy radical scissions) according to the classical lipid oxidation literature to assess contributions from the two pathways. Heating times were two hours or less to focus specifically on early initiating processes in thermal degradation.

## 2. BACKGROUND

### 2.1. Lipid Oxidation

Much scientific research has established that lipid oxidation proceeds by a free radical chain mechanism (Uri 1961); (Frankel 1962); (Scott 1965); (Brodnitz 1968); (Kochi 1973); (Frankel, Neff et al. 1979); (Frankel 1984); (Frankel 1991); (Nawar 1984), (Chan and Coxon 1987) and thus generally proceeds in three steps: initiation, propagation and termination (Figure 1).

Although lipid oxidation occurs rapidly and often seems to be “instantaneous”, as a reaction it is not spontaneous. Since oxygen (triplet state) cannot add directly to ground state double bonds (singlet state), initiators such as metals, light, heat, or other free radicals are required to generate initial radicals,  $L^{\bullet}$  (Reaction 1, Figure 1) which then react almost instantaneously ( $k > 10^9 \text{ L mol}^{-1} \text{ sec}^{-1}$ ) with oxygen to form lipid peroxy radicals,  $LOO^{\bullet}$  (Figure 1).

In the propagation stage, free radical chain reactions become established as peroxy radicals abstract hydrogen atoms from nearby lipid molecules to produce hydroperoxides,  $LOOH$ , and generate new acyl radicals,  $L^{\bullet}$  (Figure 1). The acyl radicals then add oxygen and repeat hydrogen abstractions to establish a free radical chain reaction that continues indefinitely (Figure 1). H abstraction by  $LOO^{\bullet}$  is rather slow ( $k = 36\text{-}62 \text{ L mol}^{-1} \text{ sec}^{-1}$ ) and selective, abstracting only hydrogens with low bond energy, such as allylic  $-\text{CH}_2-$ , thiols, phenols (Howard and Ingold 1967). Thus, peroxy radicals are the main chain carriers only at the very beginning of the chain reaction, and their relatively slow, specific reactions dictate kinetics and pathways during early stages of oxidation.

Radical chains are also propagated and branched by lipid alkoxy radicals (Figure 1) formed by the decomposition of lipid hydroperoxides by heat, metals or ultraviolet light. Alkoxy radicals reaction rates are several orders of magnitude greater than peroxy radicals and their reactions are more specific, so they are the main chain carriers later in the chain reaction after peroxy radicals and hydroperoxides have accumulated. Alkoxy radicals they are responsible for rapid rate period of lipid oxidation and introduction of multiple secondary reactions.

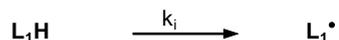
In the termination stage, a wide range of non-radical products such as alkanes/alkenes, aldehydes, ketones, epoxides, and dimers are formed. Radicals recombine with each other or alkoxy radicals undergo  $\alpha$  and  $\beta$  scission reactions (Nawar 1984).

Figure 1 shows the traditional thinking about lipid oxidation reactions. However, the actual process of lipid oxidation is much more complex than merely a series of hydrogen abstractions. Multiple alternate reaction pathways (addition, cyclization and scission) compete with hydrogen abstraction for each major intermediate (Schaich 2005); (Figure 2), rerouting radicals and generating a more complex mixture of products than implied by the simple free radical chain. Consideration of multiple competing pathways can explain complicated oxidation kinetics and varied mixtures of products during lipid oxidation, enable more accurate evaluation of the extent of oxidation. The situation becomes even more complex at high temperatures ( $>100$  °C) where thermal energy is sufficient to break bonds. Such thermal scissions can impose still additional degradation pathways that form products different from autoxidation.

This study aimed to explore how high temperatures (100 – 180 °C) shift the balance between these various reactions and whether, at high temperatures, normal autoxidation

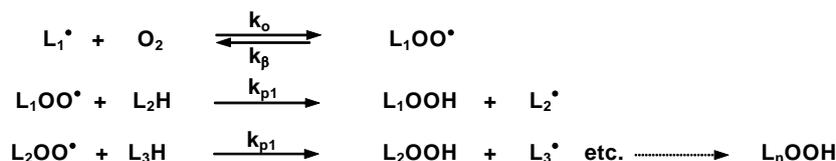
free radical chains proceed but with Arrhenius acceleration or, instead, other degradation mechanisms such as thermal scissions become dominant. (Schaich 2005); (Figure 2). For

**Initiation (formation of ab initio lipid free radical)**

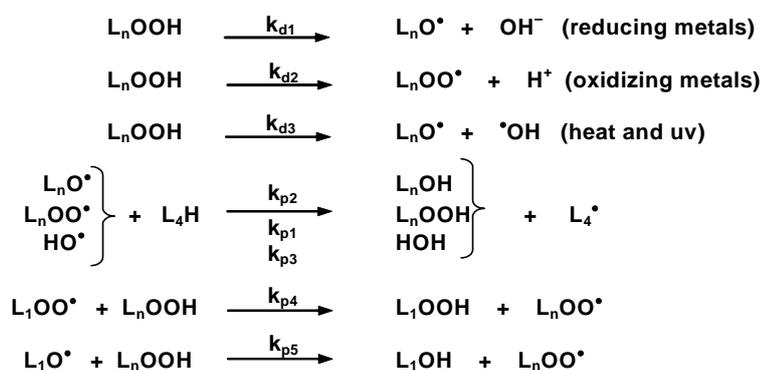


**Propagation**

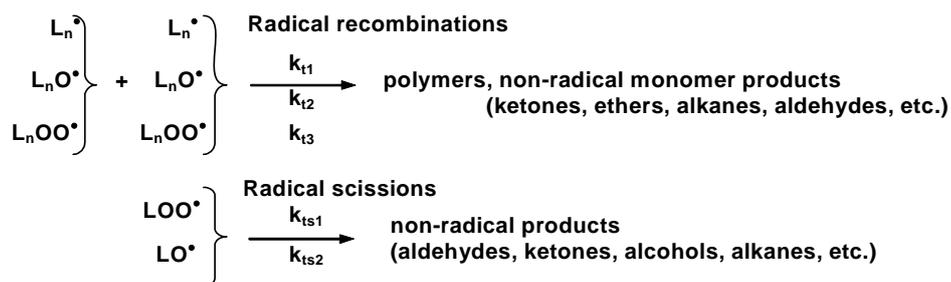
*Free radical chain reaction established*



*Free radical chain branching (initiation of new chains)*



**Termination (formation of non-radical products)**



i - initiation; o-oxygenation;  $\beta$ -O<sub>2</sub> scission; p-propagation; d-dissociation; t-termination; ts-termination/scission

**Figure 1. Sequence of free radical reactions in lipid oxidation described by classical theory (Schaich 2005).**

## INTEGRATED SCHEME FOR LIPID OXIDATION

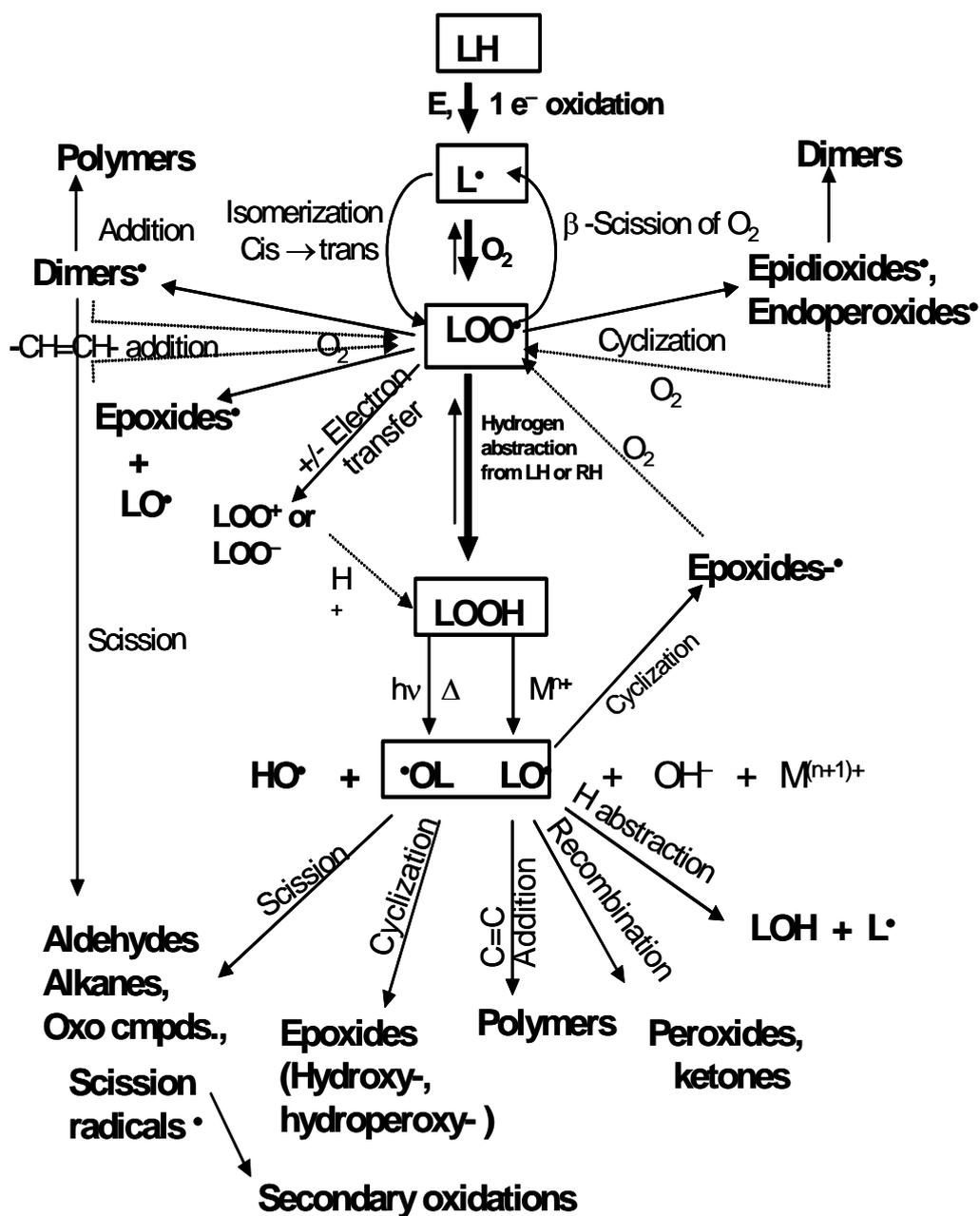


Figure 2. Integrated theory of lipid oxidation showing alternate reactions that compete with hydrogen abstraction (Schaich 2005).

current study, we are aiming at discover higher temperature may impose a thermodynamic influence on lipid oxidation, whether the carbon chain are thermally cut off to form radicals or it goes through peroxy group formation. Detailed discussions concerning three steps of lipid oxidation: initiation, propagation, termination are listed below.

### 2.1.1. Initiation

Initiation of lipid autoxidation has been discussed in detail in other references, particularly (Schaich 2005). Except where thermal scission radicals may abstract hydrogens from unsaturated fatty acids and thus initiate autoxidation chains during heating, initiation processes are not a part of this thesis so will not be discussed further here. Rather, emphasis will be given to propagation and termination reactions that determine specific products.

### 2.1.2. Propagation.

Propagation is the process of establishing a free radical chain reaction and keeping it going by a series of hydrogen abstractions. Two major radicals are involved in propagation. The first is  $\text{LOO}^\bullet$  which abstracts hydrogens to generate hydroperoxides and new radicals:



As autoxidation just gets started,  $\text{LOO}^\bullet$  is the main chain carrier. H abstraction by  $\text{LOO}^\bullet$  is very slow ( $k=36\text{-}62 \text{ L mol}^{-1} \text{ sec}^{-1}$ ) (Howard and Ingold 1967) and selective, abstracting only hydrogens with low bond energy, such as allylic  $-\text{CH}_2-$  next to double bonds (Kochi 1973).  $\text{LOO}^\bullet$  reactions dictate kinetics and pathways during early stages of oxidation.

Hydroperoxides decompose in light, heat, and the presence of metals, generating  $\text{LO}^\bullet$  radicals that are more reactive and less selective than  $\text{LOO}^\bullet$  (Bors 1987). With light

and heat energy, hemolytic scission of hydroperoxides yields two radicals, lipid alkoxy and hydroxyl:



Alkoxy radical reaction rates are several orders of magnitude faster than peroxy radicals and hydroxyl radical rates are even faster, so these radicals are responsible for rapid rate period of lipid oxidation. This process is often called “branching” because it generates additional radical chains at different rates and with different products than original  $\text{LOO}^\bullet$  reactions.

Alkoxy and hydroxyl radicals become the main chain carriers later in the chain reaction.

As was shown in Figure 2, addition, cyclization and scission reactions compete with H abstraction to determine the kinds of radicals and products resulting from  $\text{LO}^\bullet$  reactions. Ultimately, radicals are always transferred between molecules by hydrogen abstraction, but the original  $\text{LOO}^\bullet$  or  $\text{LO}^\bullet$  may not be the propagating radical, and the product mix is much more complicated than implied by the simple free radical chain. Accounting for multiple competing pathways will be important for sorting out thermal from autoxidation products, so these “unconventional” pathways are considered in more detail in the sections below.

#### **2.1.2.1. Chain Propagation by $\text{LOO}^\bullet$**

Peroxy radicals are the chain carriers in early stages of lipid oxidation. Competing reactions of peroxy radicals include (Schaich 2005):

- a. Atom or group transfer (H-abstraction)
- b. Rearrangement/Cyclization
- c. Addition to double bonds
- d. Disproportionation

- e. Beta-scission
- f. Recombination
- g. Electron transfer ( $\text{LOO}^\bullet + \text{e}^- \rightarrow \text{LOOH}$ )

The first three reactions are most important of these in terms of final products.

#### 2.1.2.1.1. Atom transfer by $\text{LOO}^\bullet$ (Hydrogen Abstraction)

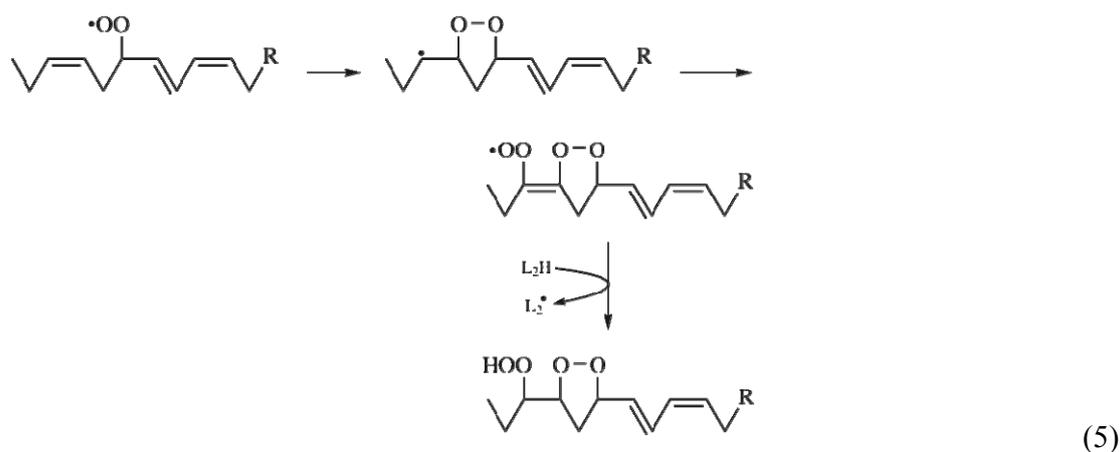
Hydrogen abstraction is the most important free radical chain reaction in lipid oxidation. Peroxyl radicals initially formed at any site on a fatty acid pass the unpaired electron to adjacent lipid molecules by abstracting hydrogens from an allylic position or a hydroperoxide and the process repeat itself indefinitely until the chain is intercepted. (Reaction 3, 4)



More radicals are formed after hydrogen abstraction, it will continue to abstract hydrogen repeatedly. Theoretically, it is an indefinite process.

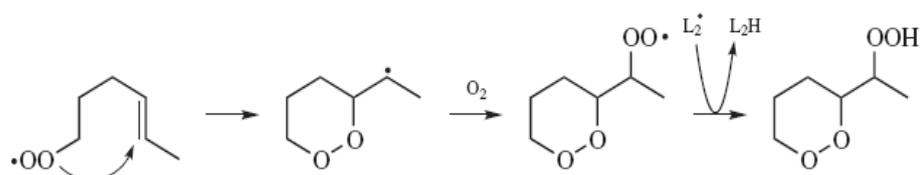
#### 2.1.2.1.2. Rearrangement/Cyclization of $\text{LOO}^\bullet$

Hydrogen abstraction dominates the theoretical process of propagation, however, there are other reactions compete with and supplement H abstraction for propagating the chain, these alternative reactions change both the kinetics and product mix of lipid oxidation. When abstractable hydrogen is not immediately available, peroxyl radicals find pairing electrons by adding to nearby carbon double bonds, cyclization occurs and form more complex products. The most important internal rearrangement or cyclization of  $\text{LOO}^\bullet$  proceeds by 1, 3-addition of the peroxyl radical to the neighboring *cis*-double bond, attach the beta carbon to form 5-exo ring of oxygen. (Reaction 5)



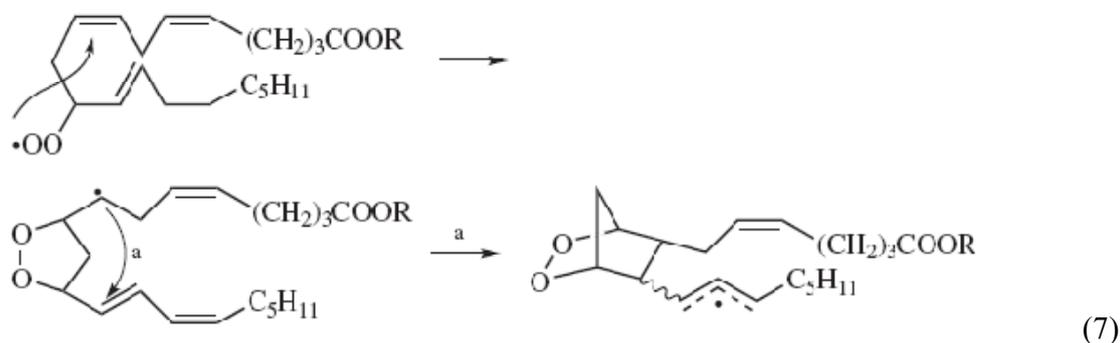
However, radicals formed during 1,3-addition can undergo being attacked by singlet oxygen to form hydroperoxide and further generate radical by scission of hydroperoxide.

Besides 1,3-addition by peroxy radical, 1,4-addition occurs on fatty acid with four or more double bonds. Although initial cyclization of  $\text{LOO}^\bullet$  via 1,4-addition to form endo cyclization to a 6-oxo ring is kinetically unfavorable (Porter, Funk et al. 1976), both 6-oxo exocyclic peroxide (Reaction 6) and endoperoxides (Reaction 7) has been observed as secondary oxidation or rearrangement products in arachidonic acid oxidation (Porter 1986).



<sup>1</sup>EPA: eicosapentaenoic acid, 20:5 $\omega$ 3; DHA: docosahexaenoic acid, 22:6 $\omega$ 3.

(6)

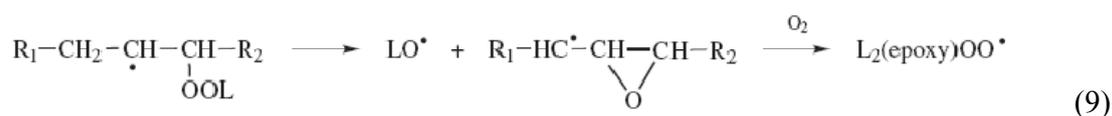


### 2.1.2.1.3. Addition of LOO• to double bonds

Peroxy radicals are quite specific in their addition preferences and it competes with external hydrogen abstraction. In early stages of oxidation, LOO• adds to double bonds to form an initial dimer complex (Reaction 8), which then reacts further to generate new radicals.



The LOOL' then undergoes beta scission to release epoxy alkyl and alkoxy radicals that continue to propagate the chain reaction (Reaction 9)



This presents an interesting analytical problem that epoxides are major products of lipid oxidation and derive from both peroxy additions and alkoxy cyclization. Consequently, it may be difficult to determine the mechanism that is operative in a given reaction system because both pathways contribute the formation of epoxide.



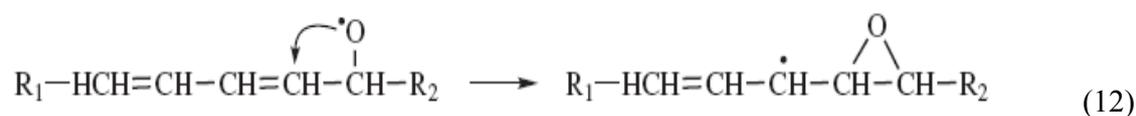


Fatty Acid	$k \times 10^{-6} \text{ L mol}^{-1} \text{ s}^{-1}$	
	Nonpolar Solvent	Aqueous Solution
Oleic	3.8	68
Linoleic	8.8	130
Linolenic	13.0	160
Arachidonic	20.5	180

**Table 1. Rate Constants for H -Abstraction from PUFA by t-BuO<sup>•</sup> in Various Solvents (Bors 1987). Reactivity of LO<sup>•</sup> is Comparable (Bors, Tait et al. 1984).**

#### 2.1.2.2.2. Rearrangement/ Cyclization of LO<sup>•</sup>

As peroxy and alkoxy compete with each other for hydrogen abstraction during propagation stage, the system may be hydrogen deficient and hydrogen accessibility become limited to alkoxy radical, thus, double bond site becomes more accessible to alkoxy radicals which carbon chains align on the surface. Cyclization/rearrangement occurs under some conditions that limited hydrogen accessibility exists, oxygen pressure is low and the system is at room temperature or lipid molecules are in high polarity solvents or aligned in monolayers, the dominant reaction shifts to internal rearrangement or cyclization in which LO<sup>•</sup> add to a beta double bond in 1,2 cyclization, forming epoxide and epoxyalkyl radicals (Reaction 12)

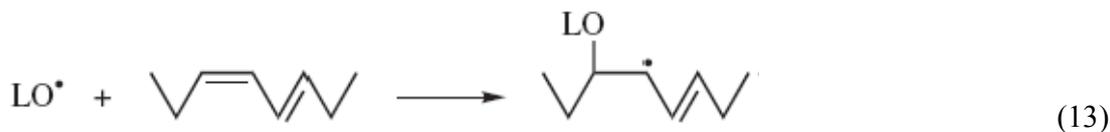


### 2.1.2.2.3. Addition of LO<sup>•</sup> to Double Bonds

Alkoxy radicals have unusually strong preference for allylic attack, so intermolecular H abstraction or internal cyclization will dominate as long as allylic/bis-allylic hydrogens are present. In absence of allylic/ bis-allylic hydrogens and by conjugation, alkoxy addition is favorable. (Table 2, Reaction 13)

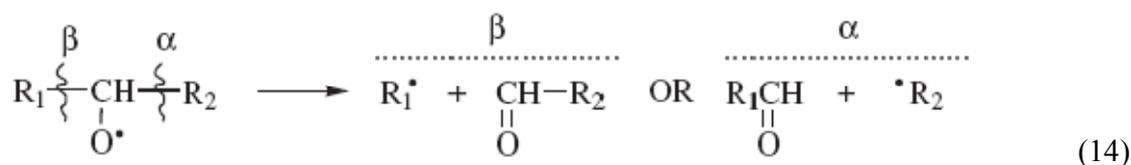
Alkene	Abstraction (%)	Addition (%)
R-CH=CH-R (trans)	95	3-4
R-CH=CH-R (cis)	83	17
R-CH=CH <sub>2</sub>	97	3
R <sub>2</sub> -C=CH <sub>2</sub>	83	17

**Table 2. Effect of Alkene Structure on Preference for Addition vs. Abstraction by t-BuO<sup>•</sup> Radicals at 40C (Kochi 1973).**

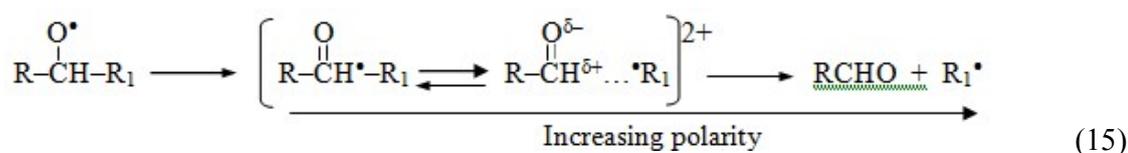


### 2.1.2.2.4. Alpha & Beta-Scission of LO<sup>•</sup>

Beta-scission of alkoxy radicals leads to scission of the C-C bond on either side of the LO<sup>•</sup> group to yield a mixture of carbonyl products and free radicals: aldehydes, alkanes, and oxo-esters (Nawar 1984). However, scission products are volatile thus so characteristically associated with rancidity and bad flavor of lipid oxidation. The simple mechanism of beta-scission of alkoxy group is illustrated below: (Reactions 14)



The reaction above either generates two acyl radicals which proceed propagation and two aldehydes which contribute rancid flavor of lipid. Besides, beta-scission is favored by polar solvent that support the formation of polar transition state that facilitate transformation of non-polar alkoxy radicals to polar aldehyde products (Reaction 15)



Solvent polarity increases relative rates of beta scission reactions over H abstraction is illustrated in Table 3

	H Abstraction	$\beta$ -Scission	
	$k_a \times 10^{-6} \text{ M}^{-1}\text{s}^{-1}$	$k_\beta \times 10^{-5} \text{ s}^{-1}$	$k_a/k_\beta \text{ M}^{-1}$
$\text{CCl}_4$	1.1	2.6	4.5
$\text{C}_6\text{H}_6$	1.2	3.7	3.2
$\text{C}_6\text{H}_5\text{Cl}$	1.1	5.5	2.0
$(\text{CH}_3)_3\text{COH}$	1.3	5.8	2.3
$\text{CH}_3\text{CN}$	1.2	6.3	1.9
$\text{CH}_3\text{COOH}$	1.3	19	0.7

**Table 3. Solvent Effects on Rates of H Abstraction and Beta-Scission of Cumyloxy Radicals ( $\text{CumO}^\bullet$ ) (Avila, Brown et al. 1993).**

### **2.1.3. Termination**

Termination of lipid oxidation is a tricky process, from a practical standpoint, the lipid oxidation chains probably never totally stops. In addition, a specific radical may abstract hydrogen or cyclization/rearrangement instead of radical combination to stop the reaction, even there is few amount of radicals, the termination process cannot totally eliminate radicals in the lipid oxidation environment. Net oxidation slows down when hydrogen abstraction accessibility is becoming limited or radical quenching process exceed the rate of new chain production, but it still would be difficult to stop entire oxidation process. Free radicals terminate to form non-radicals products by four major ways:

- a. Radical recombinations
- b. Cleavage reactions when large protons sources are present to stabilize products
- c. Co-oxidation with other molecules (e.g. protein)
- d. Eliminations

Radical recombinations include peroxy and alkoxy radicals combinations. However, the radical recombinations of peroxy and alkoxy radicals would release oxygen molecule which has strong ability to attack other radicals. Theoretically, termination process can be regarded as quench the radicals without any other reactive molecule generated.

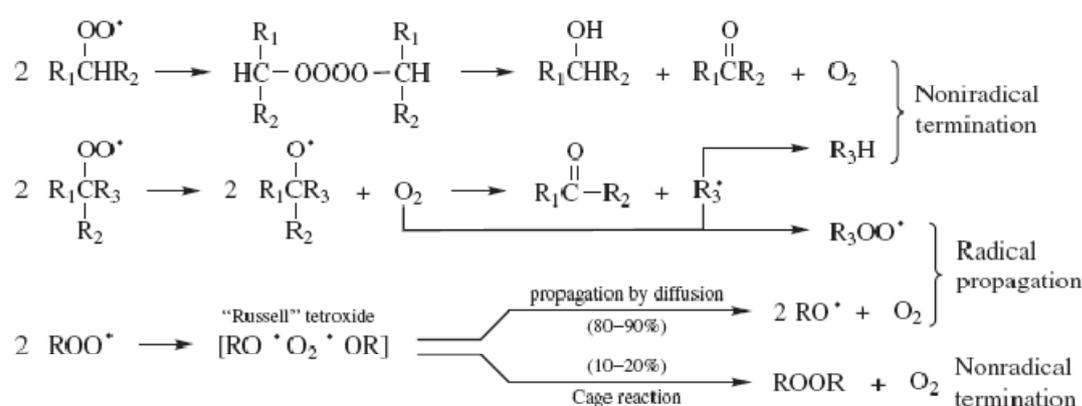
#### **2.1.3.1. Radical Recombinations**

The number of possible reaction of radical recombination is limitless and unpredictable. Due to the broad range of oxidation products detects in lipid oxidation from

literature, however, recombination is specific and follow patterns of favorable recombination.

### 2.1.3.1.1. LOO<sup>•</sup> Recombination

Peroxyl radicals are found to recombine rapidly to form wide range of products including alcohols, ketones, alkanes, acyl peroxide and peroxy radicals. (Figure 3)



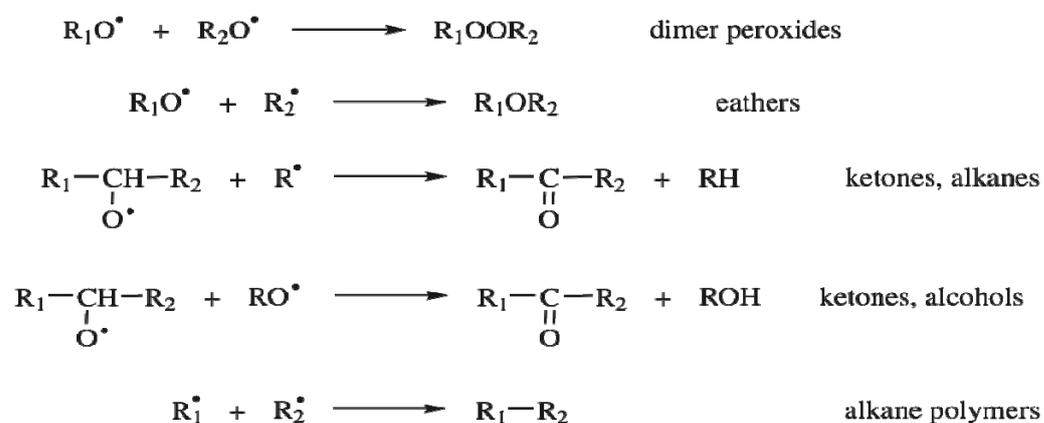
**Figure 3. Propagating radicals and non-radical products formed from recombination of peroxy radicals during lipid oxidation (Schaich 2005).**

There are several factors influencing the peroxy recombination reaction, depending on the nature of peroxy radical, the solvent and the temperature (Lindsay, Howard et al. 1973). The concentration of peroxy in lipid oxidation environment and limitlessness of hydrogen abstraction facilitate the recombination reaction. In addition, there is considerable controversy that peroxy recombination would release singlet oxygen. From reaction in Figure 4, as long as oxygen pressure is not limited, peroxy radical is more active as a propagating reaction than in termination to non-radicals. "Russell" tetraoxide intermediate proposed as the mechanism for peroxy radical recombination remains controversial. If exists, it will release singlet oxygen, however, some studies claimed to

detect singlet oxygen from lipid hydroperoxides (Howard and Ingold 1968). Releasing singlet oxygen may interrupt the radical recombination reactions.

### 2.1.3.1.2. $\text{LO}^\bullet$ and $\text{L}^\bullet$ Recombination

From wide range of products formed in literature and history lipid oxidation research, a wide variety of alkoxy and alkyl radical recombinations have been proposed to explain lipid oxidation products observed in model reaction system. Alkoxy and alkyl recombinations outlined below to provide a pathway to non-radical products (Figure 4).

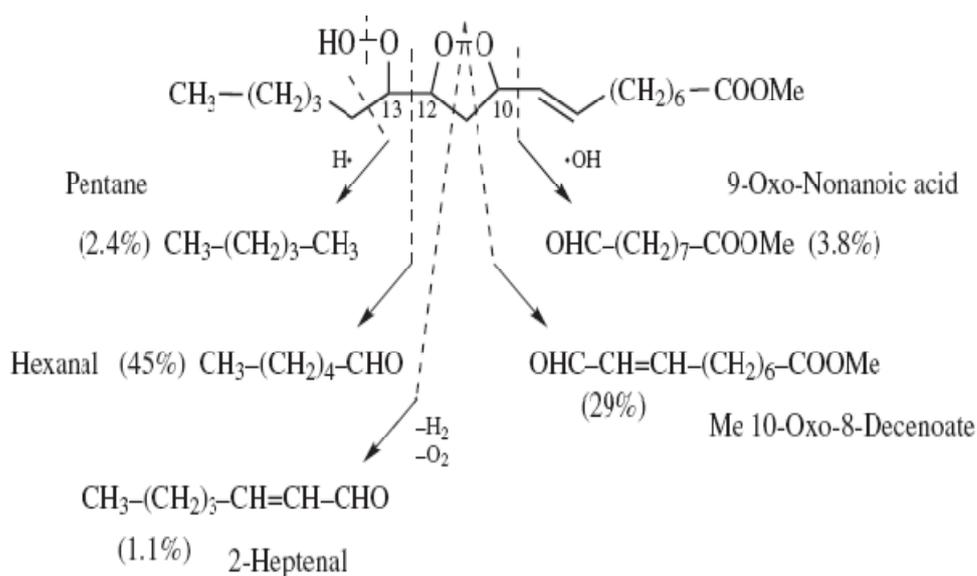


**Figure 4. Products formed via recombination of various intermediate radicals in lipid oxidation (Schaich 2005).**

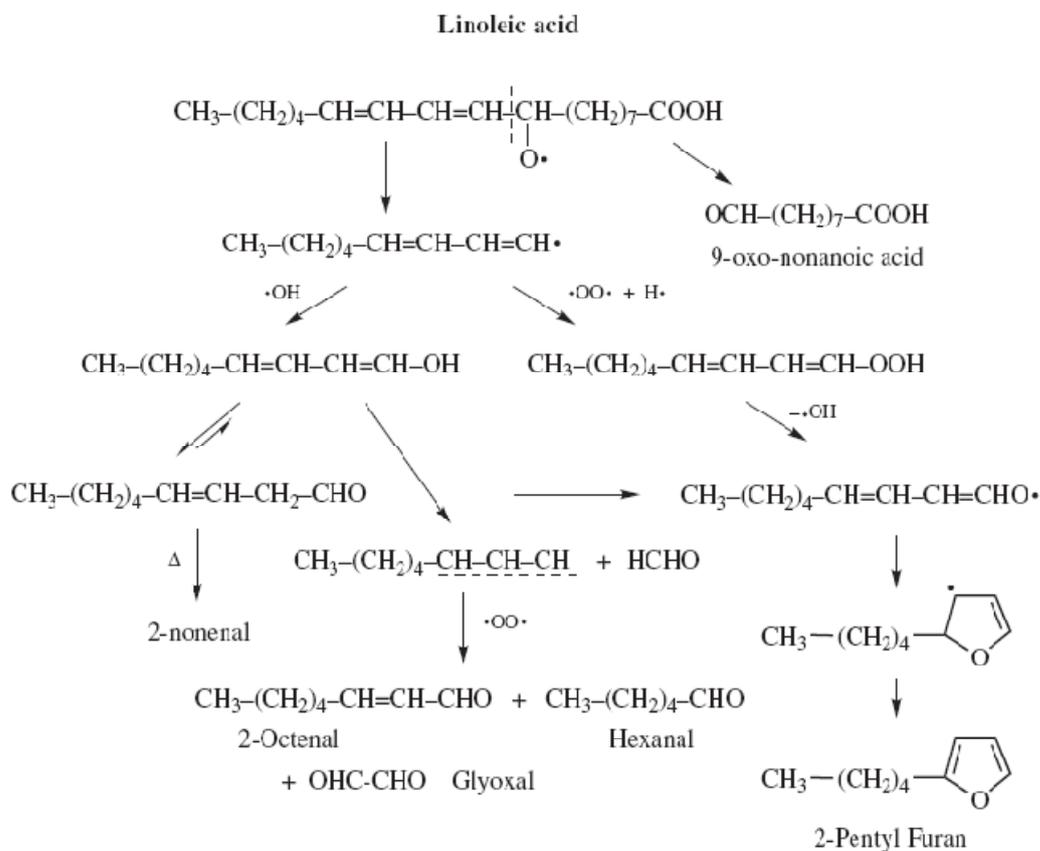
Obviously, recombination leads to polymers. However, recombination of the fragment radicals formed in alpha and beta scissions of alkoxy radicals generate low levels of volatile compounds, new formed ketones may undergo scissions to increase the volatile concentration. This may be an explanation for continuous flavors.

### 2.1.3.2. Other Pathways of Termination

As discussed above, beta-scission of alkoxy radicals are the major source of aldehyde products in lipid oxidation—a major aldehyde product and a propagating radical are formed via scission of the initial alkoxy radical in a fatty acid. The propagating radical may undergo radical recombination to form non-radical compound or hydrogen abstraction series reaction to generate various flavors. Scission products and pathway is too complex to illustrate here, however, scission of intermediate products make important contributions to the total mix of compounds generated during lipid oxidation, shown here for scission reaction of epoxidides and linoleic acid and esters. (Figure 5,6)



**Figure 5. Decomposition of epoxidides formed during photosensitized oxidation of linoleate increase yields of major aldehydes and also produce longer chain aldehydes (Frankel 1985).**



**Figure 6. Oxidation and subsequent scission of radicals released in scissions of initial alkoxyl radicals augment some of the original scission aldehydes, although by different routes (Frankel 1985).**

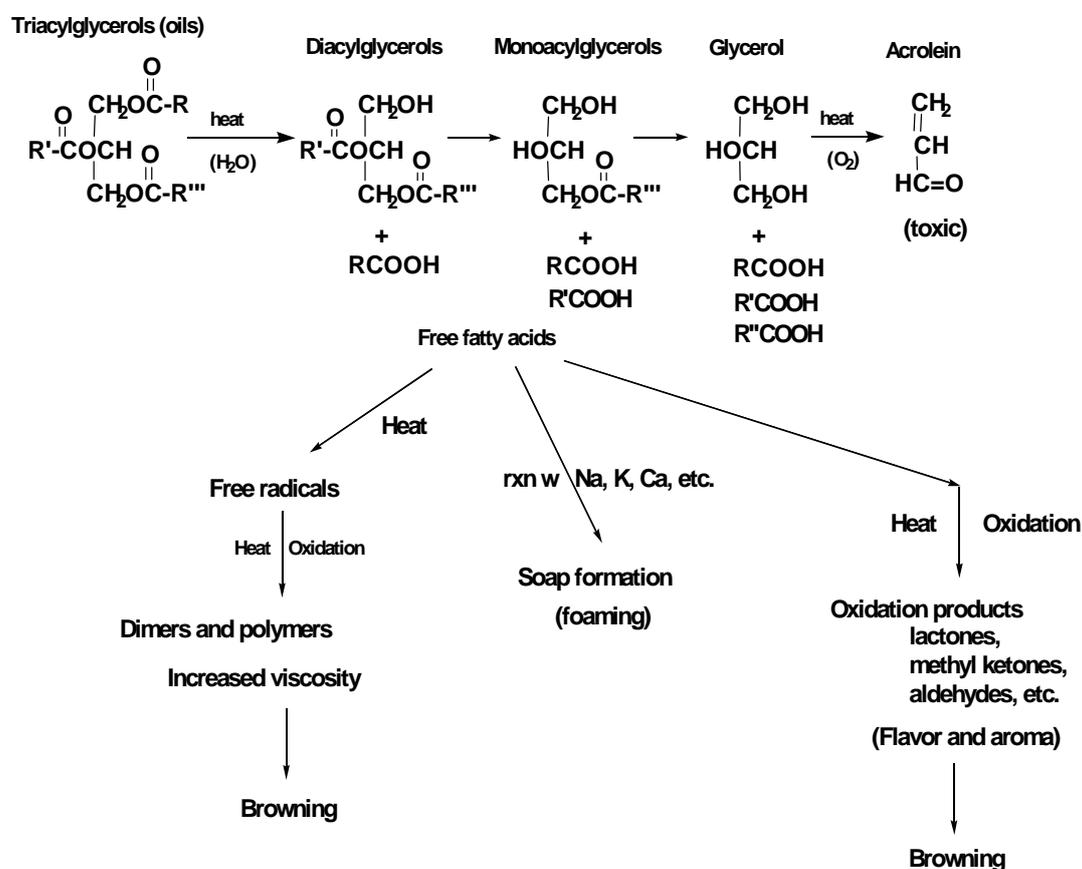
## 2.2. Frying Chemistry/Thermal Degradation of Lipids

Lipid oxidation which occurs at higher temperature 100°C or above is considered as frying process. Frying chemistry is generally complex which incorporate two key processes: autoxidation and thermal scission. Arrhenius effects of heat on reaction kinetics, and the presence of water (introduced with foods) that facilitates hydrolysis. Although frying chemistry of oils has been studied extensively (Kamal-Eldin 1997); (Shen, Fehr et al. 1997); (Saguy 2003); (Coni, Podesta et al. 2004); (Frankhauser-Noti 2006), the detailed mechanisms of thermal degradation remain poorly understood and highly controversial, with several schools of thinking.

Global mechanism about frying process is illustrated below (Figure 7), which triglyceride is cleaved by heat at release three different free fatty acids. Upon higher temperature than normal oxidation conditions, free fatty acids are likely to generate free radicals, which initiate the chain reactions by high temperature. However, our data showed that there are preferential scission positions on free fatty acid chain when free fatty acids remains on triglyceride, or scission spontaneously occur at different position at both free fatty acid chain or ester position of triglyceride, as we only measured the volatile compounds from frying. Here list possible pathway of frying mechanism of free fatty acid: Free fatty acids are quite reactive and go on several possible ends.

- 1) They oxidize to a variety of small products, including aldehydes, methyl ketones, and lactones that are characteristic flavor and aroma compounds (Kamal-Eldin 1997).
- 2) They polymerize to dimers, trimers, and higher polymers, with and without oxygen bridges that increase viscosity of oils (Nawar 1984).
- 3) They react with any soft metals present to form soaps that contribute to foaming

and formation of off-flavors.



**Figure 7. Generalized scheme for oil degradation during frying (Schaich 2008).**

As data showed in our experiment is different from global mechanism illustrated, the generalized scheme for oil oxidation was brought by commercial frying or in research settings that model commercial frying, where heating is under abusive conditions. Commercial frying institute concludes a frying curve with heating time. (Figure 8, Table 4)

	CHEMICAL REACTIONS		
	I	II	III
<b>Dominant Reactions:</b>	Hydrolysis to FFA's, mono-di-acylglycerols	Oxidation, scission, some polymerization	Secondary oxidations, scissions, polymerization, cyclization
<b>Major Effects:</b>	1) Increase in surfactant properties	1) Oxidation of unsaturated FA's	1) Glycerol oxidation to acrolein (sharp odors, toxic)
	2) Decrease in smoke point	2) LOOH decomposition ↓ aldehydes, ketones, lactones, etc.	2) Extensive polymerization → increase in viscosity
	3) Increase in metal chelation (oxidation catalysts)	3) Volatile scission products → odors	3) Increased scissions and oxidation → oil breakdown
	4) Increase in soap formation	4) Non-volatile products → flavors	4) Formation of toxic products
		5) Browning	
		6) Limited crosslinking	

Table 4. Major effects of frying process change with time (Blumenthal 1991).

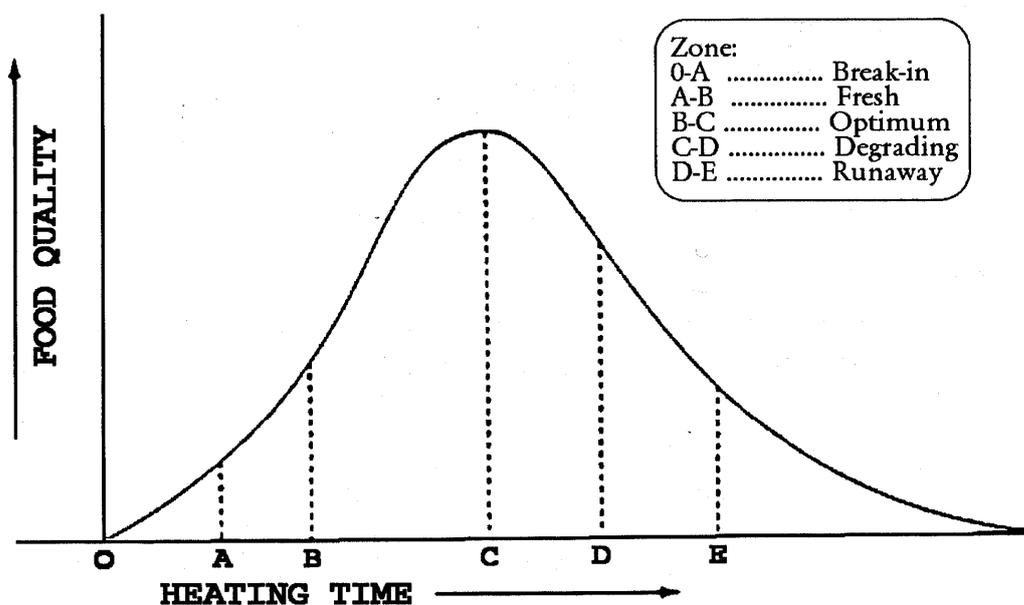
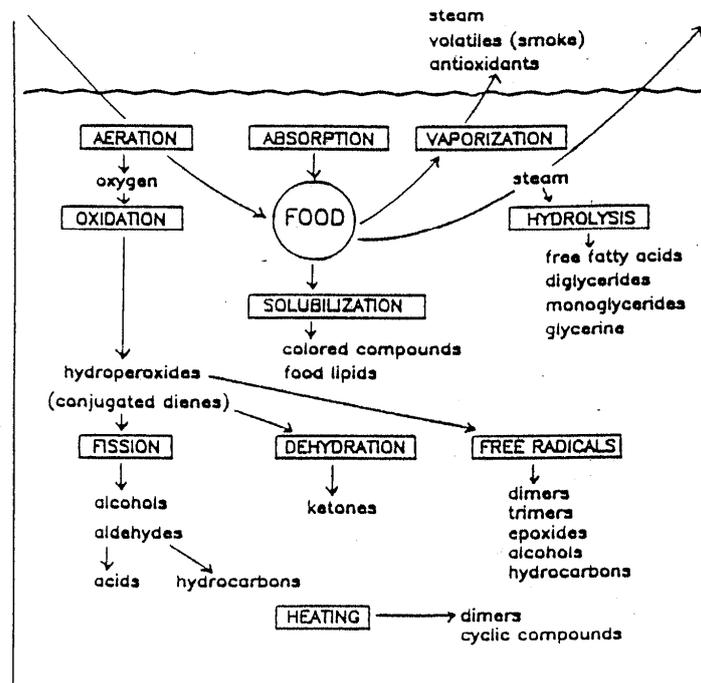


Figure 8. Frying curve developed by Michael M. Blumenthal, Copyright Libra Laboratories, Inc. [www.libralabs.com](http://www.libralabs.com)

### **2.2.1. Basic frying process in Foods**

Deeping frying food in hot oil has, for hundreds of years, been a very popular cooking method because it is fast, convenient, creates interesting flavors and textures. Frying is a complex process in which many physical and chemical changes occur together in the hot oil and food sink in it. These processes are summarized below (Figure 9). Frying food in hot oil can contribute for desirable flavor, improving functionality of food. However, some changes are degradative, leading to breakdown of the oil. The physicochemical state of oil during frying is constantly changing and the balance between desirable and degradative changes shifts from positive to negative as frying time and temperature increases. Understanding the mechanism of oil quality change is critically important for both industry and science area. Knowing and controlling the quality of oil during frying is crucial for food quality and mainly influence on consumer's health issues. Based on frying food in hot oil, there are major three major stages of frying (Schaich 2008).

- a. Break-in Phase, new oils have no fatty acids and they are totally hydrophobic, while food surfaces are hydrophilic and wet. So oil can barely contact with food surface and hardly penetrate into food matrix. In this stage, frying is slow and heat transfer between food and hot oil is limited.



**Figure 9. Schematic diagram of physical and chemical changes occurring simultaneously in frying oil and fried foods (Fritsch 1981).**

- b. At early stage of oil frying, free fatty acid is generated by heat. They act as surfactant to increase the surface contact of oil with food. The presence of surfactant increase the contact area between food and oil, and heat transfer is efficient between food and oil. During this period, the positive secondary reaction dominate, oxidation and scission to form flavor compounds and initiate browning, cyclization/rearrangement/polymerization of oil occurs but still at slow rate. During optimum frying stage, unsaturated fatty acids are oxidized and decompose into aldehydes, ketones, lactones etc. Volatile compounds offer desirable flavor, e.g. hexanal, 2, 4-decadienal, (E, E)-. Browning between protein and reducing sugar offers desirable texture, flavor and exterior features.
- c. Extended heating time will combine with increase hydrolysis as food release water into oil matrix and molecules formed from second optimum step will generate more

complex compounds mixture, e.g. cyclizations/ rearrangement. In addition, large amounts of radicals from thermal scission of oil molecules begin to accumulate, the oxidation chain reaction would be more complicated and hard to identify the quality of oil. Viscosity of oil increase due to accumulation of polymer. Toxic polymer, acrolein from glycerol accumulates as frying process extends.

### **2.2.2. Basic mechanism of lipids degraded at high temperature**

In the thermal degradation of lipids, it is often difficult to separate the specific individual effects of heat scission, hydrolysis and autoxidation. They share many same end products, however, their initial stage and consequent pathways are different. Basic research with model lipid systems, however, has contributed much to our understanding of the mechanisms involved in the thermal degradation of lipids. Merrit and Nawar made outstanding contribution to frying chemistry: thermal energy preferentially cleaves C-C bonds alpha, beta and gamma to the carboxylic acid group in saturated fatty acid and double bonds for unsaturated fatty acids (Nawar 1984), although products from radical scissions all along the chain have been observed (Yu 1997). However, preformed hydroperoxide during autoxidation of unsaturated fatty acids decompose in heat to alkoxy radicals, which may abstract hydrogen, rearrange to epoxides or decompose via alpha- or beta- scission reactions to form a variety of carbonyl compounds, acids, and alkanes. Alpha-scissions occur at the bond closest to the carboxylic end of the fatty acid, and beta-scissions occur at the bond on the methyl terminal side. In linoleic acid which can form hydroperoxide and alkoxy radicals at both 9- and 13- position, at least eight products may be produced under different conditions, however, according to result from previous frying result, hexanal, 9-oxo-nonanoic acid, octanoic acid, 2,4-decadienal, (E, E)- are first

stable products within short time exposure to high temperature. Based on volatile compound analysis in our thermal desorption system, hexanal and short chain aldehyde and unsaturated aldehyde are major compound in early stage of frying, in addition, cyclic compound is another marker during early stage of frying, e. g, furan,2-pentyl. However, based on our data showed that 2,4-decadienal, (E, E)- displayed on GC-MS chromatograph at very late stage. The result will discuss in following section.

Whatever the mechanisms and reaction pathways differ from each other, the kinetics of degradation increase dramatically with temperature. At room temperature, initiation by trace concentrations of metals, etc. and chain reaction of lipid oxidation are relatively slow, so oxidation has a very long induction period and research under room temperature takes several days to collect the volatiles and analyze the residue molecules in oil. In thermal degradation, high temperature would cleave on C-C bond to form radicals at early stage. Each scission forms two free radicals that can add oxygen to form peroxy radicals, or perhaps add  $-O^{\bullet}$  to form aldehydes or epoxides directly. However, due to activation energy of hydrogen abstraction is lower than for beta-scission, the peroxy radicals are prone to generate alkoxy radicals. Thus, wide range of radicals and radical concentration may be maintained for a long heating time. Challenges are present in understanding the specific mechanism of frying and tracing the products generated from frying. Due to large amounts of radicals formed, the reaction pathways are more complicated than autoxidation under moderate temperature and high temperature may alter the distribution and concentration of products (Nawar 1984). Since, as mentioned above (Nawar's data), thermal scission occurs randomly at all positions of acyl chains, aldehydes of a greater variety than in autoxidation, particularly short chain aldehydes, have been

reporte (Gillat 2001). Pentane, acrolein, pentanal, hexanal, heptenal and octenal are commonly reported, heptanes, octane, 2- decenal, (E)- and 2-undecenal, (E)- are reported as unique frying products (Warner and Gupta 2003) . Based on our data, main alkanal and alkenal distribute between C-4 and C-10 under air, same chain length of alkane/alkene volatiles and be trapped in adsorbent under nitrogen.

High levels of epoxides are also typical in frying oils. Methyl trans-9, 10-epoxystearate and methyl cis-9, 10-epoxystearate were observed in methyl oleate while methyl trans-9,10-epoxyoleate, methyl cis-9,10-epoxyoleate, trans-12,13-epoxyoleate and cis-12,13-epoxyoleate were also detected from methyl linoleate (Frankhauser-Noti 2006); (Marmeasat 2008). Under heating conditions, the amount of epoxide formation was proportional to the heating time (Frankhauser-Noti 2006).

Dimers and polymers also form during heating (Neff, Warner et al. 2000); (Marmeasat 2008) because the radical load can be extremely high and the radicals are too short lived to migrate so they connect with whatever is nearby. Current industrial practice aims to minimize the concentration of oxygen by flushing with nitrogen to decrease the generation of dimers/polymers. Dimers and polymers form from linear radical recombinations between molecules, but radical reactions within molecules lead also to cyclic and bicyclic products (Dobson 1997) in much higher levels than present during autoxidation at lower temperatures.

Besides formation of dimers/polymers which influence consumer's health and food quality, high temperature frying can lead to antioxidant ineffectiveness. Although diminished antioxidant effectiveness has most often been attributed to decomposition of antioxidants at high temperatures (Pellegrini 2001); (Pokorny 1987), the very high radicals

load from thermal scission and hydroperoxide decomposition also contributes to extensively “consume” capacity rapidly (Reblova 2006). Temperature is an important indicator of loss antioxidant ability, for example, Reblova found that the antioxidative activity of gamma tocopherol decreased as temperature increased from 80°C to 150°C, alpha tocopherol activity remained constant from the temperature range of 80°C to 110°C, and both tocopherols were totally ineffective at 150°C (Reblova 2006). Similar results were reported by Tomaino (Tomaino 2005). De Maria (De Maria 2000) found that while quercetin and 5-caffeoylquinic acid (5-CQA) were not effective in stabilizing soybean oil under heating conditions, quercetin decreased oxidation significantly, and 5-CQA also protected the oil but less effectively.

In summary, the high energy from frying makes thermal degradation of oils and fats very different from autoxidation in initiation mechanism, the ways to propagate free radicals, final products, and kinetics (Naz, Siddiqi et al. 2005). Although thermal degradation and autoxidation share some of end products, the pathways are basically different. Thus, studying thermal degradation requires different approaches than autoxidation at lower temperatures. Because of the multiple pathways, altered product distributions, and high concentration of volatile products which do not remain in the oil (Nawar 1984), analysis may not concentrate on one single product and should be concerned as product map changing with time/temperature. In addition, collecting all the products would be difficult due to extreme high kinetics under frying temperature. Traditional methods of frying research typically use long heating times (days to even weeks) in open fryers with little or no control over headspace, while monitoring hydroperoxides, total polars, and free fatty acids. However, open frying with such a long

time would lose a large amount of volatile and ignore the frying matrix changes from initial stage to termination stage. Under these conditions, sorting out reaction pathways and early versus late products is theoretically difficult. Research approaches of analyzing frying chemistry are discussed in below sections.

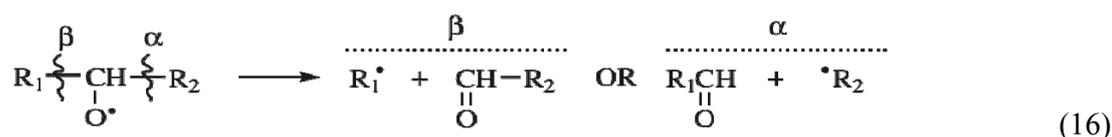
### **2.3. Methods for detecting and following the kinetics of lipid degradation**

Due to extreme complex kinetics under high temperature frying, measuring single group of compounds would be inaccurate here. In addition, higher temperature may alter the pathways and compound distribution from initial stage, both autoxidation and thermal scission occur to generate wide range of free radicals including peroxy, alkoxy, acyl radicals, however, two pathways may share same middle products and their existence may enhance or stop other reactions. Thus, the best way to monitor frying chemistry change in compounds variety and concentration is collecting a sequence of volatiles versus time and temperature. The classic method to analyze the compounds generated from frying process is HPLC-MS or GC-MS for both non-volatiles and volatiles. However, during frying process, hydroperoxides are highly unstable and hard to trace. The main goal of monitoring the process of frying corn oil is exploring the mechanism between autoxidation and thermal scission. Hydroperoxide would be an important middle compound existing during frying. Secondary compounds are major target in this project in order to plot a map of secondary volatile compounds versus time and temperature.

It is well-documented that hydroperoxides are highly unstable and can easily break down to secondary products, particularly aldehydes (Ellis, Gaddis et al. 1966; Ellis, Gaddis et al. 1968; Frankel 1982; Frankel 1987; Esterbauer and Zollner 1989). Aldehydes therefore are another class of major products measured when studying lipid oxidation

(Claxson 1994; Kamal-Eldin 1997; Moreno 1999; Schaich 2005; Smith, King et al. 2007; Guillen 2009), although the techniques are less well defined and more problematic.

Scission of alkoxy radicals is one of the major reactions that form aldehydes. Table 1 has already shown that the O-O bond in hydroperoxides has very low bond energy (157 kJ/mol), so it readily decomposes to alkoxy radicals when exposed to heat, UV radiation, or reducing metals. The alkoxy radicals then undergo either alpha or beta scission reactions to form alkanes plus terminal alkoxy radicals that transform to aldehydes (Schaich 2005). (Reaction 16)



Short chain secondary products are volatiles that can be trapped and analyzed by GC-MS, long chain secondary product would stay in oil thus HPLC is a feasible instrument to analyze. Nevertheless, combination of HPLC and GC may offer a specific map of compounds, scission positions or rearrangement/cyclization patterns.

### 2.3.1. High Performance Liquid Chromatography

HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column, and a detector that provides a characteristic retention time for the analyte. The detector may also provide other characteristic information (i.e. UV/Vis spectroscopic data for analyte if so equipped). Analyte retention time varies depending on the strength of its interactions with the stationary phase, the ratio/composition of solvent used, and the flow rate of the mobile phase. It is a form of liquid chromatography but smaller column size, smaller beads inside column and higher pressures. The basic interaction mechanism of analyzing lipid

chemistry is differential retention time resulted from polarity of fatty acid. Since there are many ways to manipulate separation parameters (polarity of the mobile phase and stationary phase, oxidized lipid compounds, flow rate, etc.), choosing appropriate parameter is key step in analyzing lipid oxidation compounds. Both reverse phase and normal phase methods have been reported (Palmer 1984; Kaufmann 2001; Uran 2001). High performance size-exclusion chromatography is widely used in the separation of polar and non-polar fractions from oils and fats (Summo 2008). Conjugated dienes can absorb UV light under 234nm. However, other chemical structures are hard to detect running HPLC without derivatization. Schulte reported a method by reacting carbonyl groups in aldehydes and ketone with 2,4-dinitrophenylhydrazine (DNPH), then measure the concentration of derivatized compound at 370nm (Schulte 2002). Mass spectrometer coupled with HPLC is used to identify chemical compound with specified peaks (Kerwin 1996; Schneider 1997; Sjoval 1997). Other detectors like Electrochemical detector, which discriminate products by its redox potential; evaporative light scattering detector (Makinen 1996) suspended the solute into particular matter by atomized gas and photomultiplier detect the light passing through the atomized gas/particle mixture and results are generally based on Rayleigh scattering the response should be proportional to the mass of solute present; as a consequence, it is sometimes referred to as the mass detector.

### **2.3.2. Gas Chromatography**

Gas chromatography is a common type of chromatography used in analytic chemistry for separating and analyzing compounds that can be vaporized without decomposition. In some situations, GC may help in identifying a compound (match of retention time or mass spectrum). In preparative chromatography, GC can be used to

prepare pure compounds from a mixture (Pavia 2006).

Compared to high performance liquid chromatography, the component and mechanism of GC is different. In gas chromatography, the mobile phase is carrier gas (inert), usually there are three types of gas used in GC: helium, nitrogen, hydrogen. Hydrogen has lowest molecular weight which would result in optimum resolution. However, hydrogen is very reactive. Thus, scientists usually use helium and nitrogen for carrier gas. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a glass or metal tubing called column. There are two major types of column: packed column, capillary column. Packed columns are 1.5 – 10 m in length and have an internal diameter of 2 – 4mm. The tubing is usually made of stainless steel or glass and contains a packing of finely divided, inert, solid support material (e.g. diatomaceous earth) that is coated with a liquid or solid stationary phase. The nature of the coating material determines what type of materials will be most strongly adsorbed. Thus numerous columns are available that are designed to separate specific types of compounds. Capillary columns have a very small internal diameter, usually at range of millimeters and lengths between 25- 60 meters. In our project, we use Equity<sup>TM</sup>-5 fused silica capillary column which have statistics of 60m×0.32mm×0.001mm. Most capillary columns are made of fused-silica with polyimide outer coating. Equity<sup>TM</sup>-5 fused silica capillary column consists 5% (Phenyl)-Methylpolysiloxane which displays mainly non-polar interactions with compound inside column.

In a GC analysis, a known volume of gaseous or liquid analyte is injected into the GC injector which is kept at extreme high temperature to ensure volatilization, usually using a microsyringe/ solid phase microextraction fibers/ gas source switching system such

as thermal desorption unit). As the carrier gas flushes the analyte molecules through the column, the interaction between analyte and capillary column will inhibit the analyte going through the column at normal speed. The different analyte would have various time scales to reach the detector of GC, called retention time. As temperature in GC oven start to increase at a fixed rate, the binding analyte would volatilize earlier and later depending on the strength of adsorption on the coating material. Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column. A detector is used to monitor the outlet stream from the column. Generally, substances are identified (qualitatively) by the order in which they elute from the column and by the retention time of the pure analyte in the column under the same temperature program.

#### **2.3.2.1. Samplers/Autosamplers**

The autosampler provides an easy way to inject a sample automatically into the inlets. Manual injection is feasible and low-cost, however, automatic injection provides better reproducibility and time-optimization. Comparison of three major samplers system is discussed below.

##### **2.3.2.1.1. Syringe injection**

Syringe injection can divide into liquid injection and headspace injection. Liquid injection usually use syringe from ten micro-liters to ten milli-liters to absorb small volume of analyte and inject into inlets of GC directly. The liquid analyte then undergo volatilization due to high temperature of inlet, inlet is set to 250°C in our experiment. The negative effect of syringe injection is lost of analyte during absorption from small vials to injection into inlets and some analyte may stay on syringe needle to influence the

quantitative result from GC-MS. In order to keep the reaction system with constant chemical concentration, after absorbing certain volume of analyte, replenishment is necessary, however, the system reaction may change at longer time. Especially during time sequence analysis, liquid injection is not an ideal method. When analyzing liquid sample, it is still a feasible way to inject sample, e.g. determination of organic compounds in water, sediments, and sewage sludge using perdeuterated internal standard by Large-Volume-Injection GC/MS (Cédric 1998).

Headspace injection is used to directly analyze the volatiles from vials. A headspace sample is normally prepared in a vial containing the sample, the dilution solvent, a matrix modifier and the headspace. Volatile components from complex sample mixtures can be extracted from non-volatile sample components and isolated in the headspace or gas portion of a sample vial. It is a fast and low-cost way to simplify the injection process compared to other injection instrumentation, e.g. fast way to characterize sensory flavor profile of espresso coffee aroma (Mateztu 2001), comparison of the volatile flavor compounds of six European cheeses by Headspace-GC-MS method (Bosset 1993). Nevertheless, headspace is also problematic that this injection process can also lose chemical compounds from absorption by syringe. In addition, according to lipid oxidation, the oxidation matrix loses certain volume of gas which would fluctuate oxygen concentration in vial thus affect the lipid oxidation rate and end products. Replenishment of oxygen (air) usually applies to minimize the negative effect. However, replenishment can lead unknown factors from air to oil matrix which may alter the final result.

#### **2.3.2.1.2. Solid Phase Microextraction (SPME)**

Solid Phase Microextraction, or SPME is a sample preparation technique used in

the laboratory and on-site. Developed in the early 1900s at the University of Waterloo by Dr. Pawliszyn's group (Pawliszyn 1999), it is simple and inexpensive technique where solvent is not necessarily used. SPME involves the use of a fiber coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent), which extracts different kinds of analytes (both volatile and non-volatile). The amount of analytes extracted by the fiber is proportional to its concentration in the sample matrix as long as equilibrium is reached with help of agitation. After extraction, the SPME fiber is transferred to the GC inlet, the high temperature of GC inlet allow vaporization of analyte. The attraction of SPME is that the extraction is fast and simple and can be done without solvents, and detection limits can reach parts per trillion (ppt) levels for certain compounds. SPME have also potential of on-site application. However, it is still inaccurate for frying analysis due to large amount of volatiles generated from frying and SPME would not be easily to monitor the concentration changes, compounds distributions and pathways alternations because it is not feasible to "store" compounds at certain period and make a sequence sample collection for further analysis. Scientific area still apply this method to analyze lipid oxidation, e.g. monitoring the oxidation of almond oils by SPME-GC-MS and ATR-FTIR (Beltrán 2010).

#### **2.3.2.1.3. Thermal Desorption (TD)-GC-MS**

Thermal Desorption Unit was invented by Dr. Thomas Hartman in Food Science Department, Rutgers University, New Jersey. Thermal desorption units sit directly on top of a GC injection port to provide for the direct desorption of both volatile and semi-volatile samples into the GC injection port and column. Due to the "short path" compared to syringe injection and SPME, these new systems overcome the shortcoming of previous desorption systems by eliminating transfer lines, which are easily contaminate the sample

and by providing for the optimum delivery of samples to the GC injector via the shortest path possible-the direct, syringe-like injection into the GC. Thermal desorption unit generally consist gas lines, pressure gauge, heating block and traps. Inside traps, by adding adsorbent material Tenax<sup>TM</sup> and Carboxen<sup>TM</sup>, volatiles can be trapped by Purge & Trap system. After collection of volatiles, the trap is a closed system which both side is covered with caps, volatiles would not be influence by other environmental factor. In case of desorption, the TD unit is connected with air and helium (carrier gas), the controller of TD will utilize air to push the trap with a needle on it into the injection port of GC, heating block will thereafter cover the trap and increase the temperature (250°C in our experiment) and flush the volatiles away from adsorbent into GC system with carrier gas. Desorption may take few minutes to totally flush all the volatiles into GC system (5 minutes in our thermal desorption system).

The advantage of Short path thermal desorption system is to minimize interference of manual operation, in addition, it is a closed system which other environmental factors may influence the final result. Scientific area has been utilized thermal desorption-GC-MS in determining the trace amounts of off-flavor compounds in drinking water (Nobuo 2001), direct sample introduction to thermal desorption-GC-MS to analyze of semi-volatile organic compound in atmospheric aerosols (Alla 2001), effect of packaging on the lipid oxidation storage stability of dehydrated pinto beans using thermal desorption unit (Hartman 1994).

### **2.3.2.2. Detector of Gas Chromatography**

The most common are flame ionization detector (FID) and thermal conductivity detector (TCD). While TCDs are essentially universal and can be used to detect any component other than the carrier gas, FID is sensitive primarily to hydrocarbons, however, FID is destructive by “burning” and cleave hydrocarbon into small pieces. Since TCD is not destructive, it can be operated in series before an FID, thus providing complementary detection of same analyte. Several GCs are connected to a mass spectrometer which acts as the detector. GC-MS has been utilized in several scientific areas, some GC-MS are connected to NMR spectrometer or GC-MS-MS use two mass spectrometers to analyze isotope. In our experiment, lipid oxidation products are mainly hydrocarbons, high sensitive FID and Mass spectrometer are used to identify volatile compounds. We use mass spectrometer to qualify MS data and compare to their retention time with FID. Although there is difference of retention time of each detector (due to vacuum parts of mass spectrum), comparison of retention time is still helpful in identifying peaks.

### **3. HYPOTHESIS & OBJECTIVES**

#### **3.1. Hypothesis**

High temperatures ( $>100^{\circ}\text{C}$ ) induce scissions of fatty acid acyl chains and the resulting radicals are important sources of primary products and initiation of secondary reactions including lipid autoxidation. In early stages of heating or with short heating periods, thermal scission rather than autoxidation will be the dominant mode of thermal degradation, even in air.

#### **3.2. Specific Goals and Objective of This Study**

1. Use purge and trap methodology to heat corn oil and collect generated volatiles under controlled conditions.
2. Heat oil samples to a range of temperatures (100, 120, 150, 180, 235 °C) under air and nitrogen.
3. Collect volatiles from each sample over time to determine the distribution, pattern, and kinetics of product generation.
4. Separate and quantitate products by gas chromatography and identify products by mass spectrometry.
5. Compare product distributions under nitrogen vs air to determine contributions of heat alone and to observe shifts induced by oxygen.
6. Compare products detected in heated oils with those normally expected from autoxidation.
7. Determine role and contribution of thermal scission processes (if any) in overall thermal degradation of corn oil.

## 4. MATERIALS & METHODS

### 4.1. Materials

Corn oil was selected for this study because it is used worldwide for frying, it is predominantly linoleic acid (which simplifies the types of products generated), and it is the most common oil added to foods for to raise linoleic acid content. The source oil used was Mazola™ corn oil (verified authentic, gift of Libra Technologies, Metuchen, NJ), which had the fatty acid composition listed in Table 5. The oil contains tocopherols added for stability.

Fatty acid percentage in corn oil	Fatty acid content
55% polyunsaturated fatty acids	98% linoleic acid (C18:2) 2% alpha-linolenic acid (C18:3)
30% monounsaturated fatty acids	99% oleic acid (C18:1)
15% saturated fatty acids	80% palmitic acid (C16:0) 14% stearic acid (C18:0) 3% arachidic acid (C16:0)

**Table 5. Fatty acid distribution in corn oil used for this study (www.mazola.com)**

Tenax TA 60/80 5.0 grams was purchased from Scientific Instrument Services, Inc. (Ringoes, NJ). Carboxen™ 20/45 mesh grams was purchased from Supelco (Bellefonte, PA). Benzene-d6 and Toluene-d8 internal standards for GC-MS analyses were purchased from Aldrich (St. Louis, MO); naphthalene-d8 was purchased from Isotec (Miamisburg, Ohio). Celite™ 545 (diatomaceous earth) was purchased at Fisher Scientific Inc. (NJ)

## **4.2. Experimental procedures**

### **4.2.1. Glassware cleaning**

Glass purge and trap tubes and containers in contact with corn oil in this experiment were cleaned with the following procedure:

- 1) Glassware was washed with Citranox detergent, rinsed three times with tap water to remove detergent residues, then three times with 18 M $\Omega$  resistivity high purity water (Milli-Q water purification system). Citranox (manufacturer and city) is a phosphate free detergent designed to bind trace metals; it is composed of a blend of alkanolamines, anionic and non-ionic surfactants and organic acids (including citric acid).
- 2) Washed glassware from 1) was soaked in potassium hydroxide-saturated denatured alcohol overnight to saponify any lipid residues. Glassware was then removed from the alcoholic KOH, then washed and rinsed as described in 1).
- 3) Glassware from 2) was then soaked overnight in 1 N hydrochloride acid (HCL) to remove trace metals, rinsed three to five times with 18 M $\Omega$  resistivity high purity water, and dried for one hour in an oven to remove moisture totally.

### **4.2.2. Oil storage and handling**

Mazola corn oil was transferred from its commercial package into labeled 500 ml glass bottles, flushed with argon for 5 minutes, sealed, and stored frozen at -20°C.

Before experiments, oil was removed from the freezer, brought to room temperature in a water bath, then sparged in conical flask with argon for 15 minutes to ensure limited oxygen concentration. To accomplish this, a Pasteur pipette connected to the inert gas line with moderate gas flow rate was placed in the conical flask which was also covered with aluminum foil to limit the light exposure of the oil. After sparging, the oil

was immediately loaded into glass purge and trap tubes containing Celite to increase the surface area of the oil, heated, and analyzed by gas chromatography and mass spectrometry.

#### **4.2.3. Purge & Trap System**

3.5 grams of Celite<sup>TM</sup> were loaded onto glass tube with one side filled with glass wool. Use shaker to tighten Celite<sup>TM</sup> compounds in the glass tube. Load 1 gram of argon-sparged oil onto the Celite<sup>TM</sup> support in the glass tube. Wait until oil dispersed into Celite<sup>TM</sup> compounds, then loaded glass tubes were attached to a Purge & Trap system (Scientific Instrument Services, Ringoes, NJ) with fittings on each end. The inlet fitting was connected to a gas supply (air or nitrogen) which maintained flow at constant rate of 40 to 50 ml/min; the exit fitting was connected to a thermal desorption trap packed with Tenax<sup>TM</sup> (50mg) : Carboxen<sup>TM</sup> (50mg) = 1:1 (Scientific Instrument Services, Ringoes, NJ) to collect volatiles. Tubes were heated to a range of temperatures (100, 120, 150, 180, and 235°C) under air or nitrogen. To limit the number of products and prevent overload, tubes were removed and replaced with a fresh tube periodically. Collection times for samples heated at 100, 120, and 150 °C were 5, 10, 15, 30, 45, 60, 90, and 120 minutes. Above 150°C, volatiles increased dramatically so collection times had to be shortened to 2, 5, 7, 10, 13, 15, 20, 25, and 30 minutes to avoid overloading the thermal desorption traps.

After heating, the oil and Celite compounds were removed into trash container with steel rod.

#### 4.2.4. Thermal desorption and GC-MS

Pretreatment of traps: After collection of volatiles, the thermal desorption traps were spiked with 1µl internal standard (benzene-d6, toluene-d8, naphthalene-d8 at 10 mg/ml), flushed with heating gas for 3 minutes, and desorbed at 20 to 30 ml/min for 1 hour at room temperature to remove excessive moisture. Traps were then capped until GC-MS analysis.

Thermal desorption: TD-2 short path thermal desorption unit (Scientific Instrument Services, Inc., Ringoes, NJ) was connected to both air and helium and pre-heated to 250°C. Loaded thermal desorption traps were connected to the thermal desorption unit via a 35mm seal-autodesorb needle, and the side with serial number was capped. The thermal desorption controller was set to 10 seconds helium flushing before injection, injection for 30 seconds, desorbed for 5 minutes with heating block closed. After injection and desorption, the trap was pulled out using air by thermal desorption system and the unit was allowed to cool to room temperature before turning off. Note that the pressure gauge of GC dropped and regained 20 psi sooner after injection, because time gap exists when injection may switch the gas flow from GC carrier gas to thermal desorption carrier gas.

Gas chromatography with mass spectrometry: Products were identified on one tube from each set by separating compounds on a Equity™-5 on a Varian 3400 gas chromatograph (Varian, Sugar Land, Texas) connected to a Finnigan MAT 8230 mass spectrometer (Finnigan, Cincinnati, OH), 80eV voltage and 25 kHz frequency. The injection port temperature was set to 250°C. To prevent loss of short chain volatiles ( $C \leq 5$ ), the initial temperature of GC was set to -20 °C for 5 min; the low temperature was

maintained by filling the GC oven with dry ice. A Varian 3400 GC (Varian, Sugar Land, Texas) was programmed to then increase the temperature to 280 °C at a rate of 10 °C /min.

Flow of separated components was routed from the GC directly into the MS for structure identification. The mass spectrometer scanned molecular weights from 35 to 350 minus 40 and 44 to exclude noise signals from carbon dioxide and argon.

#### **4.2.4.1. Determination of sample size**

Although the Purge & Trap system can load 10 grams of oil for collecting volatiles, reconciliation must be made between the collecting time and sample volume. 1, 5, and 10 gram samples were pre-tested at 150°C. 1 gram was determined to be the maximum amount of oil per tube.

#### **4.2.4.2. Determination of amount of celite compounds**

Preliminary tests were conducted to determine the amount of Celite™ required to fill the purge and trap tubes and increase the surface area of oil. 1, 3.5, 5, and 10 gram Celite were packed into purge and trap tubes. The optimum amount of Celite™ to completely fill the tube was found to be 3.5 grams.

#### **4.2.4.3. Effects of collecting time**

Preliminary experiments were conducted to determine how frequently to change the traps during oil heating to maintain a manageable number of peaks. At temperatures of 100 to 150°C, sampling times of 5, 10, 15, 30, 45, 60, 90, and 120 minutes were found to be adequate for renewing traps. However, at 180 and 235°C, increased degradation rates made it necessary to shorten collecting times to 2, 5, 7, 10, 13, 15, 20, 25, and 30 minutes to prevent overload.

#### 4.2.4.4. Effects of temperature

The heating temperature of the Purge & Trap system was varied (100, 120, 150, 180, and 235°C) to determine effects of temperature on product distribution and kinetics. 235°C was the highest temperature used because it is the smoke point of mazola corn oil. In addition, temperature series collections under 100/120/150°C are 30 minutes collection. At 180°C, the collecting time was changed to 10min, and at 235°C, the collecting time was shortened to 5 minutes to avoid overloading of volatiles.

#### 4.2.5. Identification and Quantitation of products from GC-MS data

Peak retention times and areas for each chromatogram were determined using MassLynx software. Peak components were identified from mass spectra patterns using MassLynx and NIST Mass Spectrum Database software packages.

Product concentrations in each trap were quantified by normalization of sample peak areas to peak areas of three internal standards. Peak areas of the internal standards were averaged and sample peak areas were divided by this average area and multiplied by the internal standard concentration (10 mg/ml) and volume (1 $\mu$ l):

$$\text{Peak Concentration} \left( \frac{\mu\text{g}}{\text{trap}} \right) = \frac{\text{Peak area}}{\text{Average area of 3 internal standard}} \times \frac{10\text{mg}}{\text{ml}} \times 1\mu\text{l} \quad (17)$$

## 5. RESULTS AND DISCUSSION

Thermal desorption traps were used to collect volatile products from heated corn oil under two sampling regimes: time sequences and temperature sequences. To determine changes in products as a function of temperature, samples at 100/120/150°C were heated under nitrogen or air for 30 minutes. Due to volatile overloading at temperatures >150°C, collection times were shortened to 10 min at 180°C and to 5 min at 235°C. Changes of products with heating time were determined by collecting volatiles at 5, 10, 15, 30, 45, 60, 90, and 120 minutes for temperatures up to 150 °C, and at 2, 5, 7, 10, 13, 15, 20, 25, and 30 minutes at 180 and 235°C.

In general, products formed under nitrogen are expected to be thermal products while products formed under air must be combinations of thermal and oxidation products. Oxidation products include compounds formed both by oxygen reaction with thermal scission radicals and by autoxidation chains initiated by thermal radicals.

Examples of chromatograms obtained under nitrogen and air are shown (Figures 10 and 11), respectively. Except when specific features may become important, for the remainder of this thesis, only data derived from the chromatograms (peak identities, areas, and retention times) will be reported.

### 5.1. Effect of heating temperature under nitrogen

Samples of corn oil were heated under nitrogen atmosphere to determine effects of heat without complications of secondary oxidation. Generally, an exponential increase in both peak intensity and numbers of peaks occurred between 120°C and 150°C and again between 180°C and 235°C. Under nitrogen, there were three classes of products (Tables 6-10, Figures 13-17): 1) pentane, by far the dominant volatile product, 2) a homologous

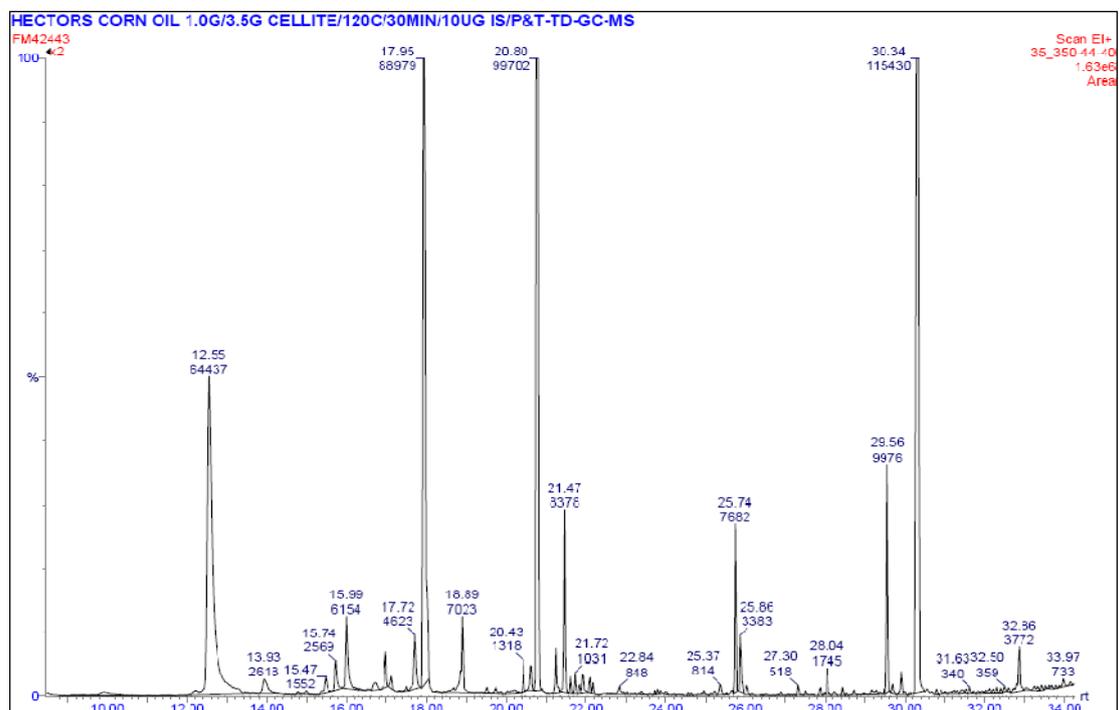


Figure 10. GC chromatogram of corn oil heated 120°C for 30min under N<sub>2</sub>.

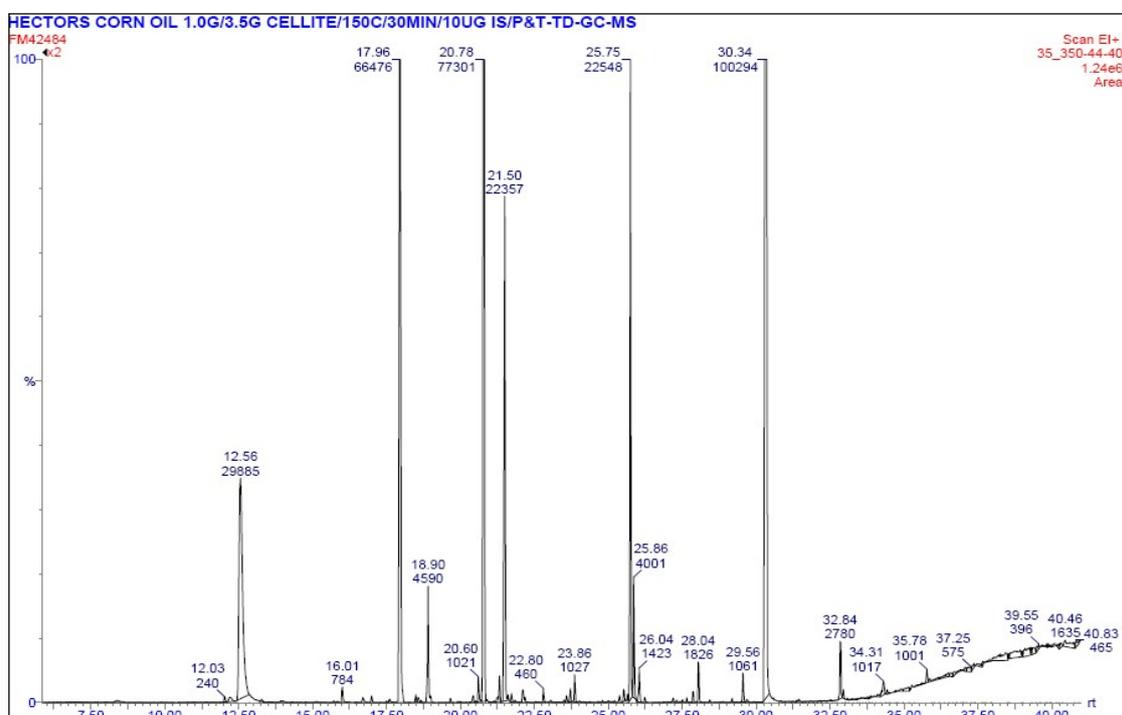
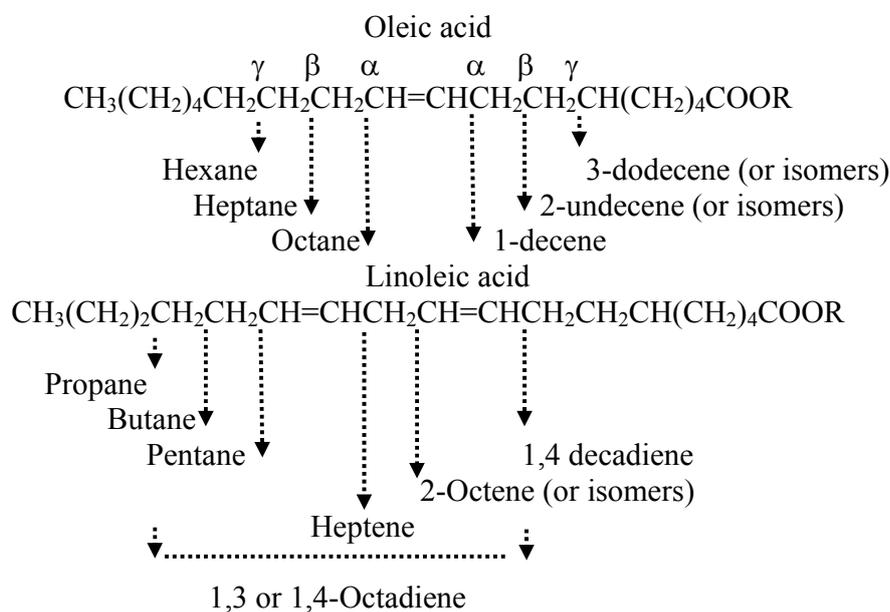


Figure 11. GC chromatogram of corn oil heated 150°C for 30 min under N<sub>2</sub>.

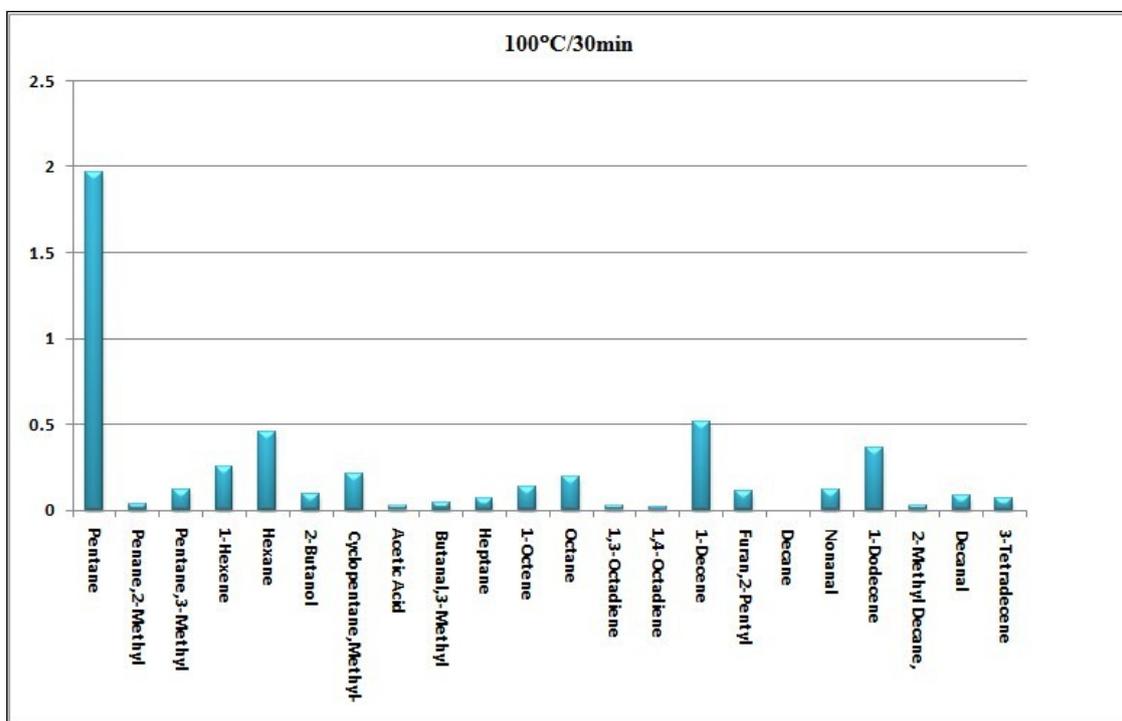
series of alkanes, alkenes, and their derivatives from two to twelve carbons in chain length, and 3) low levels of oxidation products of same chain length as the alkanes and alkenes, arising from traces of oxygen not removed by sparging.

Even at temperatures as low as 100 °C (Table 6), thermal scissions were evident in the hydrocarbon products. According to Fennema chapter (Nawar 1996), scissions preferentially occur at positions  $\alpha$ ,  $\beta$ , and  $\gamma$  to double bonds, and this pattern explains the major products observed (pentane, 1-hexene, hexane, heptane, 1-octene, octane, 1,3-octadiene, 1,4-octadiene, 1-decene, decane, and 1-dodecene). Although the dominant product, pentane, comes from linoleic acid, the next most numerous products are from degradation of oleic acid. Initial scission occurred preferentially from methyl end in linoleic acid and carboxylic end of oleic acid.



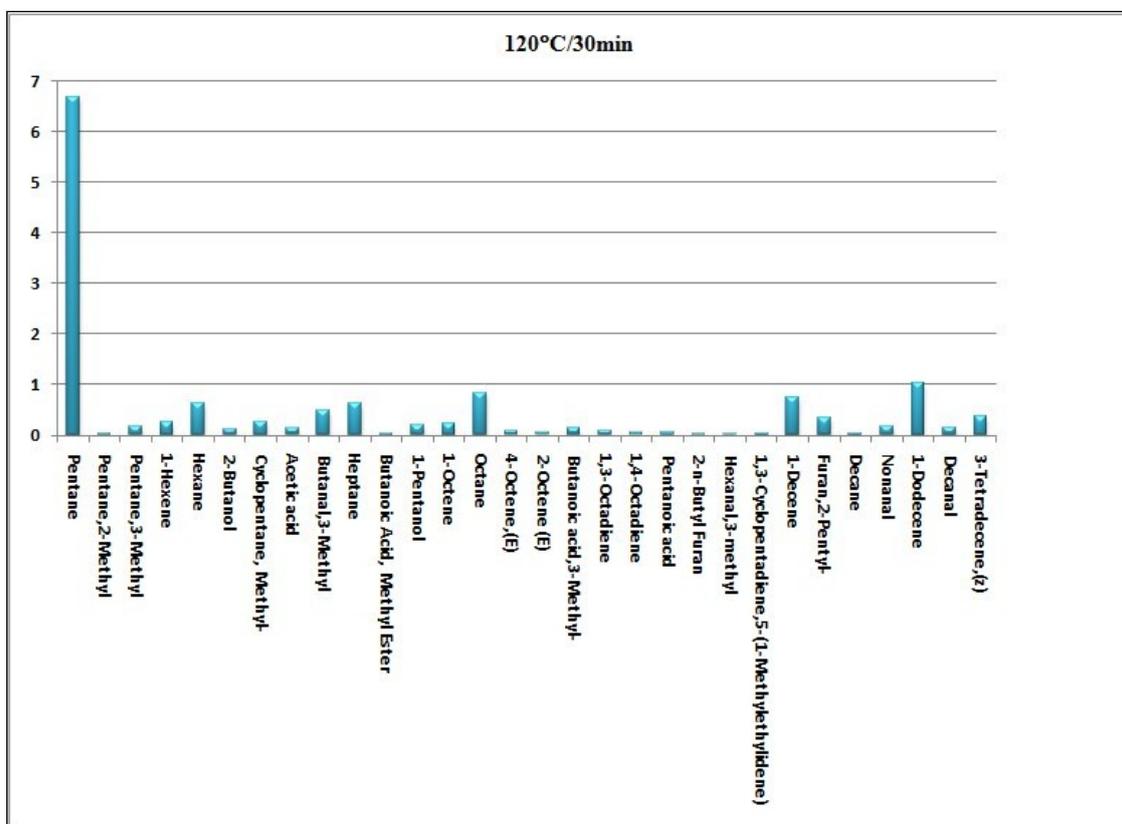
**Figure 12. Illustration of thermal scission on oleic acid & linoleic acid.**

The low levels of oxygenated products observed (2-butanol, 2-pentylfuran, nonanal, and decanal) resulted from oxidation of the thermal scission products, and were inconsistent with products expected from normal autoxidation.



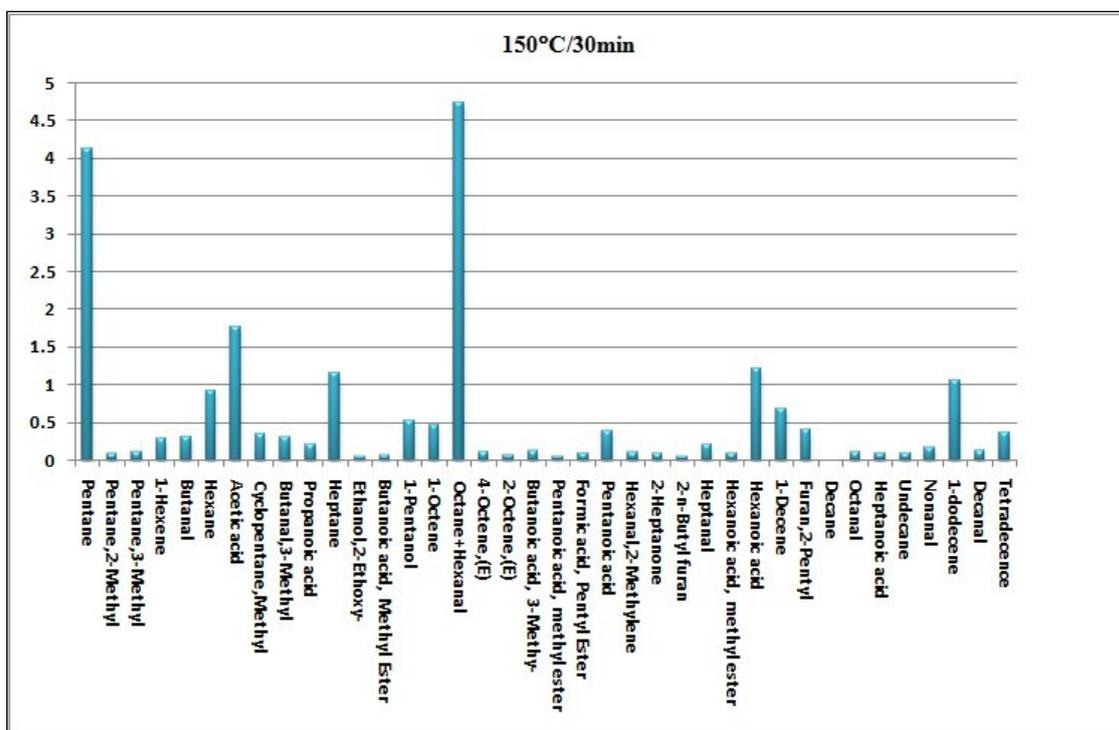
**Figure 13. Volatile product distribution in corn oil heated at 100°C for 30min under N<sub>2</sub>.**

When temperature was increased to 120°C for 30 minute, concentrations of products increased dramatically and the distribution of products became more complex (Table 7, Figure 14). Pentane remained predominant with 7 µg generated per trap. Again, a homologous series of alkanes and alkenes was observed, with carbon chain from C-5 to C-12: pentane, 1-hexene, hexane, heptane, 1-octene, octane, 1,3-octadiene, 1,4-octadiene, 1-decene, decane, and 1-dodecene. Interestingly, scission products from the methyl end of oleic acid increased more than products from the acid end. In addition, rearrangement products and branched isomers of the alkanes increased. Oxidation products such as 1-pentanol and 2-butyl furan were again present, but at low concentration.



**Figure 14. Volatile product distribution in corn oil heated at 120°C for 30min under N<sub>2</sub>.**

These patterns were maintained as temperature was raised to 150°C for 30 minutes, although concentrations of products increased dramatically and the distribution of products became more complex (Table 8, Figure 15). Pentane and octane were the major products. Other major alkanes/alkenes were 1-hexene, hexane, heptane, 1-octene, 1-decene, and 1-dodecene. Oxidation products of these and additional scission compounds accumulated to higher levels: butanal, 3-methyl butanal, 1-pentanol, pentanoic acid, hexanal, hexanoic acid, 2-heptanone, heptanal, 2-n-butyl furan, octanal, nonanal, and decanal. The oxidation products all stayed at very low concentrations ( $\mu\text{g}/\text{trap}$ ) compared to major alkanes/alkenes, resulting from traces of dissolved oxygen in the oils.

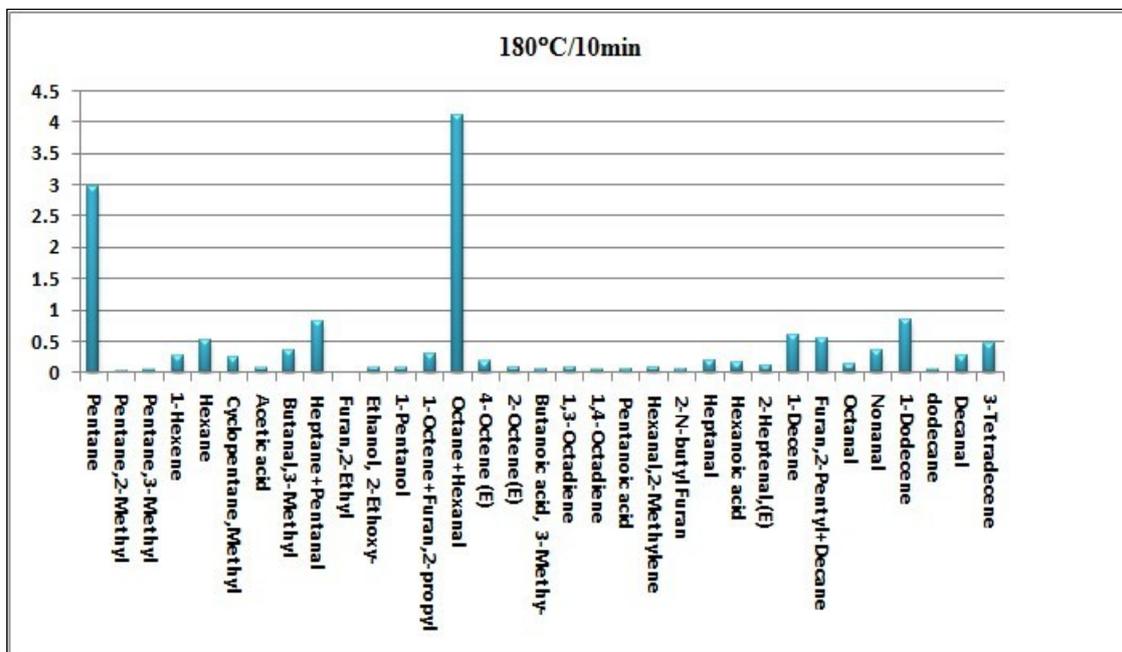


**Figure 15. Volatile product distribution in corn oil heated at 150°C for 30 min under N<sub>2</sub>.**

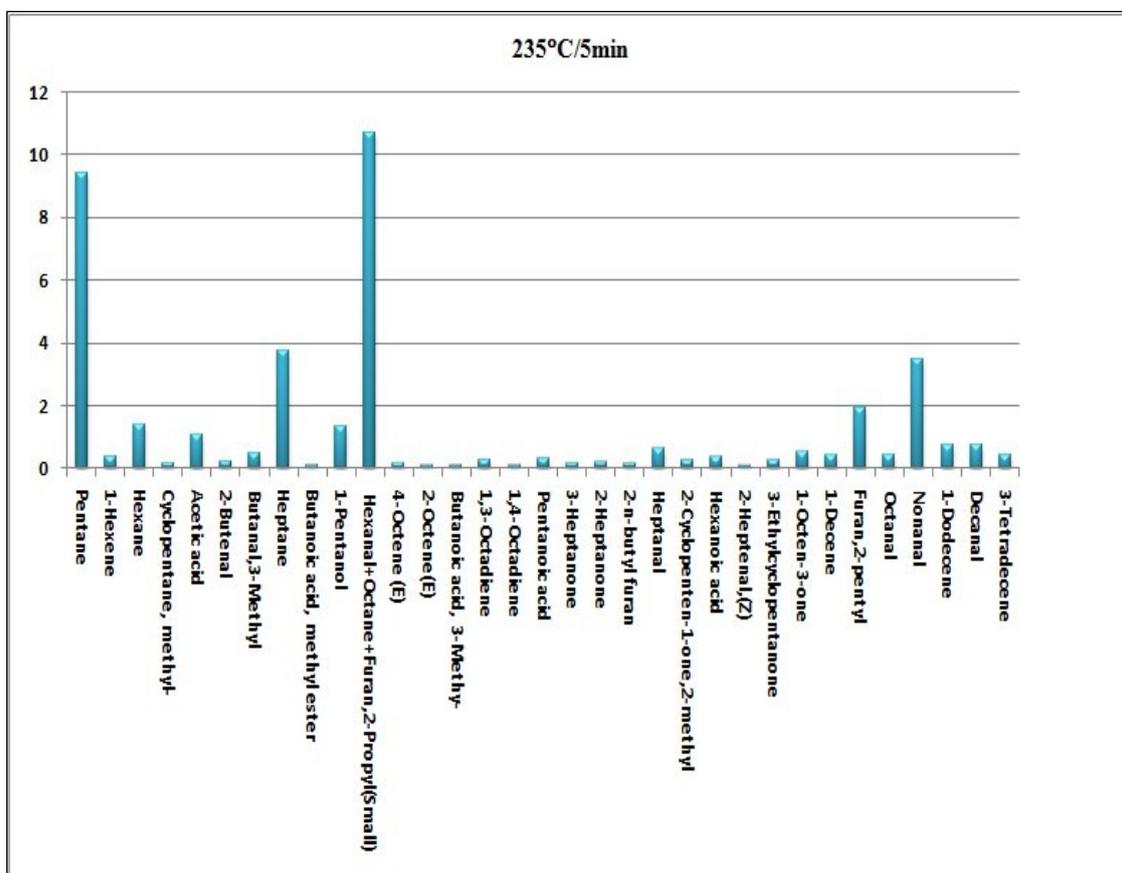
C-13 hydroperoxides from 18:2 autoxidation can undergo  $\beta$ -scission to generate pentane radicals or  $\alpha$ -scission to form hexanal, so there is some support at 150°C for the autoxidation theory. However, pentane is a primary product of thermal scission as well as a secondary product of autoxidation (derives from degraded hydroperoxides), and the hexanal detected is still a very small peak co-eluting with octane. Thus, the much higher levels of alkanes and alkenes formed document thermal scission as the predominant reaction at higher temperatures under limited oxygen.

Patterns of products remained quite similar at 180°C and 235°C (Tables 9-10, Figures 16-17), although comparable levels of products were generated in a small fraction of the heating time. Overall, the clear dominance of a homologous series of alkanes and alkenes arising from acyl chain scission points not involved in autoxidation shows that the

thermal reactions identified by Nawar and coworkers (Nawar 1984) in model lipids also occur in real food oils and provide a constant background of degradation during frying.



**Figure 16. Volatile product distribution in corn oil heated at 180°C for 10 minutes under N<sub>2</sub>.**



**Figure 17. Volatile product distribution in corn oil heated at 235°C for 5 min under N<sub>2</sub>.**

## 5.2. Time sequence of degradation under nitrogen

### 5.2.1. Oil heated at 100 °C, volatiles collected at 5/10/15/30/45/60/90/120 min

#### Summary of products function with collecting time at 100°C

As shown in the initial heating study above, major volatiles at 100°C are alkanes and alkenes, especially pentane as a dominant product. Other major non-oxidation products include hexane, heptanes, octane, 1-decene, and 1-dodecene obtained from scission of bonds  $\alpha$ ,  $\beta$ , and  $\gamma$  to double bonds. To determine the preferential sequence of scission and product shifts over time, oil samples collected over various short time periods were compared.

Generation patterns for the main alkane products are shown in Figure 18. There were two very interesting features in this data. First, all major products were present from early in the heating, indicating that they are primary products, and not derived from some other compounds. Second, pentane, already observed to be the major product appearing in heated corn oil, increased markedly in rate of production as heating time increased, while the other products increased only slowly or were relatively constant in rate of production. Thus, the relative rates here can provide an indication of the order of preference in sites for molecular scission. The preference pattern observed here is comparable to that observed reported above: 1) bond  $\alpha$  to double bond on the  $\text{CH}_3$  end of chain in linoleic acid, 2) bonds  $\alpha > \beta > \gamma$  to the double bond on the  $\text{CH}_3$  terminal side of oleic acid, 3) bonds  $\alpha > \beta > \gamma$  to the double bond on the acid end of oleic acid.

Additional products formed at 100°C are listed in Table 11 and Figure 19. With extended heating times, low levels of various oxidation products formed, including short chain alcohols and carboxylic acids (from oxidation products of aldehydes, not hydrolysis

of ester bonds), ketones, and epoxides. Long chain fatty acids and esters were released between 30-45 minutes of heating, but these products were not present after longer heating. It is not clear whether these long-chain products decomposed in the heat or were only formed under certain circumstances. Requirements for generation of long chain products needs to be investigated further.

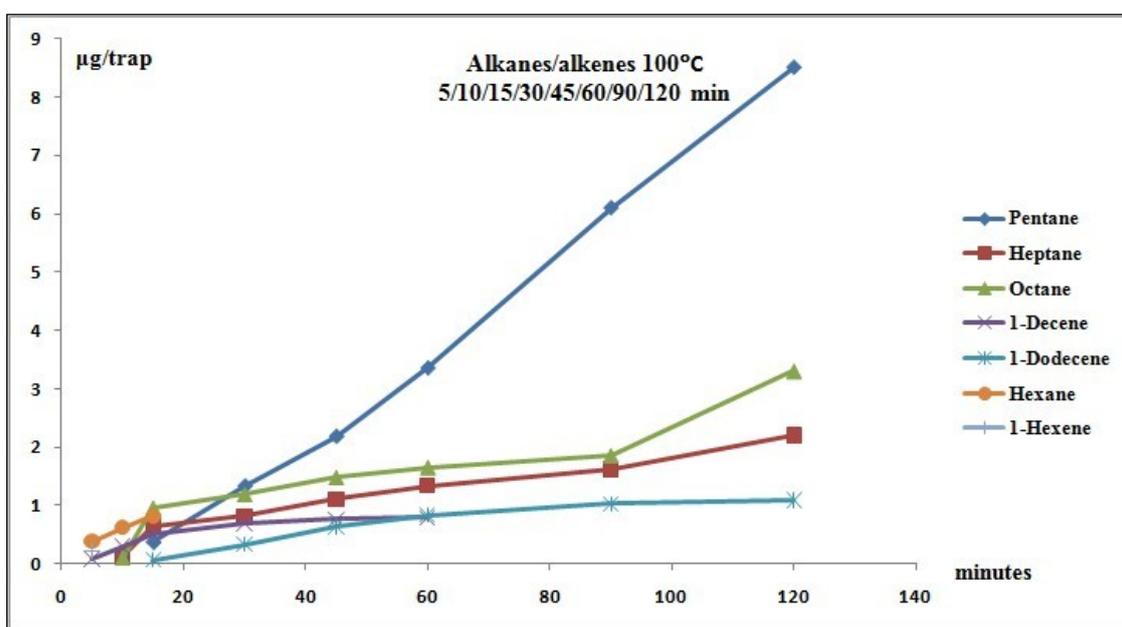
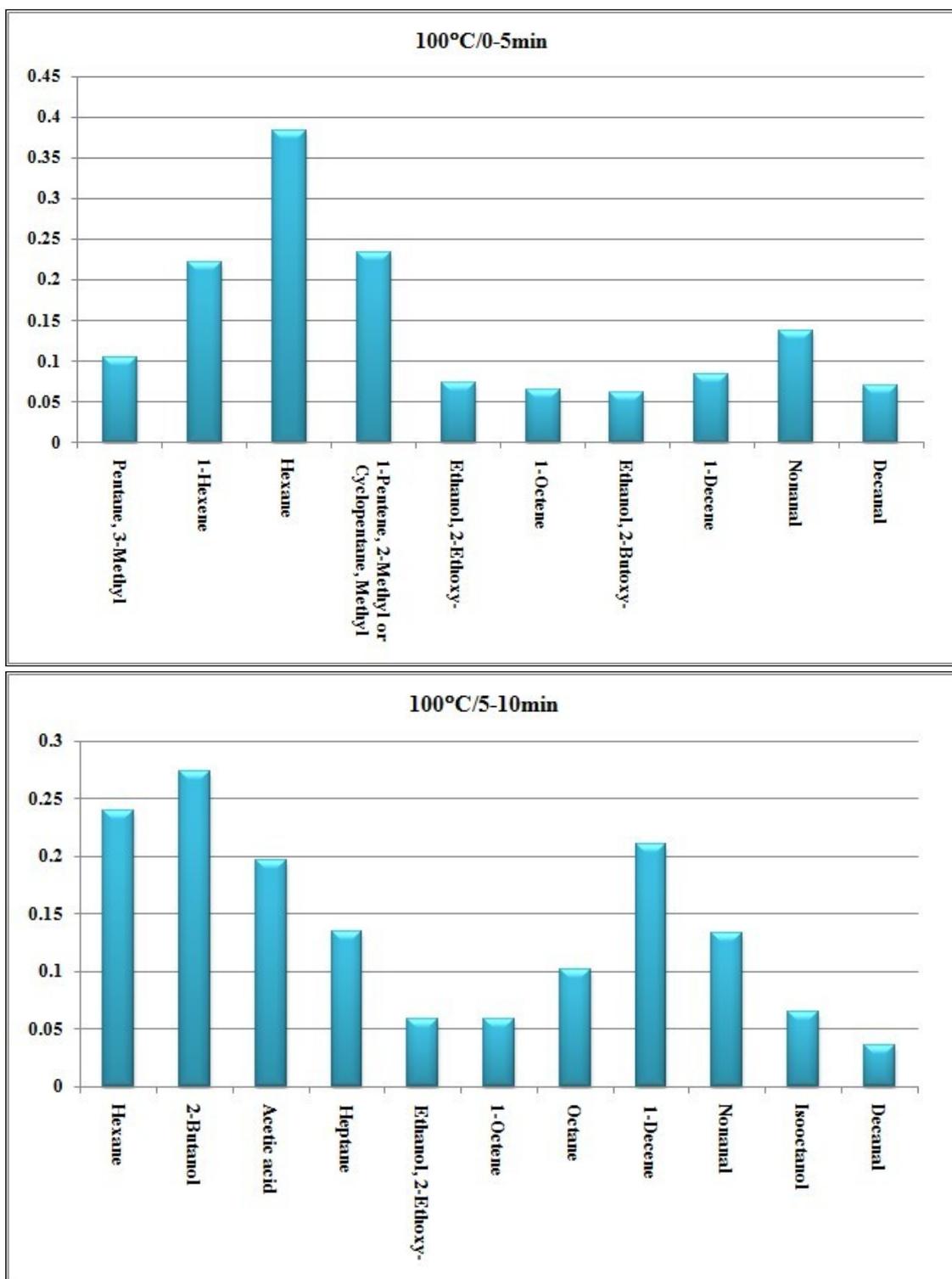


Figure 18. Alkanes/alkenes distribution under 100°C/N<sub>2</sub> function with time.



**Figure 19a.** Time sequence in development of products in corn oil heated at 100°C under N<sub>2</sub>. Samples collected (0 to 5 minutes) (top) and (5 to 10 minutes) (bottom).

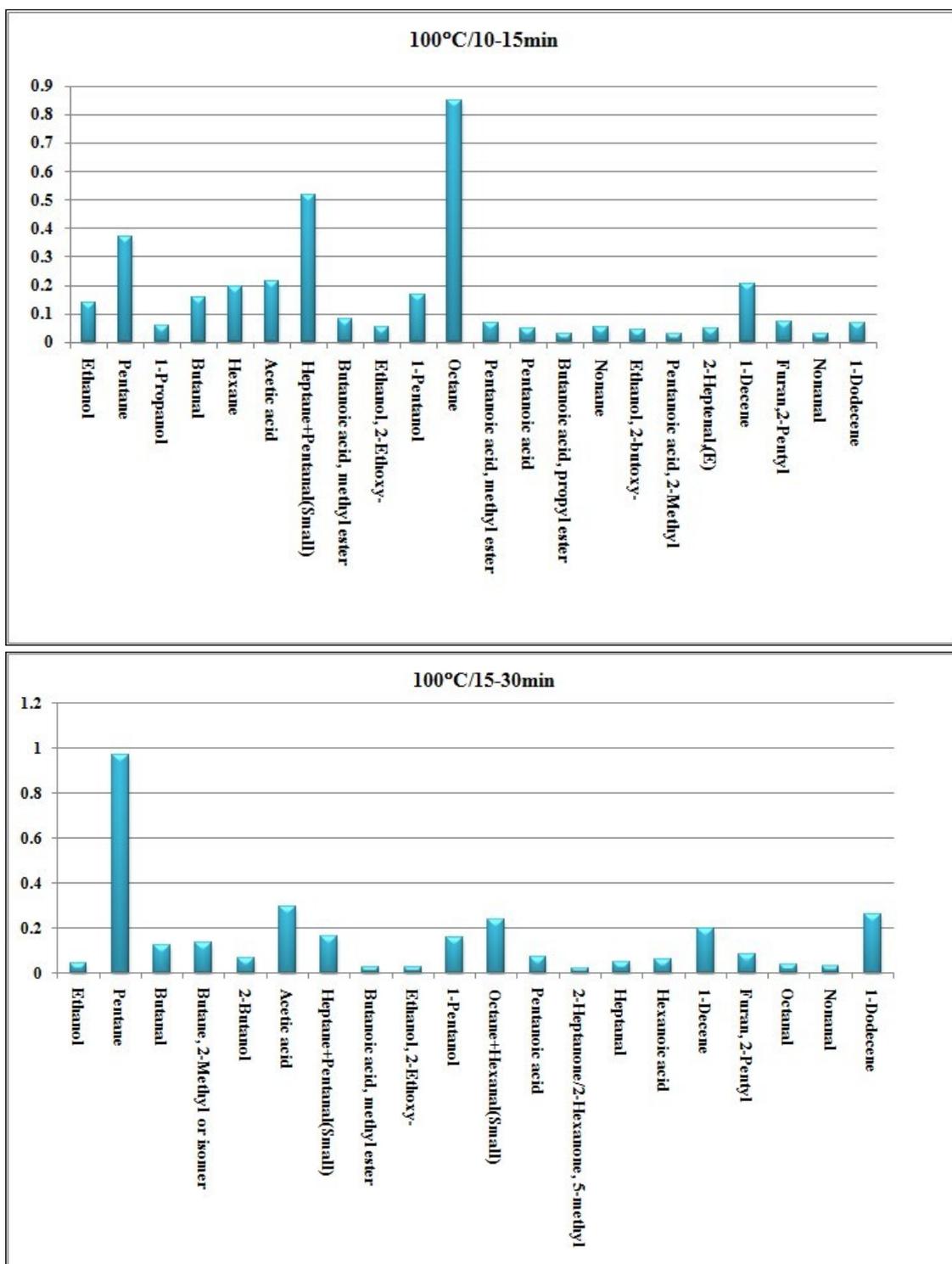


Figure 19b. Time sequence in development of products in corn oil heated at 100°C under N<sub>2</sub>. Samples collected (10 to 15 minutes) (top) and (15 to 30 minutes) (bottom).

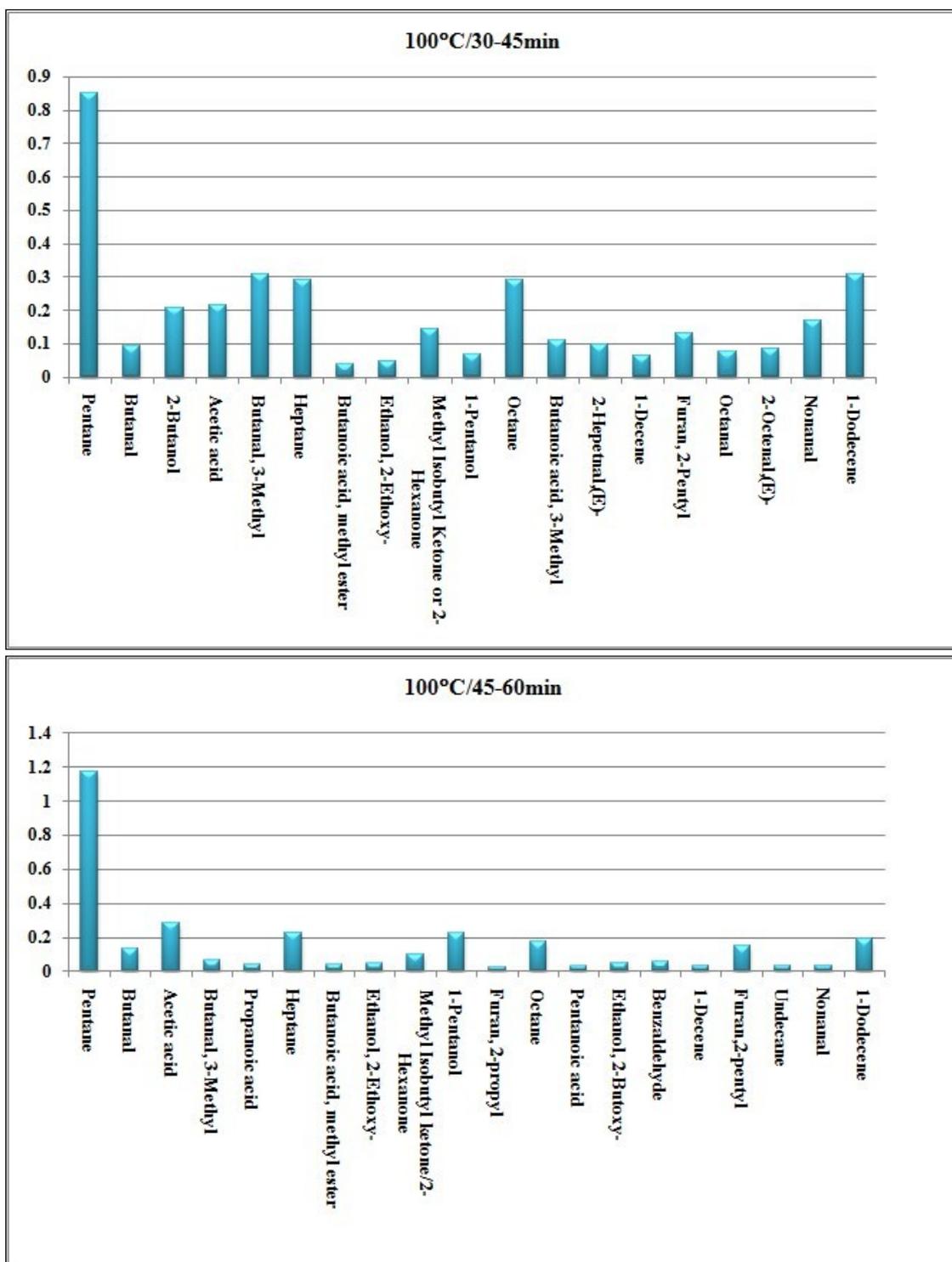


Figure 19c. Time sequence in development of products in corn oil heated at 100°C under N<sub>2</sub>. Samples collected (30 to 45 minutes) (top) and (45 and 60 minutes) (bottom).

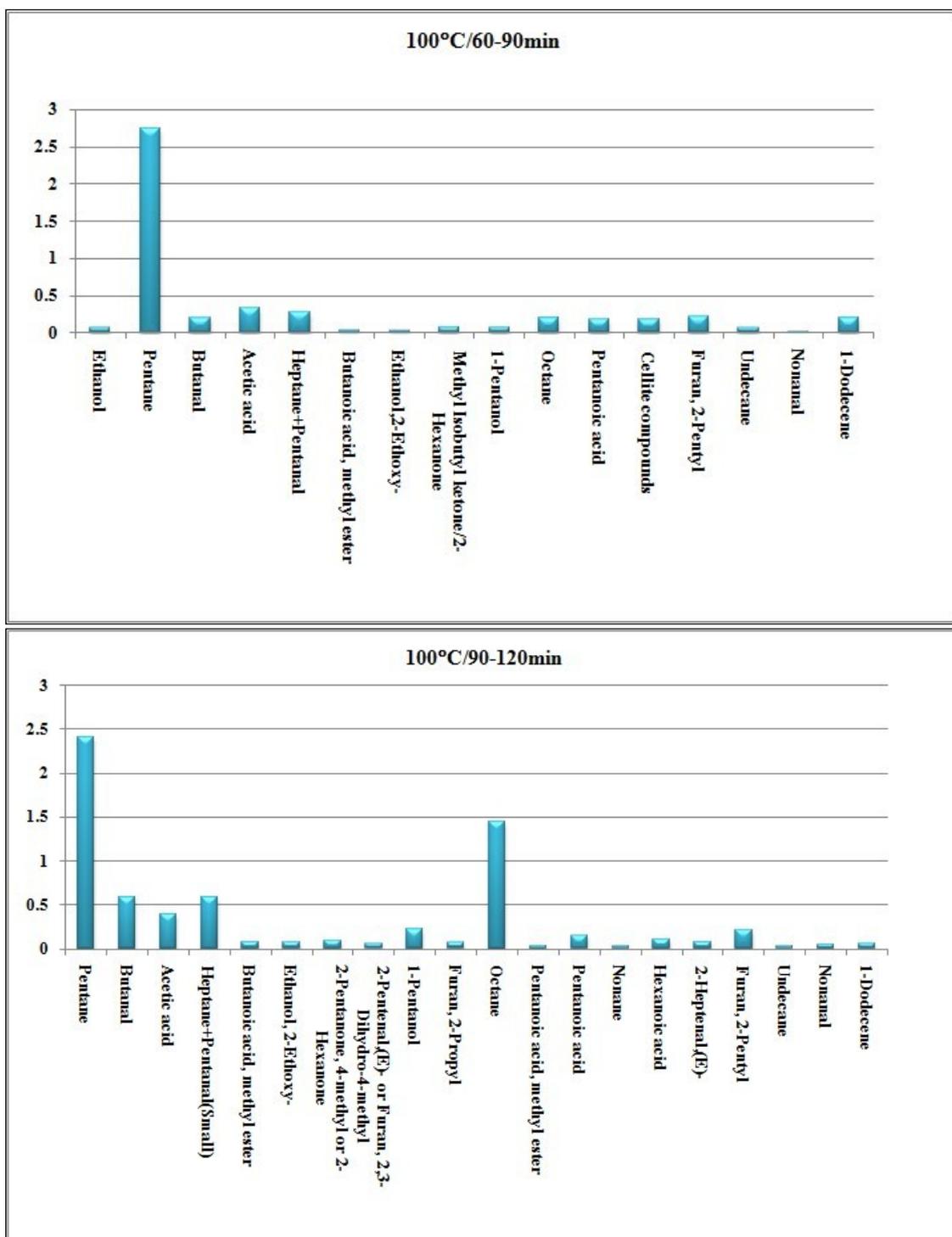


Figure 19d. Time sequence in development of products in corn oil heated at 100°C under N<sub>2</sub>. Samples collected (60 to 90 minutes) (top) and (90 and 120 minutes) (bottom).



### **5.2.2. Oil heated at 120 °C, volatiles collected at 5/10/15/30/45/60/90/120 min**

#### **Summary of products function with collecting time at 120°C**

Corn oil is heated under 120°C, at early stage, approximately 5 minutes collecting time, the GC data presents that relative equal distribution of C-5/C-6/C-10 alkanes/alkenes, such as pentane, 1-hexene, hexane, 1-decene. Other minor non-oxidation includes 1-octene and 1-dodecene. However, after 5 minutes heating, during the period of 5 to 10 minutes, pentane is a apparently predominant product in GC data and homologous series of alkanes/alkenes: 1-hexene/ hexane/ heptanes/ 1-octene/ octane/ 1-decene/ 1-dodecene. As collecting time goes up, pentane presents predominant product at every time gap, in addition, other major non-oxidation product such as octane/ heptanes are also presenting in GC data. Interestingly, hexane is a major non-oxidation product up to 15 minutes, but hexane no longer exists in late stage frying, instead, hexanal co-elute to increased amount. The possible mechanism that hexane radical is initially generated by thermal scission, then abstract hydrogen to form hexane, as collecting time goes to late stage, hexane radical can be attacked by oxygen to generate hexanal. 2-Pentyl furan is another major oxidation product under limited oxygen that stay similar trend from early stage to late frying. Alkanes/alkenes at 120°C is illustrated below, Figure 20.

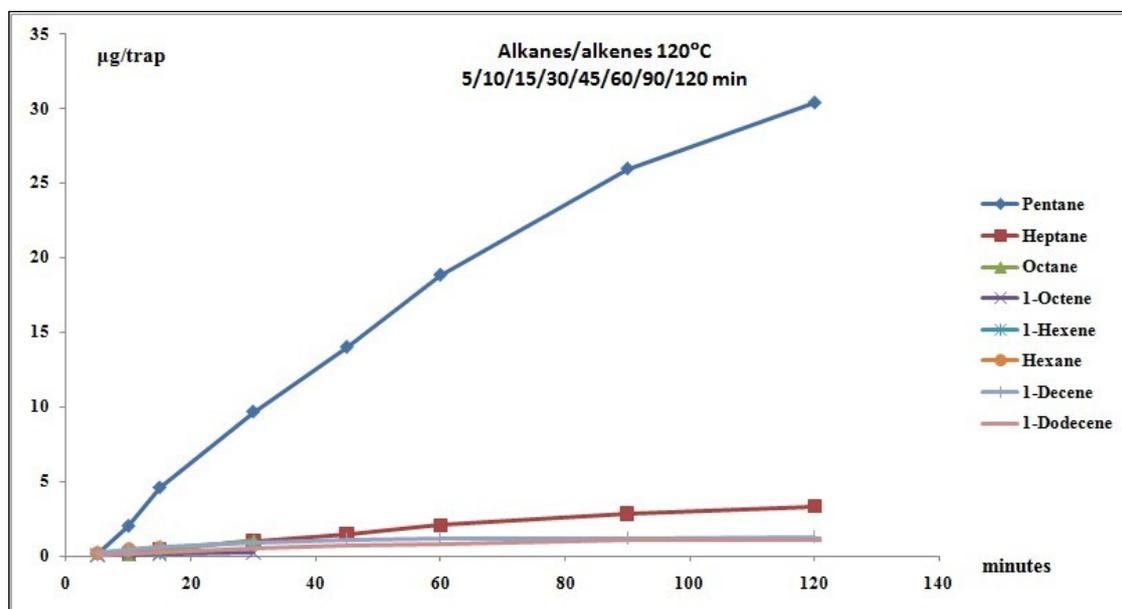


Figure 20. Alkanes/alkenes distribution under 120°C/N<sub>2</sub> function with time.

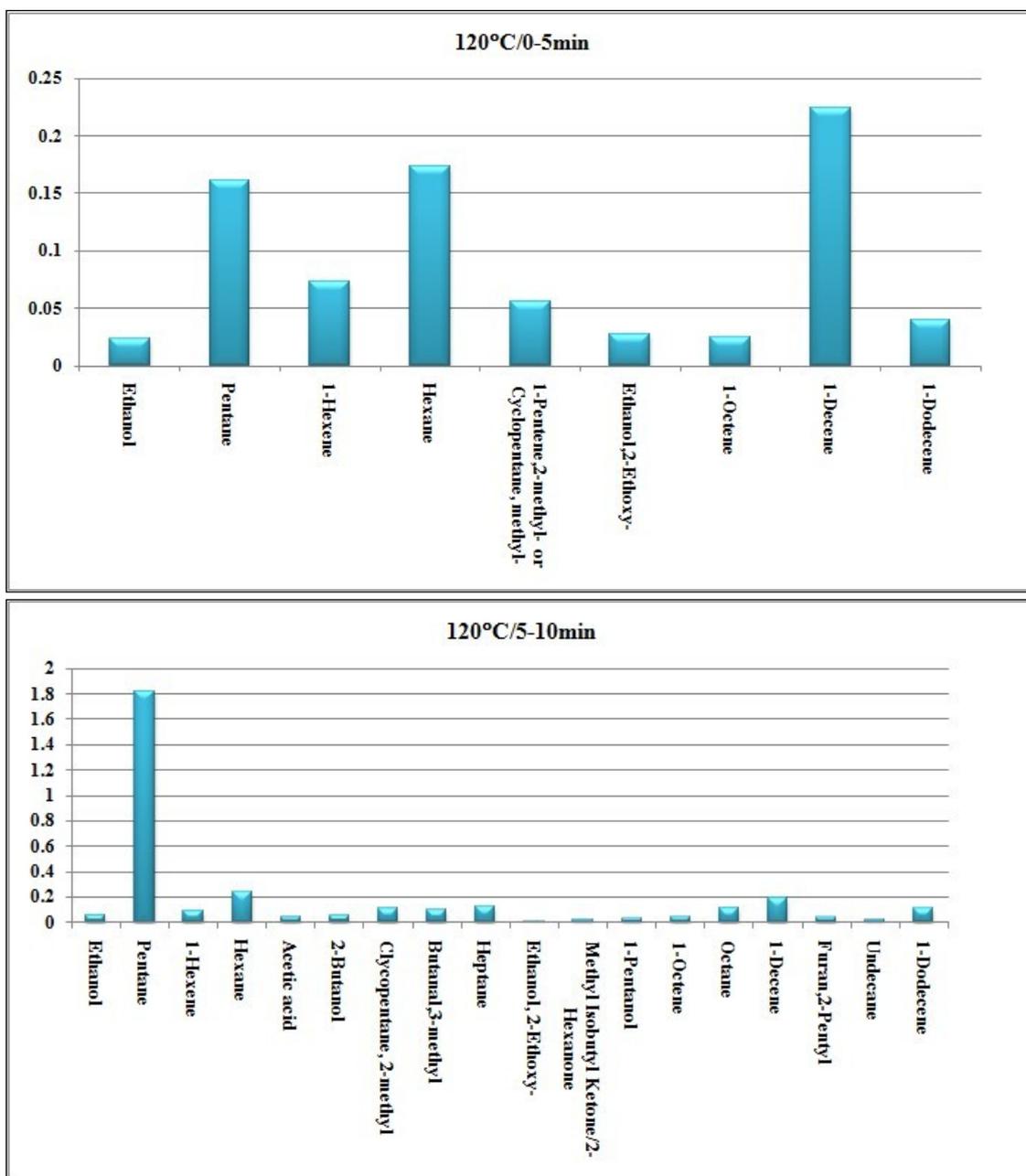
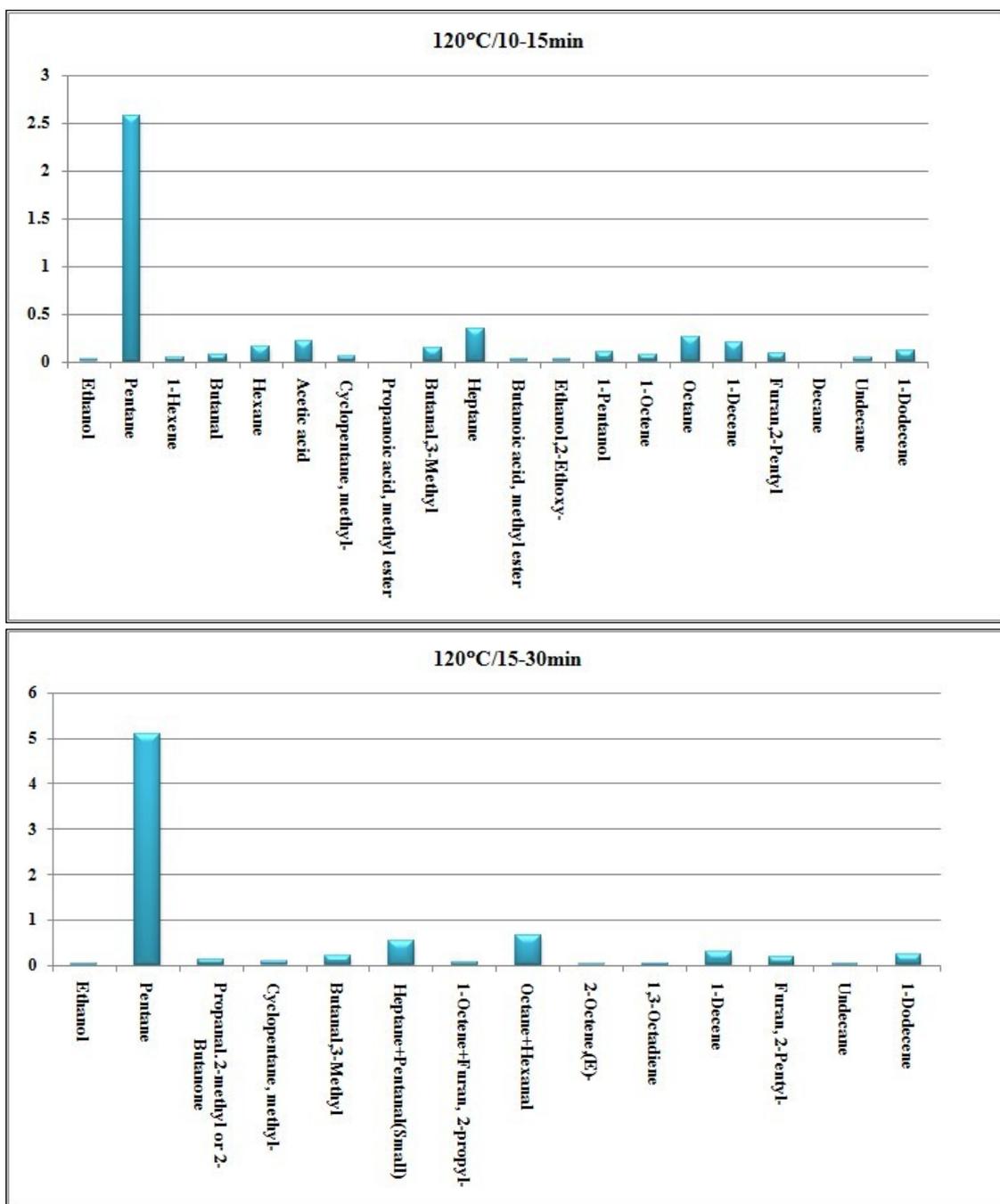


Figure 21a. Time sequence in development of products in corn oil heated at 120°C under N<sub>2</sub>. Samples collected (0 to 5 minutes) (top) and (5 and 10 minutes) (bottom).



**Figure 21b.** Time sequence in development of products in corn oil heated at 120°C under N<sub>2</sub>. Samples collected (10 to 15 minutes) (top) and (15 and 30 minutes) (bottom).

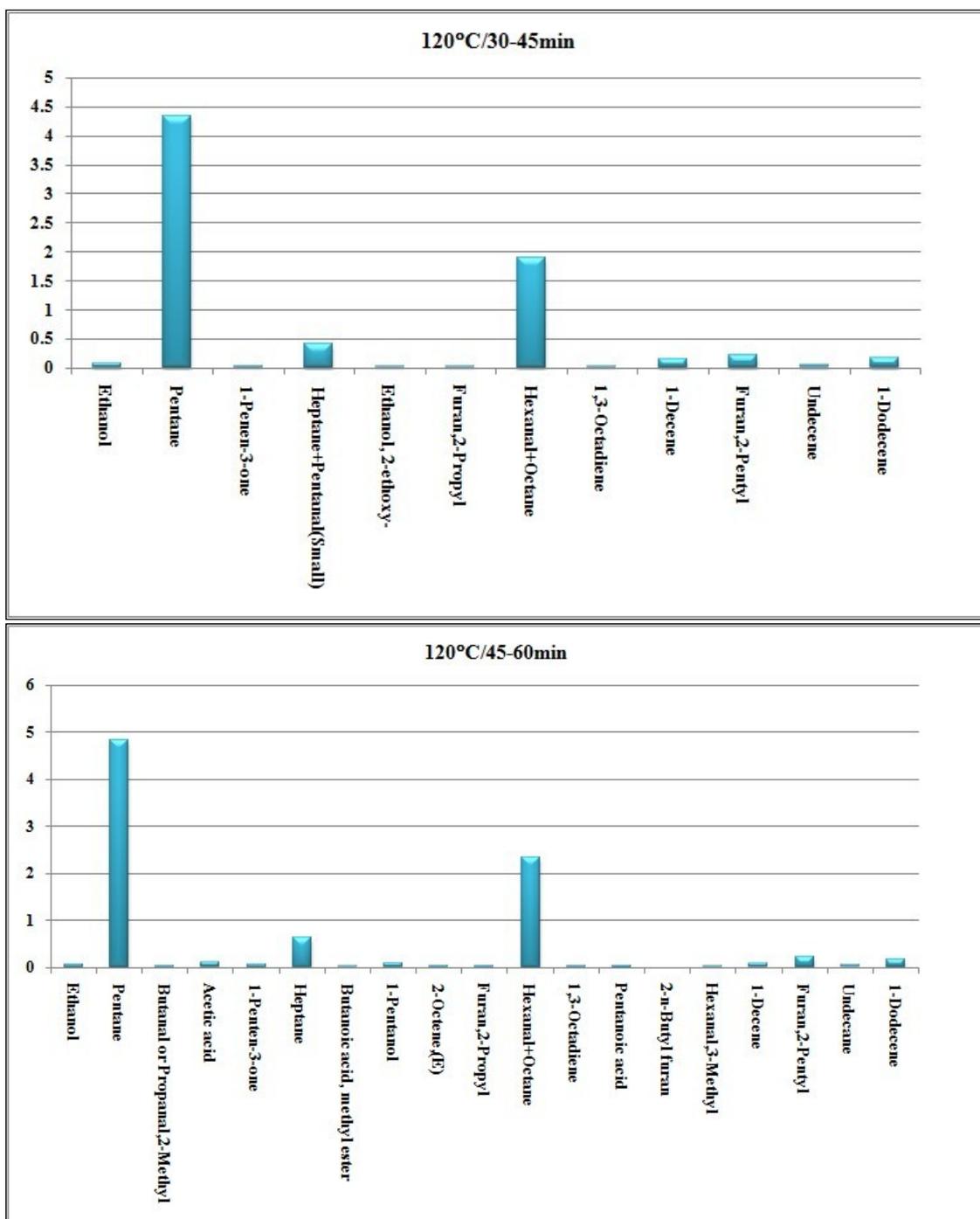
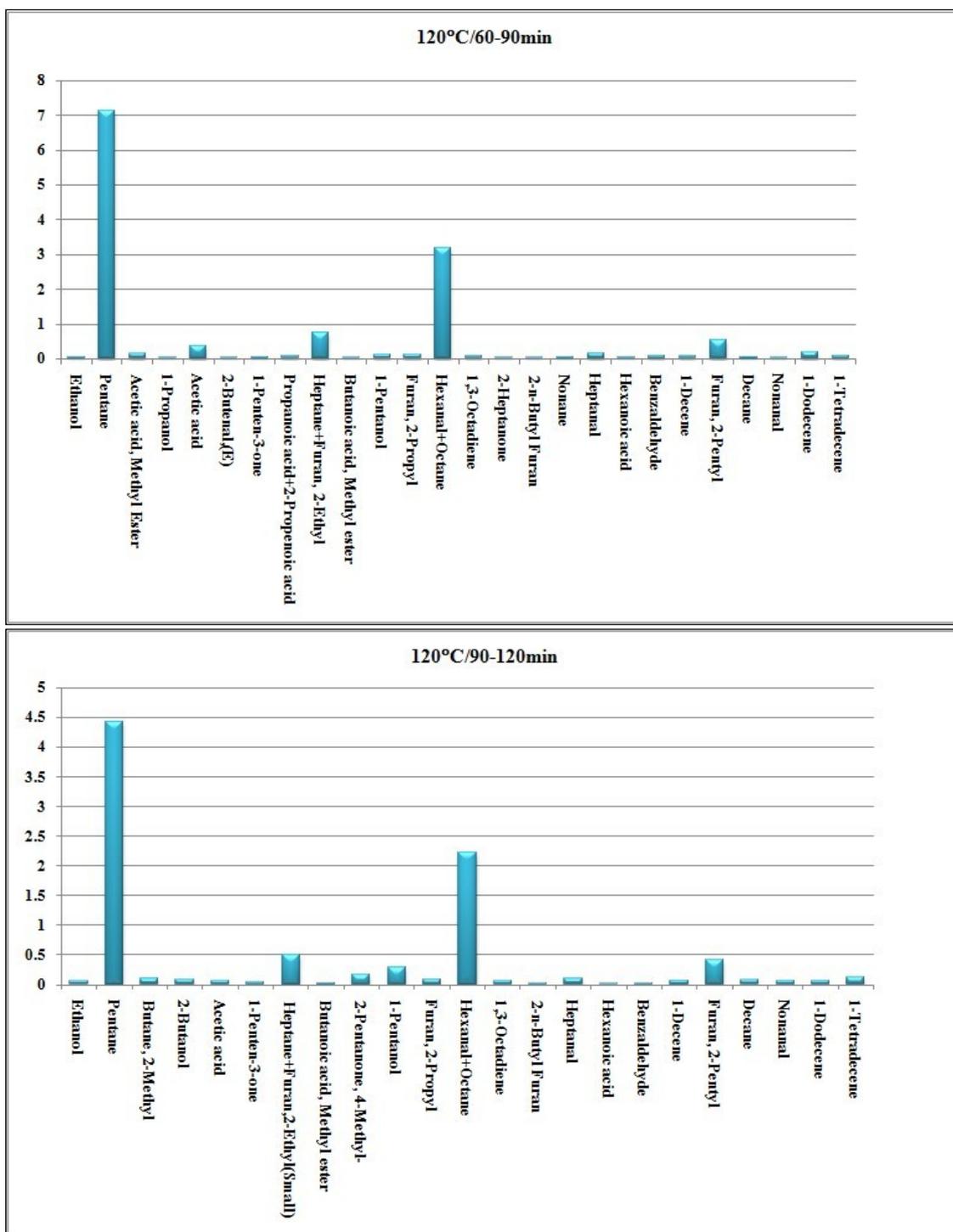


Figure 21c. Time sequence in development of products in corn oil heated at 120°C under N<sub>2</sub>. Samples collected (30 to 45 minutes) (top) and (45 and 60 minutes) (bottom).



**Figure 21d.** Time sequence in development of products in corn oil heated at 120 °C under N<sub>2</sub>. Samples collected (60 to 90 minutes) (top) and (90 and 120 minutes) (bottom).

### 5.2.3. Oil heated at 150 °C, volatiles collected at 5/10/15/30/45/60/90/120 min

#### Summary of products function with collecting time at 150°C

At early stage of frying under 150°C (0-30 minutes), non-oxidation product are dominant products such as pentane, other major alkanes/alkenes are hexane/ heptanes/ octane/ 1-decene/ 1-dodecene, minor alkanes/alkenes are 1-hexene/ 1-octene/ 2-octene, (E)-/ 1,3-octadiene/ decane. However, interestingly, during the period of 5- 10 minutes, heptanal appears and increased as heptanes formation decreases after 10 minutes. Same mechanism applies for other products, such as hexane, before 30 minutes, hexane appears in GC data but stay at low concentration, however, C-6 radical is totally formed to hexanal after 30 minutes under limited oxygen. On the other hand, under 150°C, concentration oxidation products exponentially increase, for example, during 30-45 minutes, hexanal/ 2-heptenal,(E)-/ nonanal are in comparable concentration as pentane, during 45-60 minutes, 2-octenal,(E)-/ 2-nonenal,(E)- dramatically increases, during 60-90 minutes, 2,4-decadienal, (E,E)-/ 2-decenal,(E)- apparently increase, during 90-120 minutes, 2,4-decadienal, (E,E)- increase dramatically at highest peak in GC data. Thus, 2,4-decadienal, (E,E)- which forms from alpha scission of C-9 hydroperoxide from linoleic acid will increase after 2 hours heating, C-13 hydroperoxide which forms hexanal is a preferential scission site during early frying time. Cyclization occurs after 5 minutes, 1,3-cyclohexadiene, 5-butyl/ Ethylidenecycloheptane are major non-oxidized cyclized compounds but at low concentration, other cyclized compounds are oxy compounds. Generally, alkanes/alkenes are still dominant products under nitrogen, as illustrated below, Figure 22.

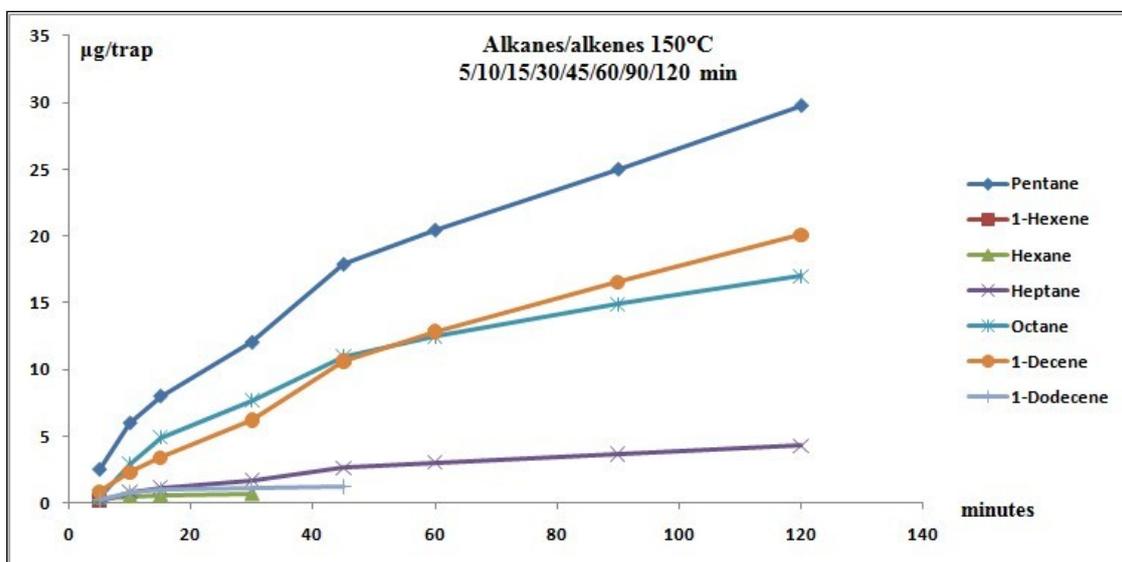


Figure 22. Alkanes/alkenes distribution under 150°C/N<sub>2</sub> function with time.

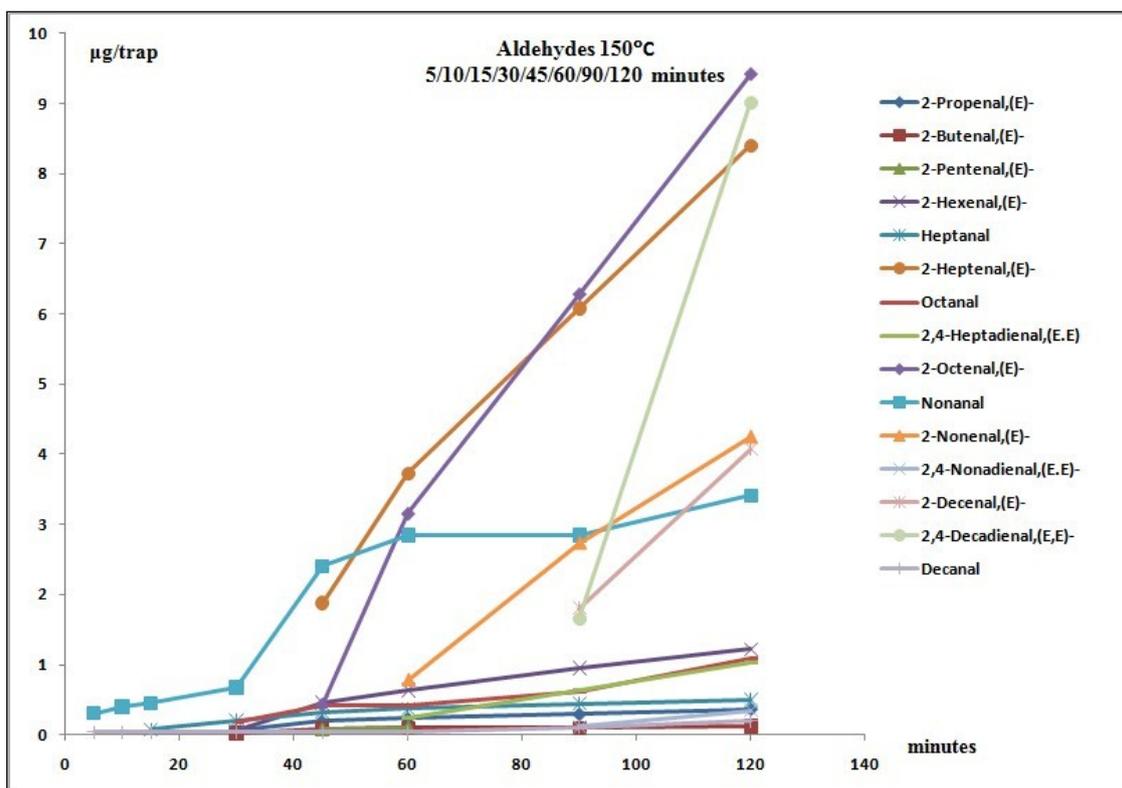


Figure 23. Aldehydes distribution under 150°C/N<sub>2</sub> function with time.

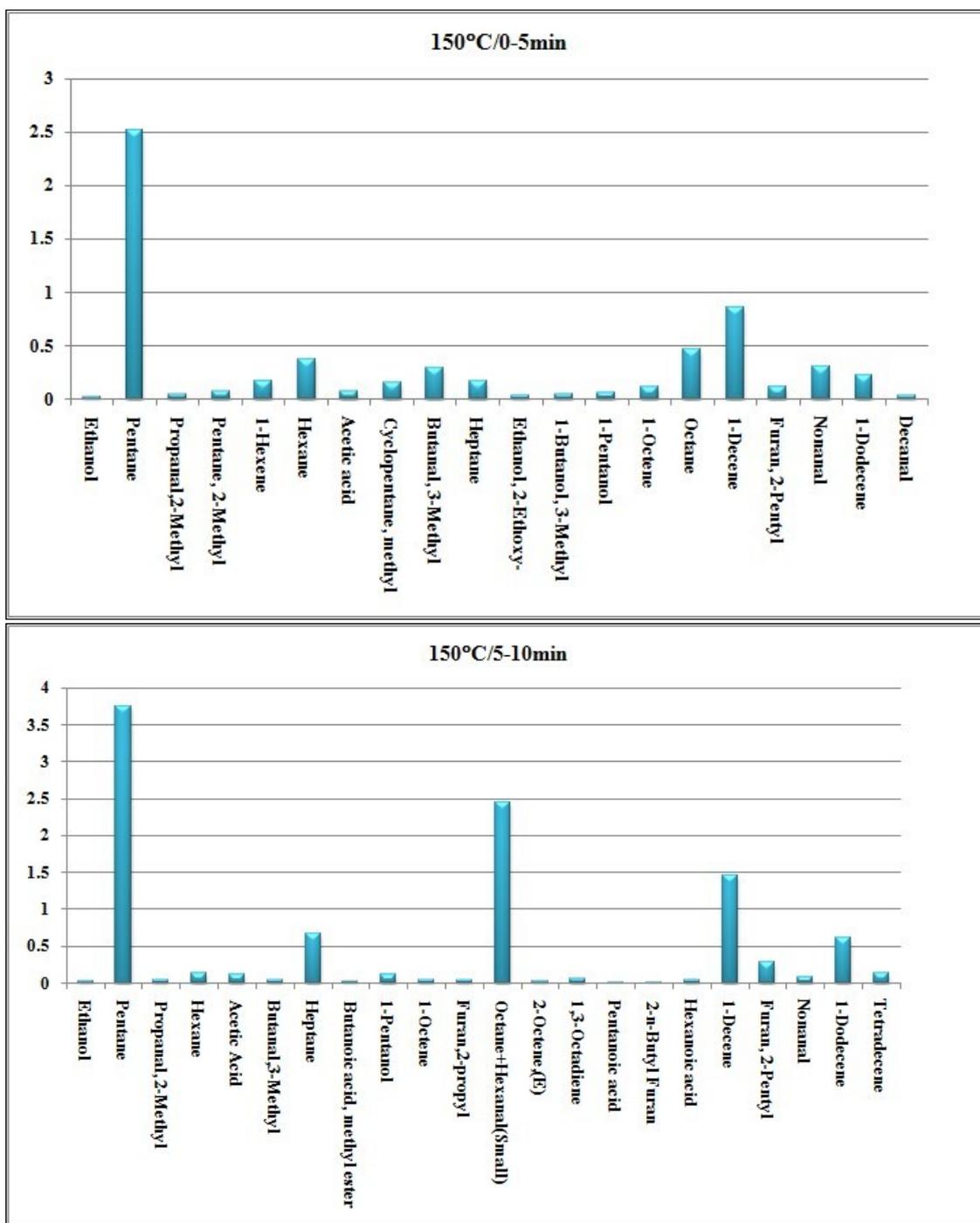
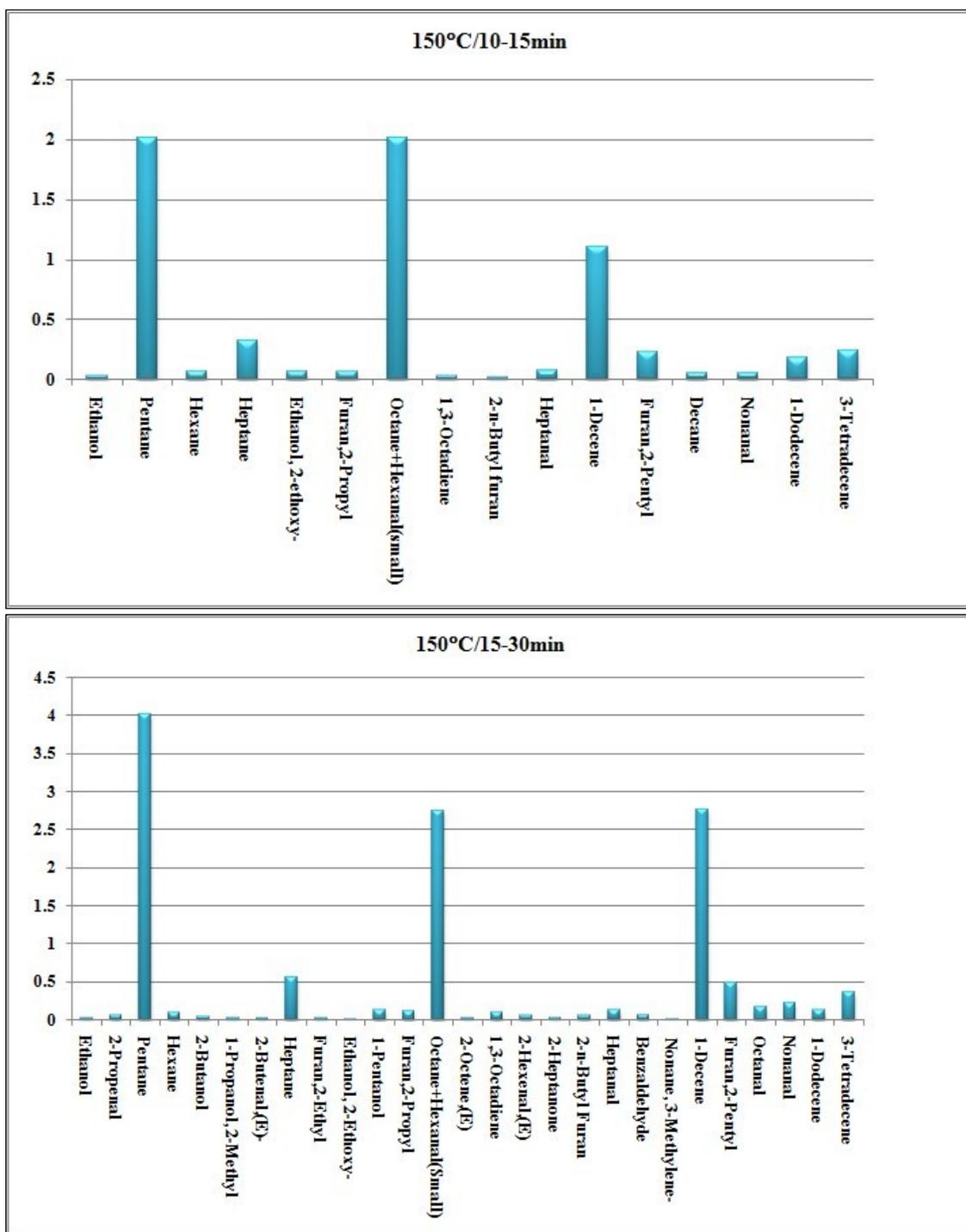


Figure 24a. Time sequence in development of products in corn oil heated at 150°C under N<sub>2</sub>. Samples collected (0 to 5 minutes) (top) and (5 and 10 minutes) (bottom).



**Figure 24b.** Time sequence in development of products in corn oil heated at 150°C under N<sub>2</sub>. Samples collected (10 to 15 minutes) (top) and (15 and 30 minutes) (bottom).

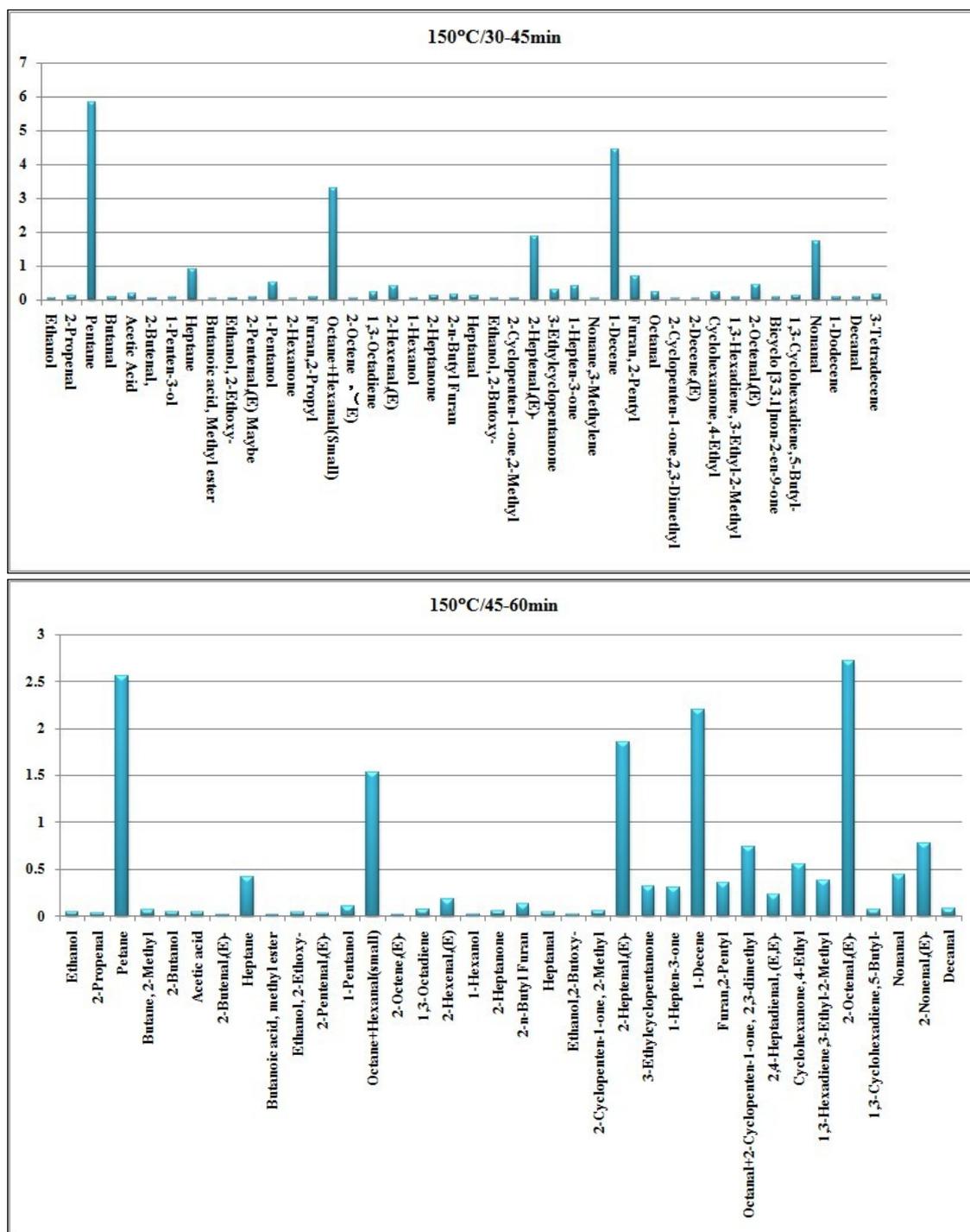


Figure 24c. Time sequence in development of products in corn oil heated at 150°C under N<sub>2</sub>. Samples collected (30 to 45 minutes) (top) and (45 and 60 minutes) (bottom).

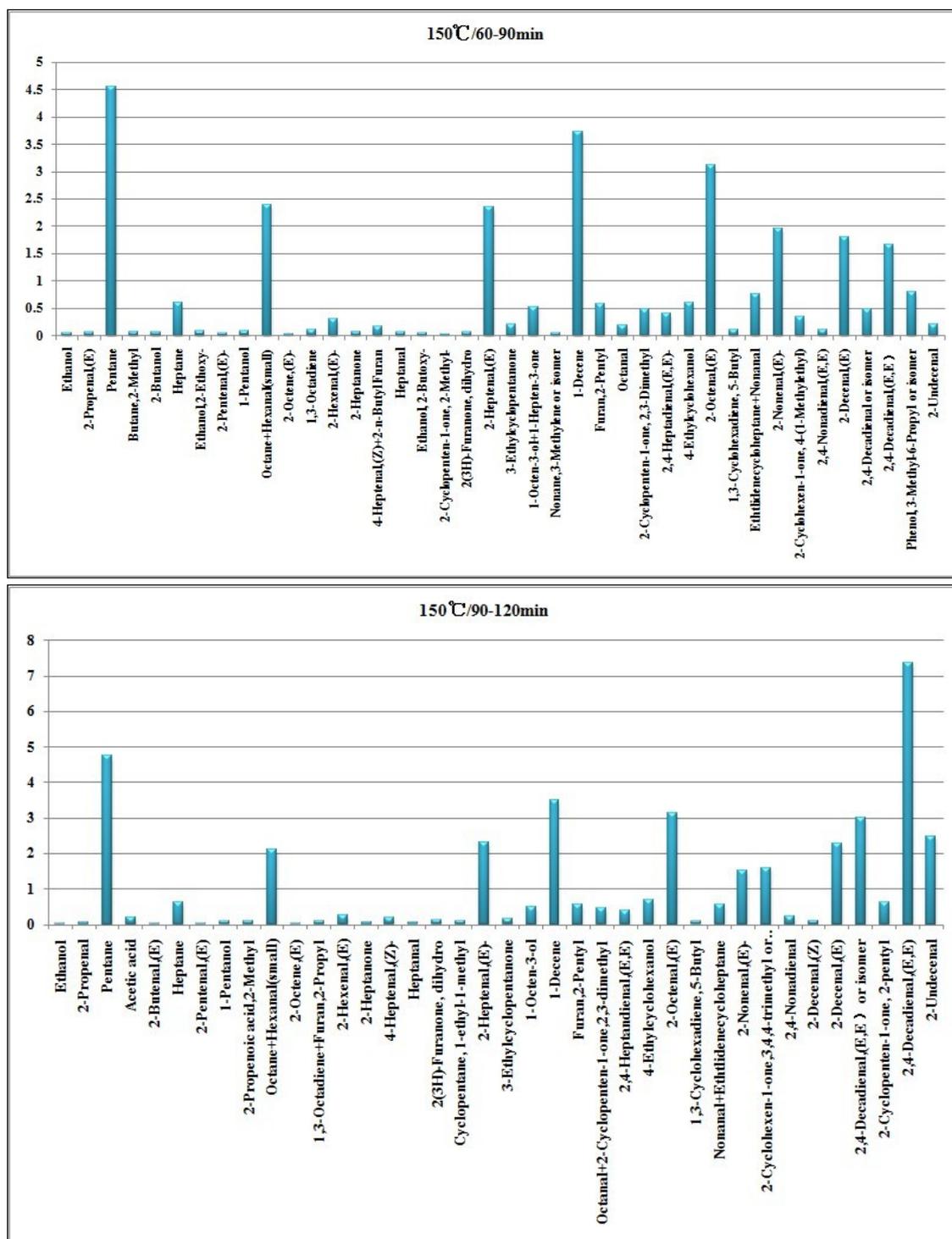


Figure 24d. Time sequence in development of products in corn oil heated at 150°C under N<sub>2</sub>. Samples collected (60 to 90 minutes) (top) and (90 and 120 minutes) (bottom).

#### 5.2.4. Oil heated at 180 °C, volatiles collected at 2/5/7/10/13/15/20/25/30 min

##### Summary of products function with collecting time at 180°C

From data analysis above, frying under 180°C is a faster progress compared to lower temperature which keeps the same pattern of product distribution. At early stage of frying, pentane become a dominant product during 0-2 minutes frying, other major non-oxidation products are hexane, 1-decene. Minor non-oxidation products are 1-hexene, heptane, 1-octene, octane, 1-dodecene. After 2 minutes frying, heptane, octane, 1-dodecene sharply increase to become major non-oxidation products, at the same time, minor alkanes/alkenes are more complex compared to early stage” hexane/ 1-octene/ 4-octene,(E)-/ 2-octene,(E)/ 1,3-octadiene/ undecane/ 3-tetradecene.

During period of 5-7 minutes, homologous series of oxidation products are volatilized but stay at extremely low concentration, except C-5/C-6 aldehydes. Pentanal/hexanal co-elute with heptanes/octane, which is possibly a major reason peaks of heptanes/ octane dramatically increases compared to 0-2 minutes. Homologous series oxidation products include C-4 to C-10 carbon chain, such as 1-butanol/ 2-butenal,(E)-/ pentanal/ 2-pentenal,(E)-/ 1-pentanol/ hexanal/ 1-hexanol/ 2-hexenal,(E)-/ 2-heptanone/ heptanal/ 2-heptenal,(E)-/ 2-heptenal,(Z)-/ 1-hepten-3-one/ 2,4-heptadienal,(E,E)-/ octanal/ 1-octen-3-ol/ 1-octen-3-one/ 2-octenal,(E)-/ nonanal/ 2-nonenal,(E)-/ 2,4-nonadienal,(E,E)-/ decanal/ cis-4-decenal/ 2-decenal,(E)-/ 2,4-decadienal,(E,E)-, the complexity of product distribution is driven by high temperature as shown in GC data. 2,4-decadienal,(E,E)- which is an important flavor volatiles forms in large amount during late stage of frying. The data of oxidation /non-oxidation products are illustrated below, Figure 26, Figure 25.

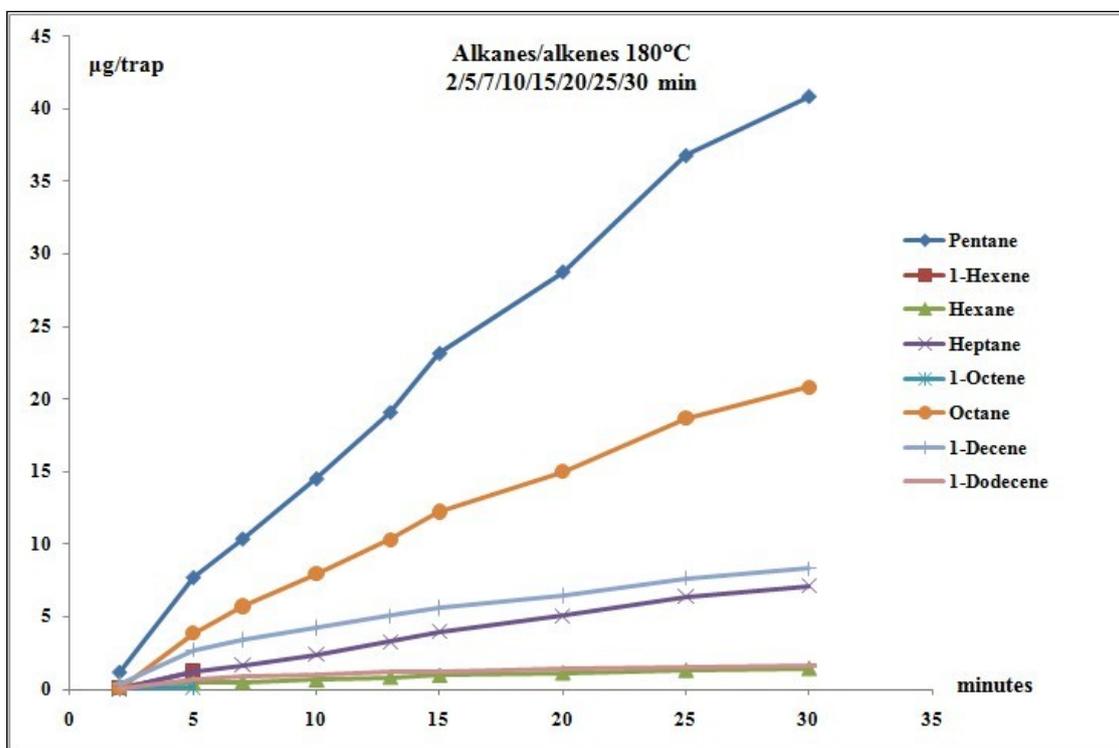


Figure 25. Alkanes/alkenes distribution under 180°C/N<sub>2</sub> function with time.

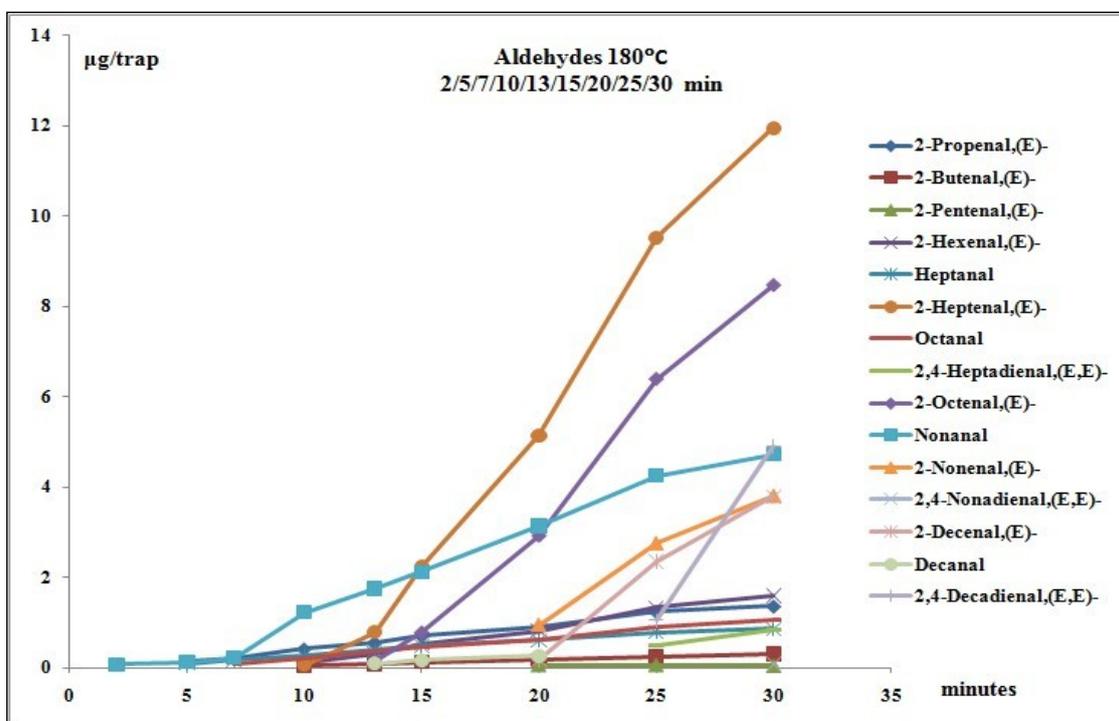


Figure 26. Aldehydes distribution under 180°C/N<sub>2</sub> function with time.

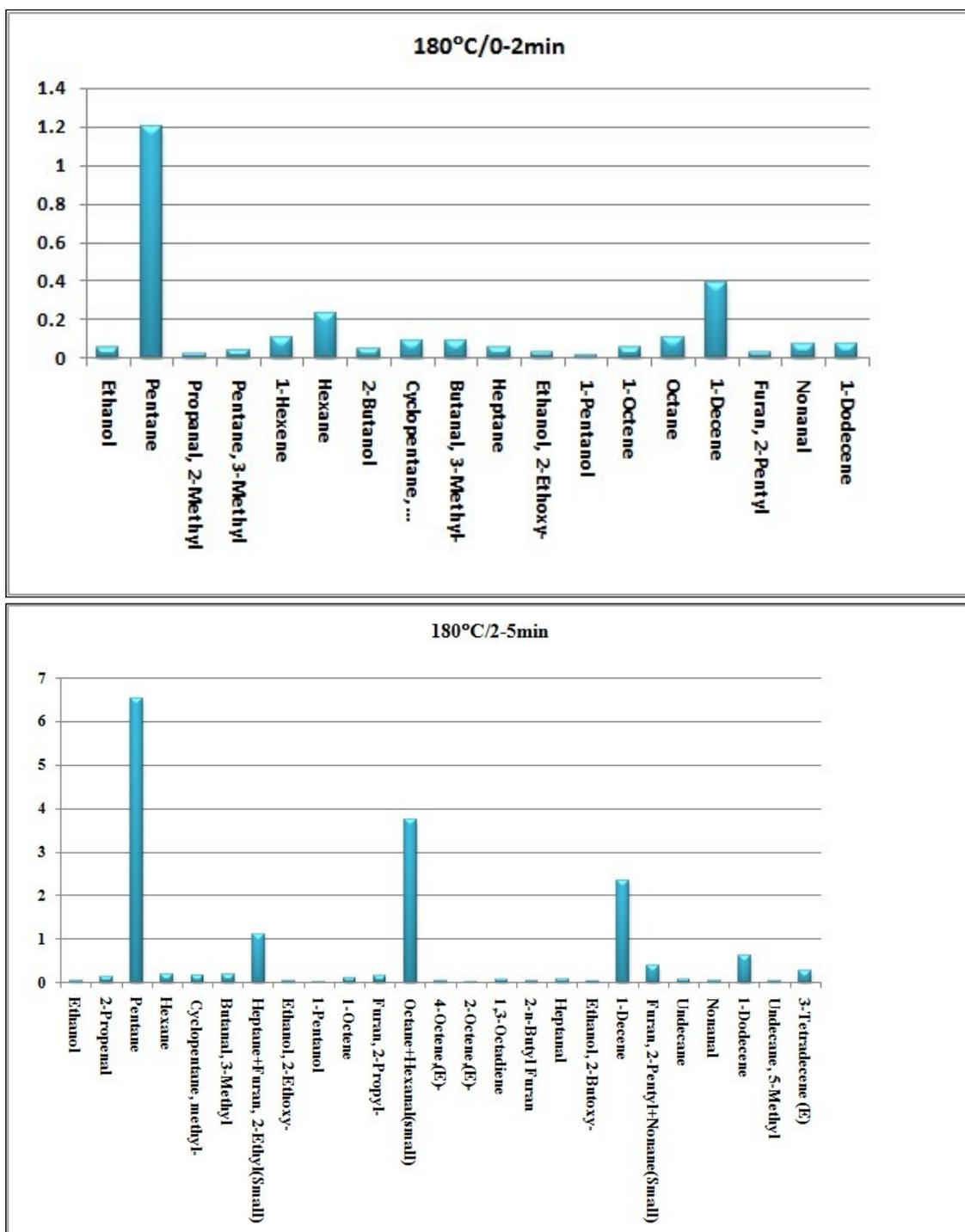


Figure 27a. Time sequence in development of products in corn oil heated at 180°C under N<sub>2</sub>. Samples collected (0 to 2 minutes) (top) and (2 and 5 minutes) (bottom).

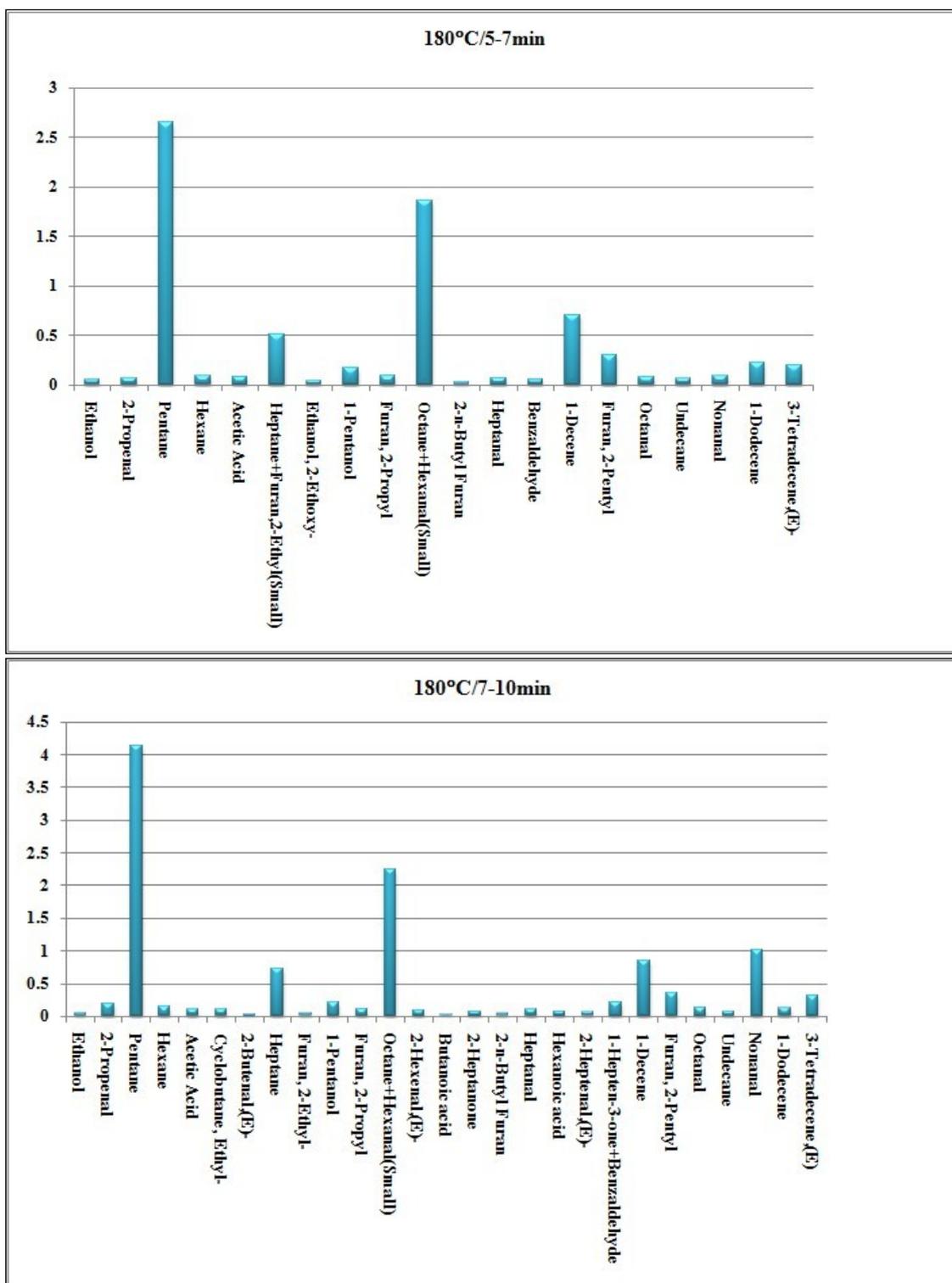


Figure 27b. Time sequence in development of products in corn oil heated at 180°C under N<sub>2</sub>. Samples collected (5 to 7 minutes) (top) and (7 and 10 minutes) (bottom).

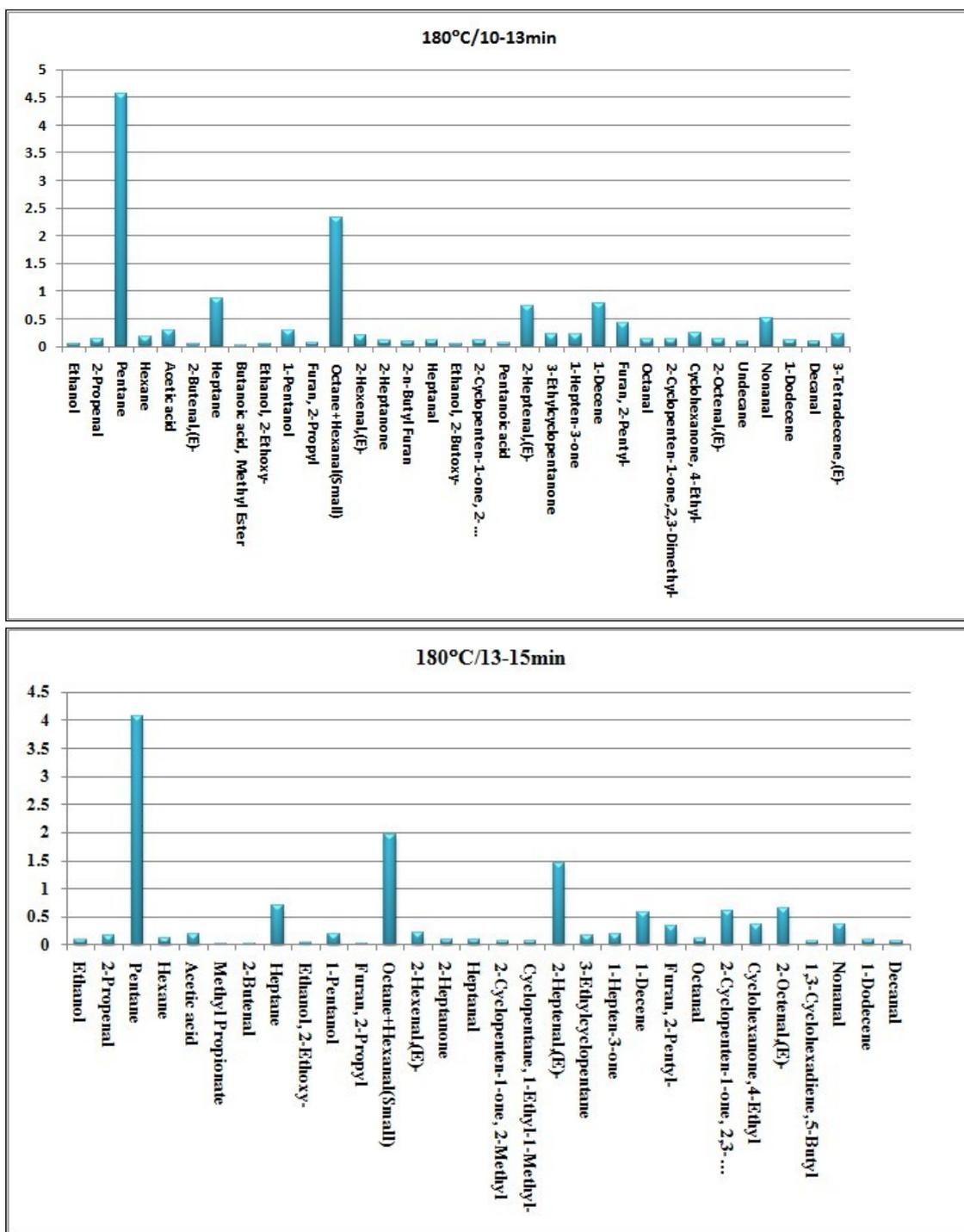


Figure 27c. Time sequence in development of products in corn oil heated at 180°C under N<sub>2</sub>. Samples collected (10 to 13 minutes) (top) and (13 and 15 minutes) (bottom).

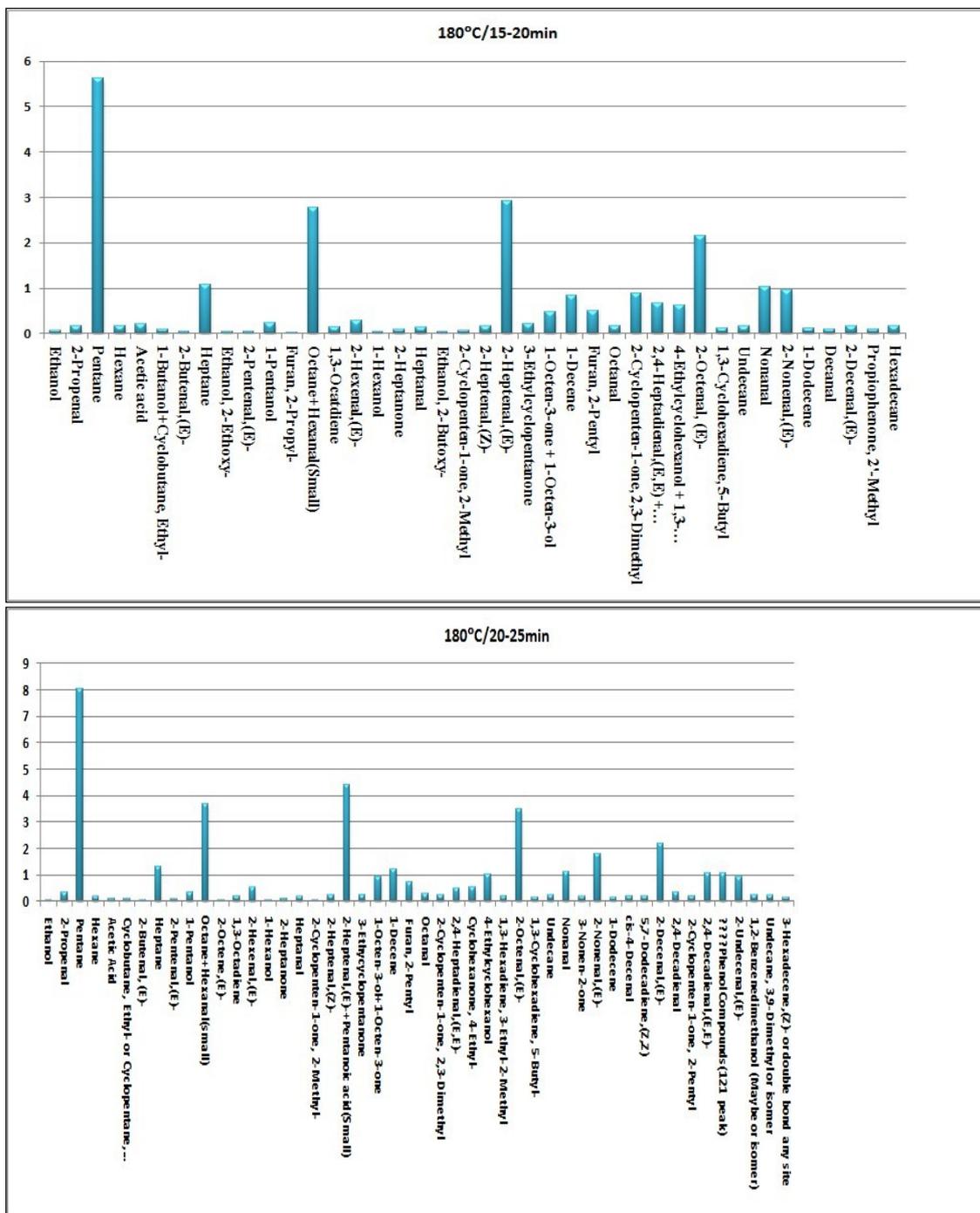


Figure 27d. Time sequence in development of products in corn oil heated at 180°C under N<sub>2</sub>. Samples collected (15 to 20 minutes) (top) and (20 and 25 minutes) (bottom).

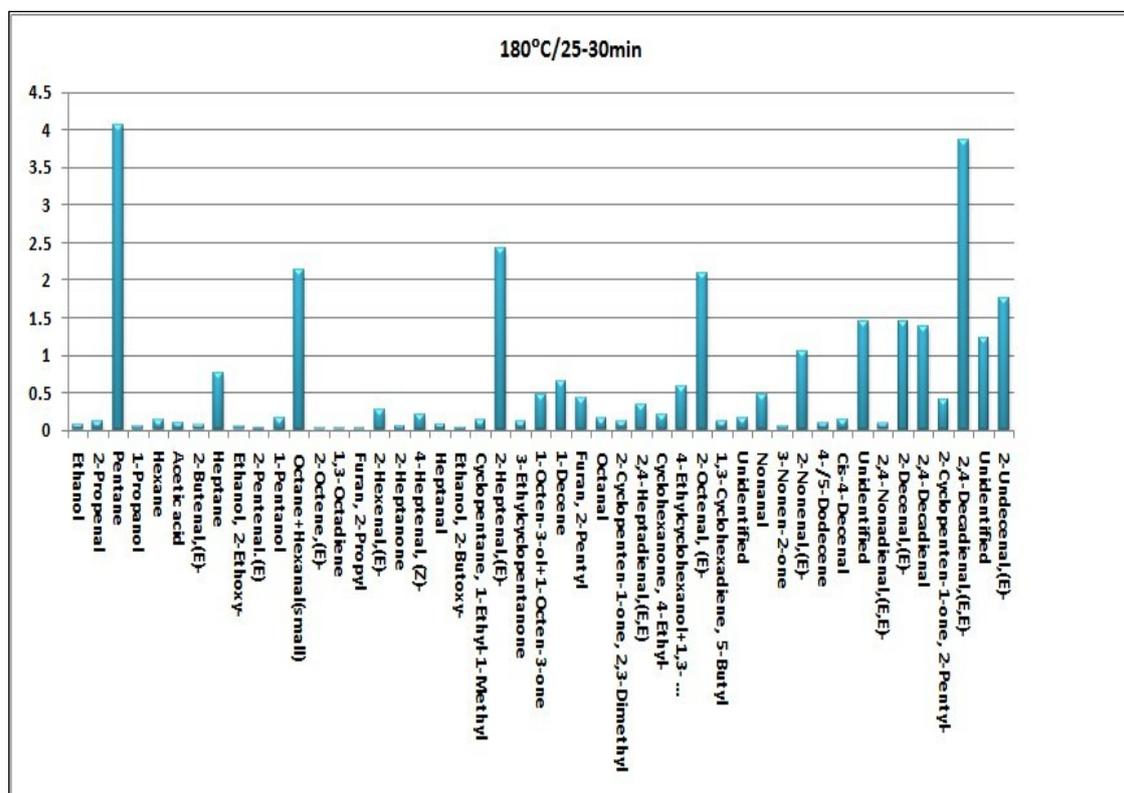


Figure 27e. Time sequence in development of products in corn oil heated at 180°C under N<sub>2</sub>. Samples collected (25 to 30 minutes).

### **5.2.5. Oil heated at 235 °C, volatiles collected at 2/5/7/10/13/15/20/25/30 min**

#### **Summary of products function with collecting time at 235°C**

Frying at 235°C is more complicated than 180°C, at early stage (up to 7 minutes), pentane/ octane/ heptane/ 1-decene are major non-oxidation products which are predominant products, however, oxidation products are still present but stay at low concentration which 2-heptenal,(E)-/ 2-pentyl furan/ nonanal are major concentration. As time reaches to 20 minutes, homologous series of oxidation products are more complex than low temperature from C-4 to C-10: 3-buten-2-one, 2-butenal,(E)-, 1-penten-3-ol, 2-pentanone, 1-pentanol, 2-hexenal,(E)-, 1-hexanol, 2-heptanone, 4-heptenal,(Z)-, heptanal, 2-heptenal,(Z)-, 2-heptanal,(E)-, 2,4-heptadienal,(E,E)-, 1-octen-3-ol, 1-octen-3-one, octanal, 2-octenal,(E)-, 2-nonenal,(Z)-, 2-nonenal,(E)-, 2-decenal,(E)-, 2,4-decadienal,(E,E)-. Cyclization also occurs at high temperature such as 2-Cyclopenten-1-one, 2,3-Dimethyl, 4-Ethylcyclohexanol, Cyclohexanone, 4-Ethyl etc. Compared to non-oxidation products, oxidation products are still at low concentration due to limited oxygen content (Figure 28, 29).

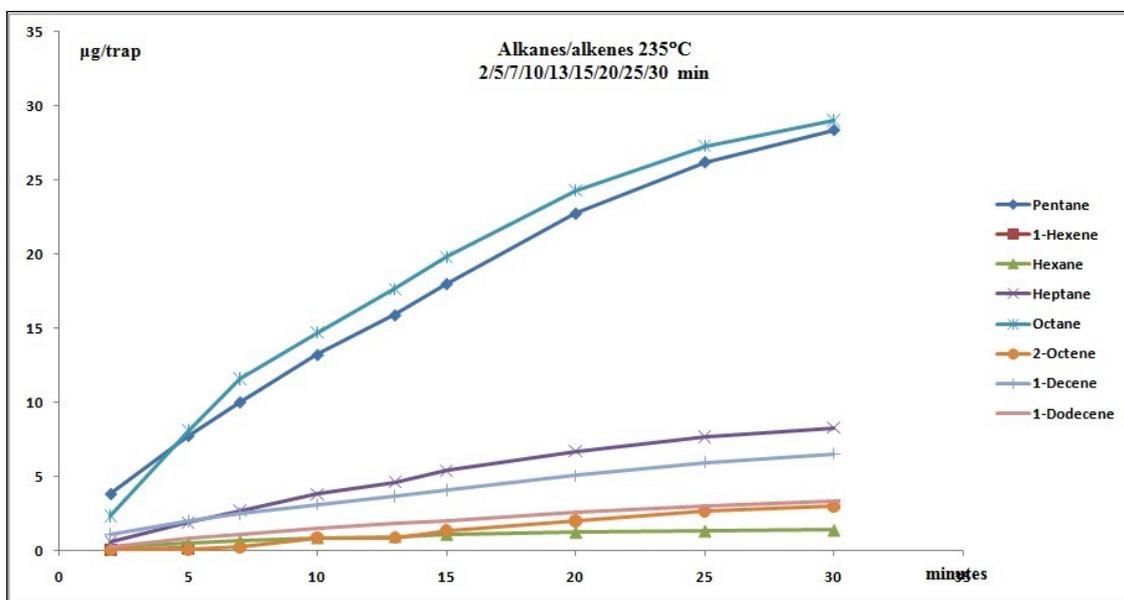


Figure 28. Alkanes/alkenes distribution under 235°C/N<sub>2</sub> function with time.

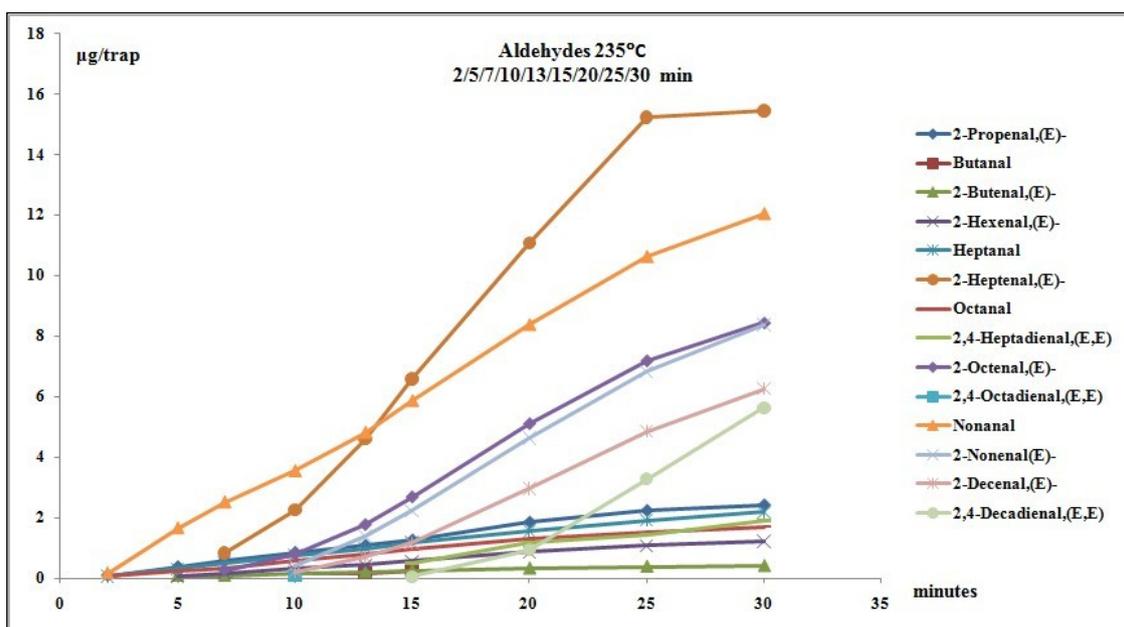
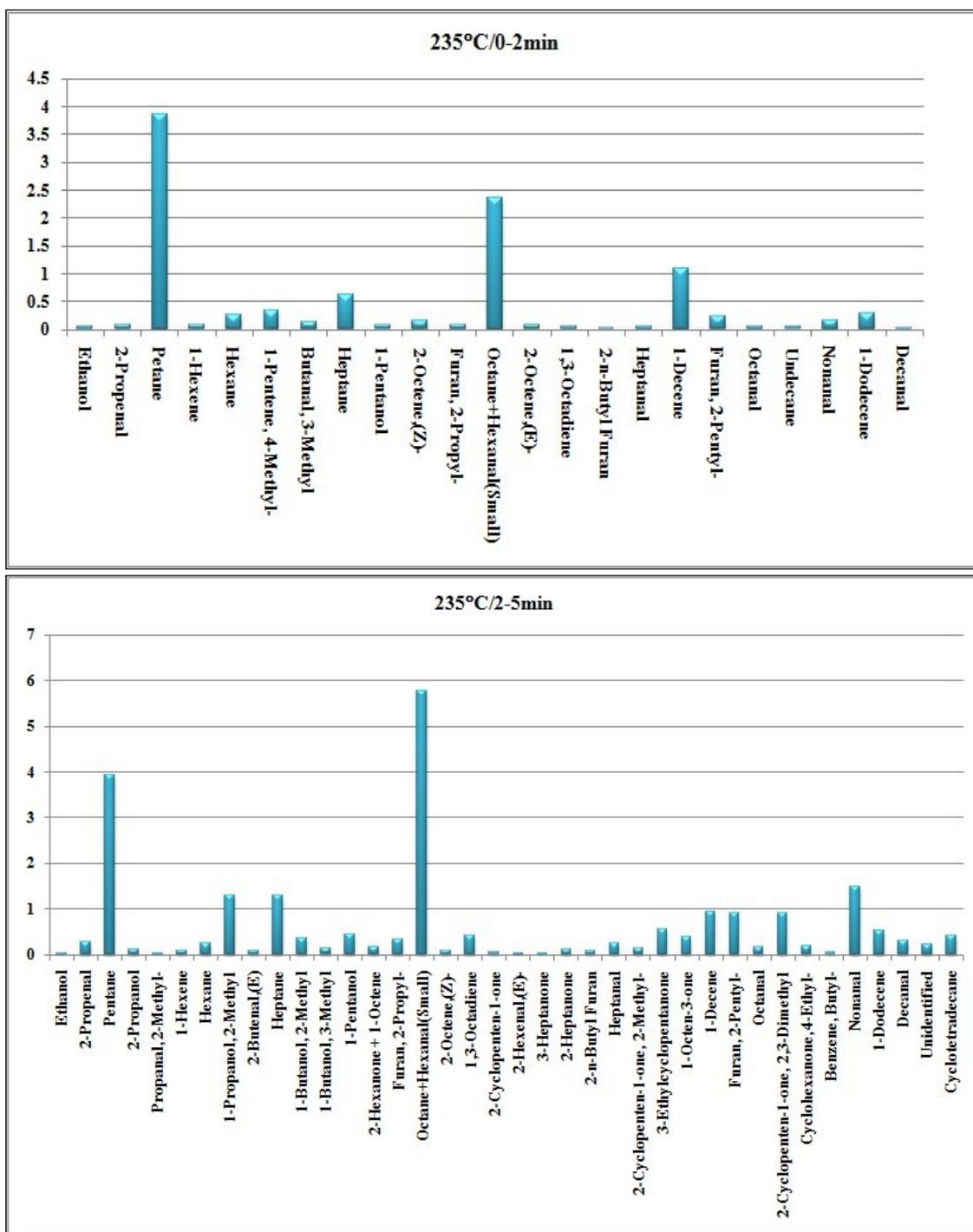
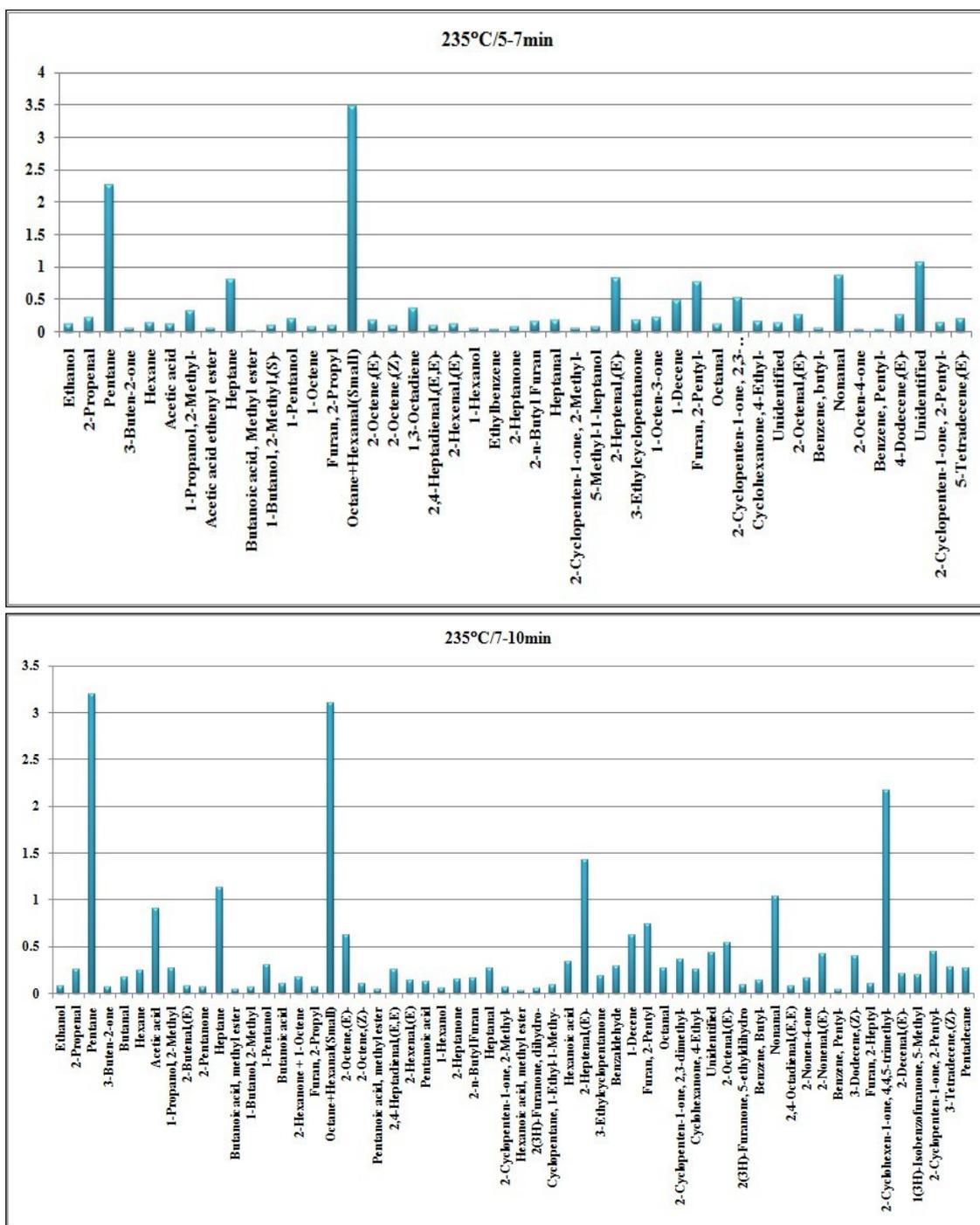


Figure 29. Aldehydes distribution under 235°C/N<sub>2</sub> function with time.



**Figure 30a.** Time sequence in development of products in corn oil heated at 235°C under N<sub>2</sub>. Samples collected (0 to 2 minutes) (top) and (2 and 5 minutes) (bottom).



**Figure 30b.** Time sequence in development of products in corn oil heated at 235°C under N<sub>2</sub>. Samples collected (5 to 7 minutes) (top) and (7 and 10 minutes) (bottom).

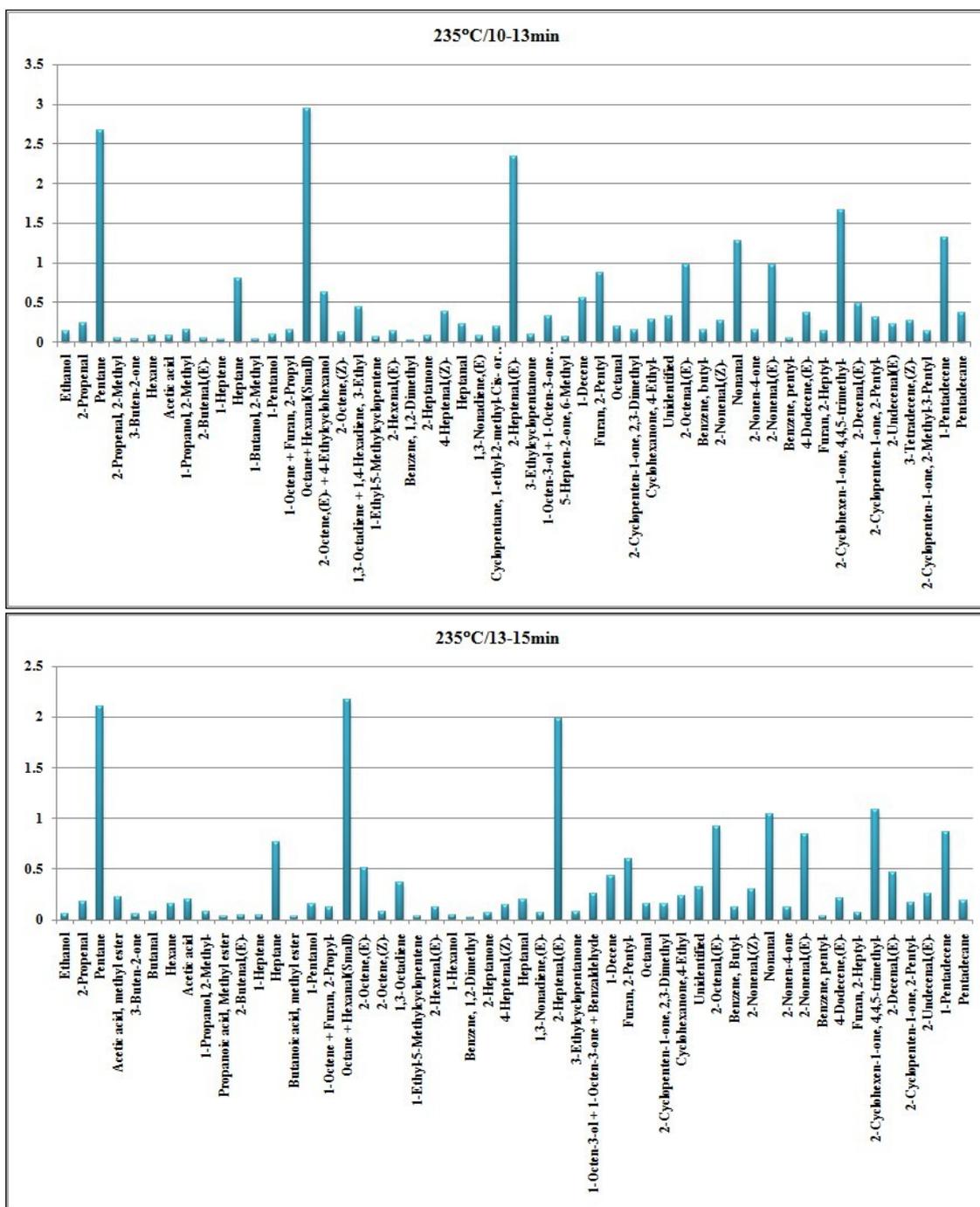


Figure 30c. Time sequence in development of products in corn oil heated at 235°C under N<sub>2</sub>. Samples collected (10 to 13 minutes) (top) and (13 and 15 minutes) (bottom).

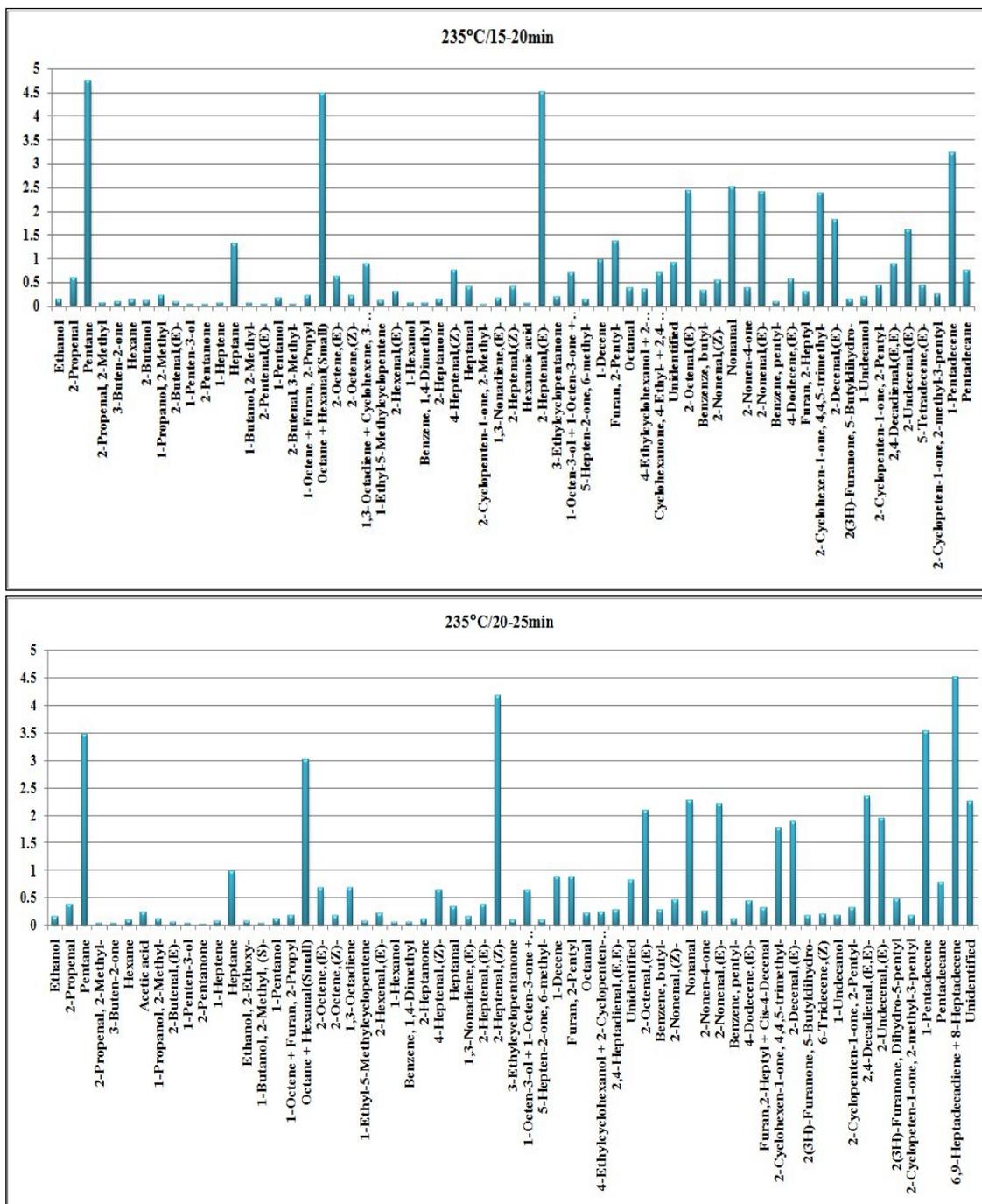


Figure 30d. Time sequence in development of products in corn oil heated at 235°C under N<sub>2</sub>. Samples collected (15 to 20 minutes) (top) and (20 and 25 minutes) (bottom).

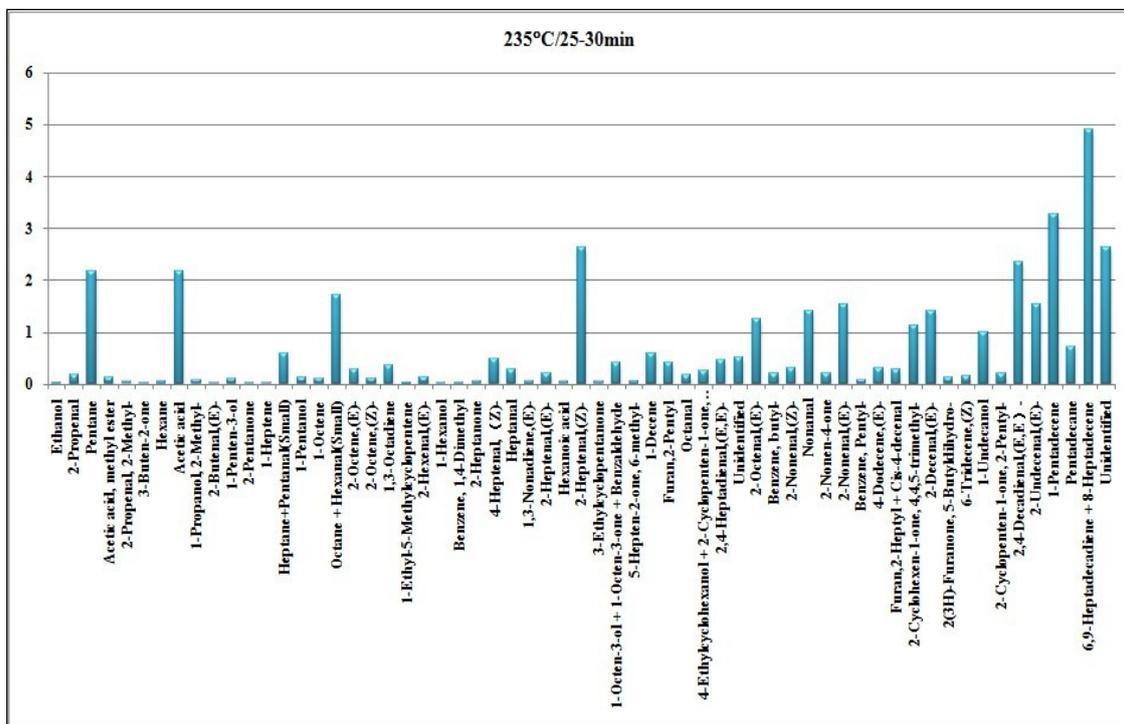


Figure 30e. Time sequence in development of products in corn oil heated at 235°C under N<sub>2</sub>. Samples collected (25 to 30 minutes).

### **5.3. Effect of heating temperature under air**

#### **Summary of temperature sequence under air**

GC data from thermal desorption method analysis of corn oil under different temperatures (100/120/150/180/235°C) displayed an exponential increase of product concentration and alternation of products distributions. Generally, at lower temperature, the major volatiles are alkanes/alkenes and slight amount of aldehydes, as temperature goes up, amounts of products are increasing with oxidation products dominance the overall concentration. C-5/C-6 are major oxidation products presented in GC chromatograph, such as pentanal/1-pentanol/hexanal. In addition, upon the reaction of oil with oxygen and thermal effect of frying temperature, homologous series of oxidation products are major volatiles and non-oxidation products such as alkanes/alkenes stay relatively lower concentration due to competition of free radical react with oxygen to generate higher amount of oxidation products. Exponential change of products concentration and distribution occurs between 120°C and 150°C as shown (Figure 31, 32)

Quatitation of GC data are carried out using benzene-d6, toluene-d8 and naphthalene-d8 with each 10 mg/mL. Above all, from temperature series under air, oxidation products are major volatiles presented in GC chromatograph with small amount non-oxidation products. Among oxidation products, besides C-5/C-6 aldehydes, other aldehydes displayed a homologous series, and cyclization reaction will increase exponentially as temperature reaches up to 180°C. Overloaded amount of volatiles at higher temperature force to shorten the collecting time to explore the early stage of frying at high temperature.

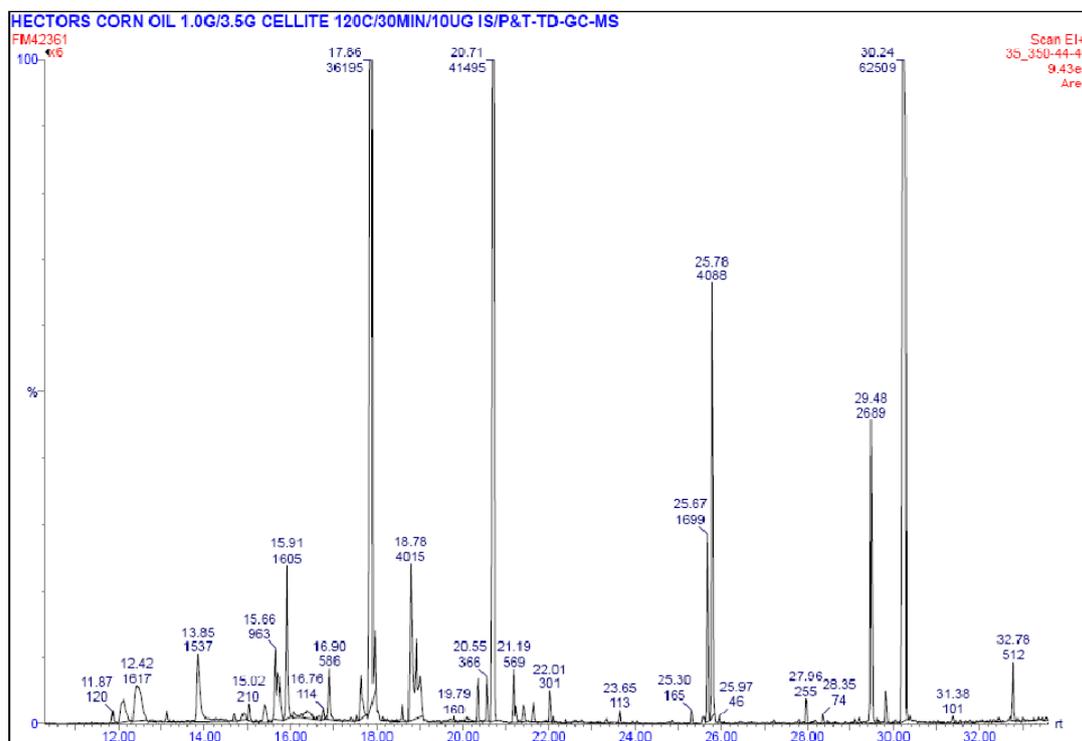


Figure 31. GC chromatograph under 120°C/30min/air.

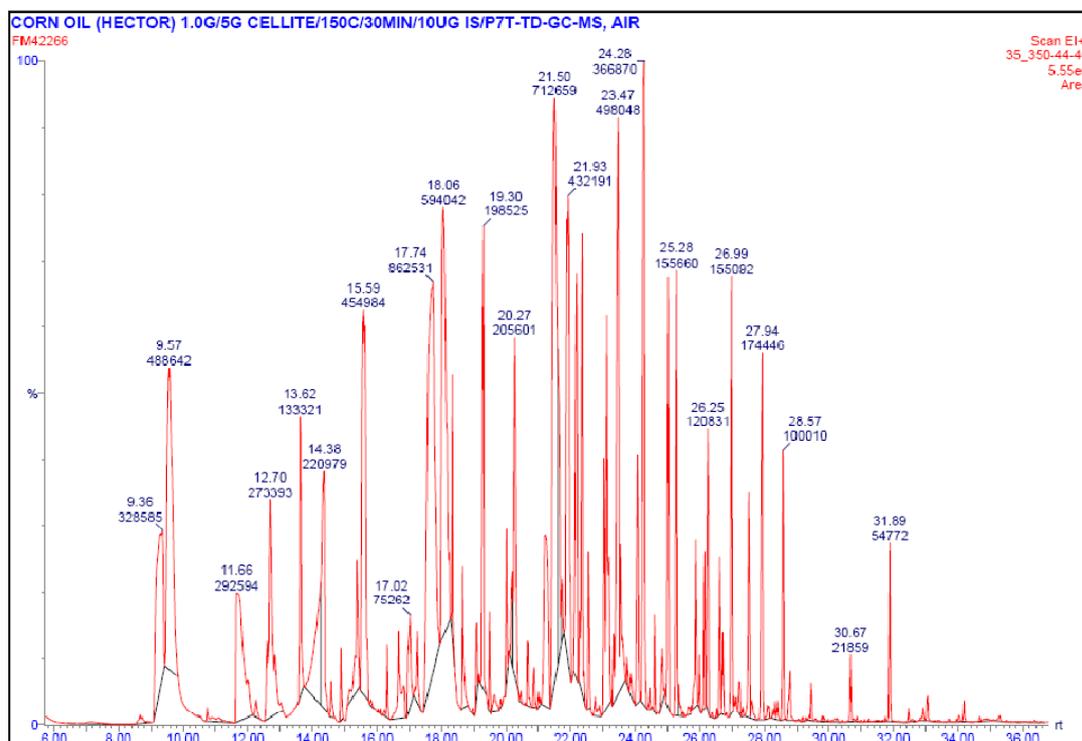
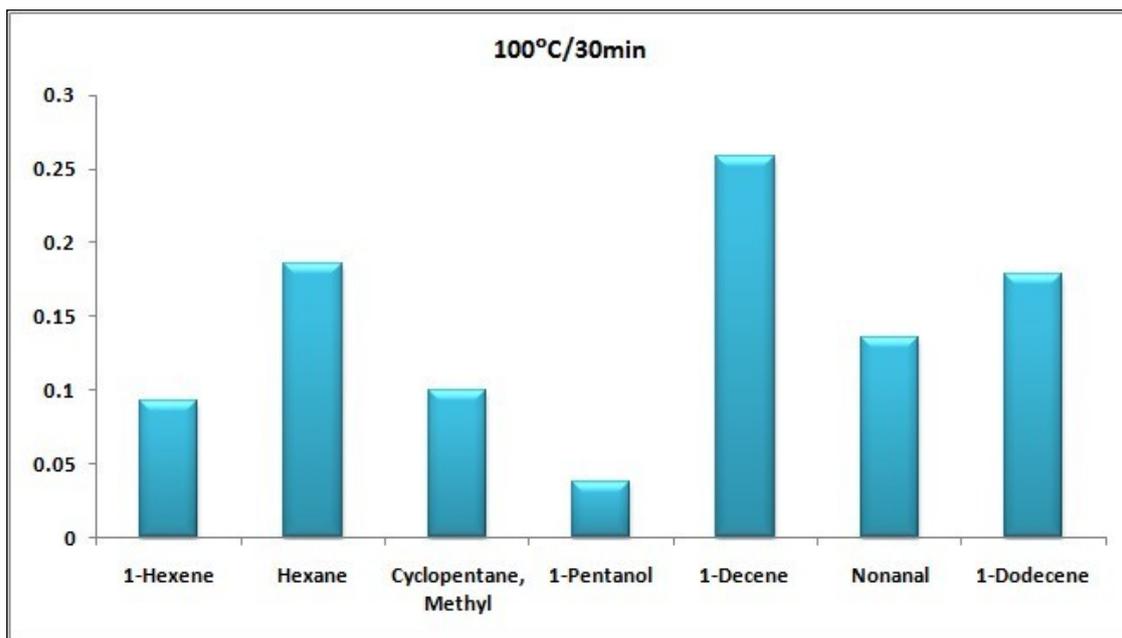


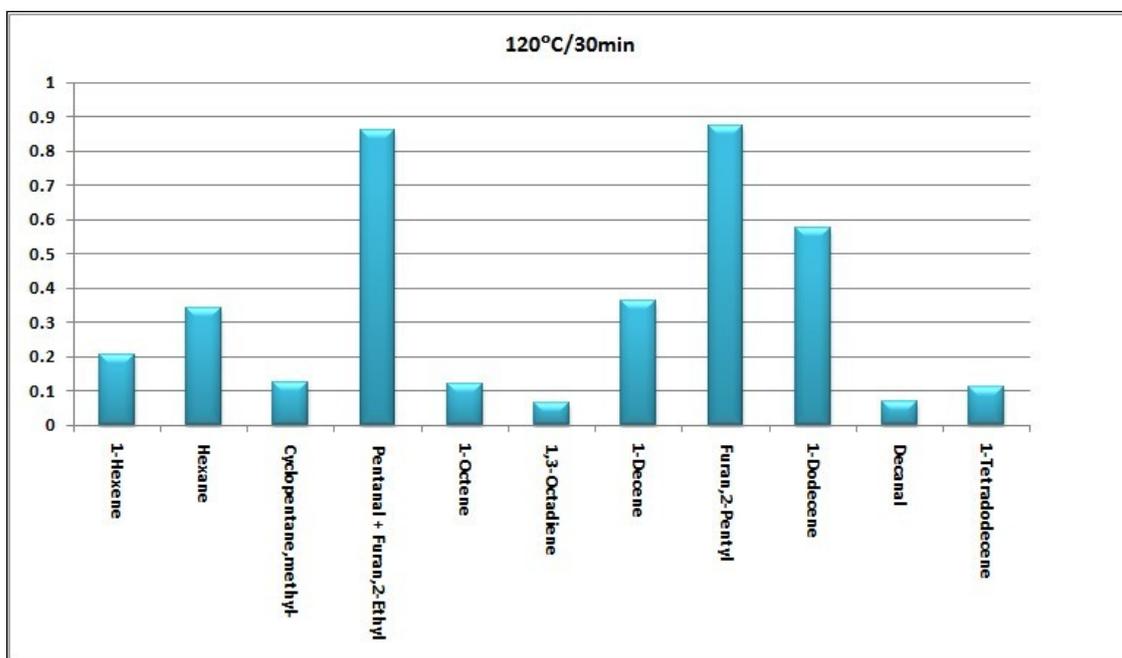
Figure 32. GC chromatograph under 150°C/30min/air.

Under air, all products increased dramatically in concentration; hexanal became the dominant product, accompanied by aldehydes of the same chain length as the original alkanes, plus ketones, lactones and other cyclized compounds. Details of these results are presented in the following sections.



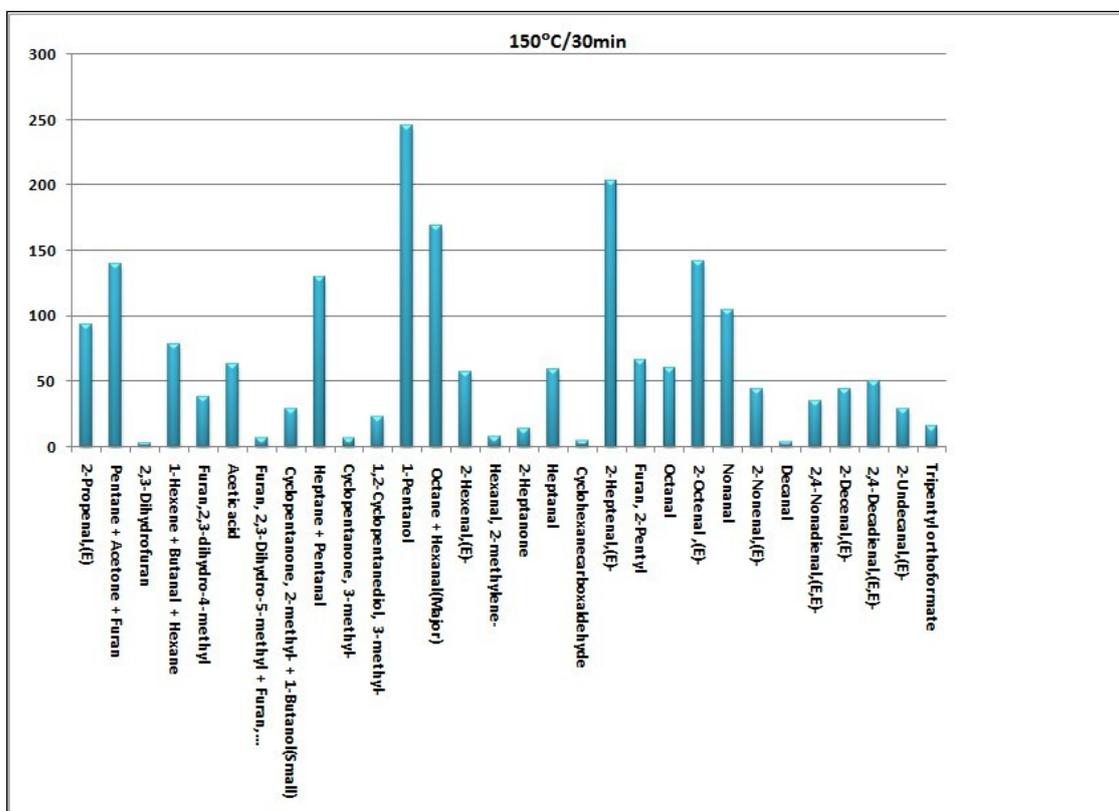
**Figure 33. Product distribution under 100°C/30min/air.**

Corn Oil was heated under 100°C for 30 minutes. Major volatiles are alkanes/alkenes and aldehydes. From Figure, 1-hexene/hexane/1-decene/1-dodecene are major long alkanes/alkenes, Nonanal is major aldehyde presented in GC chromatography. Beside above products, 1-pentanol is present as a only alcohol compounds at 100°C.



**Figure 34. Product distribution under 120°C/30min/air.**

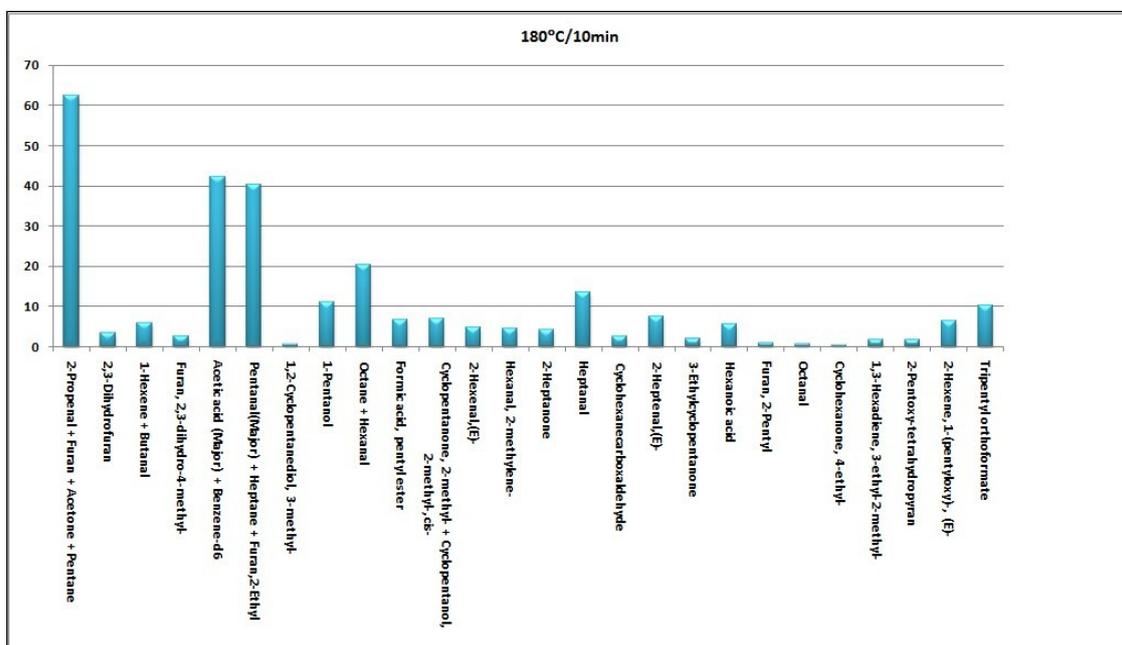
120°C heating under air for 30 minutes can generate more volatiles compare to 100°C for 30 minutes. Major volatiles are alkanes/alkenes and aldehydes dominant. Compared to 100°C, 1-hexene/hexane/1-decene/1-dodecene are also present in chromatography with increase concentration ( $\mu\text{g}/\text{trap}$ ), besides, cyclopentane-methyl which present in 100°C /30min but in lower concentration are kept in constant concentration under 120°C. In addition, 1-octene, 1,3-octadiene and 1-tetradecene are initially presented under 120°C. On the other hand, pentanal increased rapidly instead of producing 1-pentanol. Furan, 2-pentyl which is a major flavor compound contributes to frying are firstly displayed in chromatograph with dominant concentration. 10 carbon aldehyde such as decanal is also trapped but presented in low concentration.



**Figure 35. Product distribution under 150°C/30min/air.**

Mazola corn oil is heated under 150°C for 30min with constant air flow in Purge & Trap system. Compared to 120°C, large amount of products are volatilized and being collected onto short path thermal desorption trap. Products concentration and distribution are exponentially increased from 120°C to 150°C. Products distribution varies from alkanes/alkenes to aldehydes/alcohols/ketones/cyclized compounds. The highest concentration is 1-pentanol and its corresponding aldehyde (pentanal) also presents a major peak in GC data. Pentane/1-hexene/hexane/heptanes/octane are non-oxidation products which pentane is displayed on chromatograph and it co-eluted with acetone and furan and concentration of pentane stays moderate level compared to its corresponding alcohol/aldehyde. Besides non-oxidation products, oxidation products dominate the major

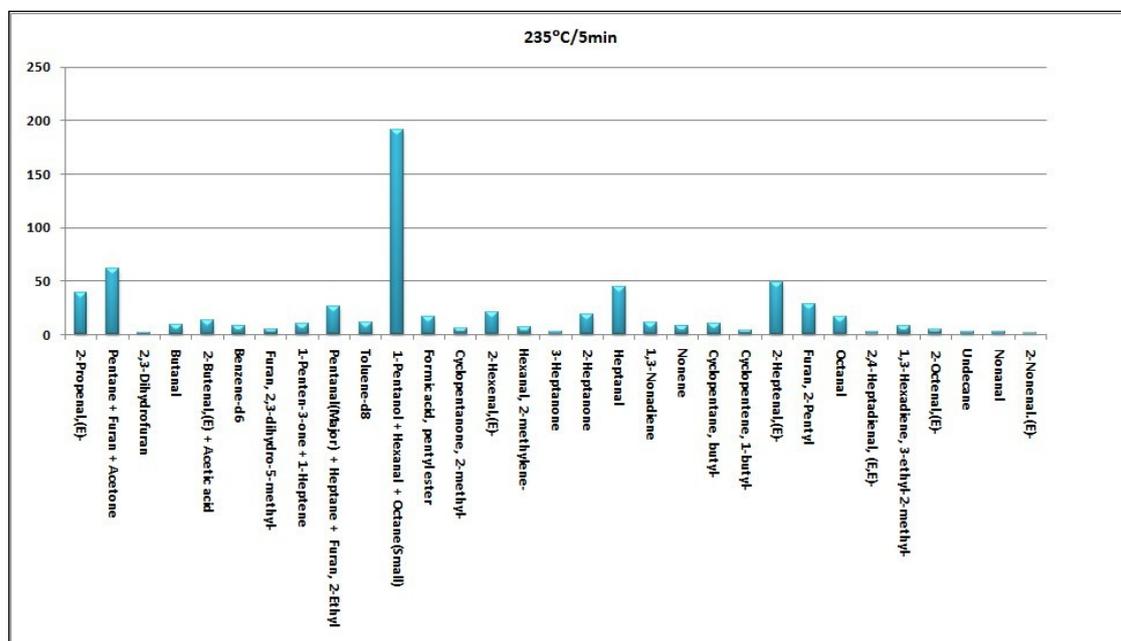
portion of volatiles. Hexanal/1-pentanol/pentanal are major oxidation products with C-5/C-6 carbon chain. In addition, oxidation products distributes from C-3 to C-12 with homologous series of aldehydes and other oxidation products such as ketones or 2,4-dienals. Two specific autoxidation products from C-9/C-13 position are hexanal/2,4-decadienal,(E,E)-. However, hexanal displays a higher concentration compared to 2,4-decadienal,(E,E)-. Above all, 150°C /30min under air can generate complex and mixed volatiles and distribution varies from alkanes/alkenes to aldehydes/ketones/cyclized compounds.



**Figure 36. Product distribution under 180°C/10min/air.**

GC chromatograph of corn oil which is heated under 180°C for 10min present a more complex products distribution compared to 150°C for 30min. Pre-experiment is tested under 180°C for 30 minutes, however, concerning on the same load of corn oil on the glass tube, 30 minutes collecting time will overload the short path thermal desorption trap and thus volatiles will escape from trap and generate strong smell in laboratory. Thus, shortening the collecting time is necessary to monitor the products distribution change. By analyzing the GC chromatograph, products distribution are similar compared to lower temperature but generate more complex compounds through cyclization, such cyclopentanone, 2-methyl-/cyclopentanol, 2-methyl-, cis-/3-ethylcyclopentanone. Beside cyclized products, aldehydes/alcohol presents largest peak area in GC chromatograph with C-5/C-6/C-7 dominance, such as pentanal/1-pentanol/hexanal/2-hexenal/heptanal/2-heptenal. Higher temperature can also generate homologous series of aldehyde from C-4 to C-12. Autoxidation products such as hexanal can undergo further oxidation pathways to

form hexanoic acid. Alkane/alkene which are possible thermal scission products are co-eluting with oxidation products, but can be still traced in GC chromatograph.



**Figure 37. Product distribution under 235°C/5min/air.**

Smoke point of corn oil is at 235°C and shortened collecting time is also applied at 235°C to avoid overloading of volatiles of short path thermal desorption trap. The compounds distribution and concentration also increased exponential between 180°C and 235°C. Data of products are shown above, C-5/C-6 oxidation products are dominant and their concentration are dramatically higher than other oxidation/non-oxidation products. Other major oxidation products includes C-7, C-8 such as heptanal/ 2-heptenal/ octanal/ 2-octenal, (E)-. Non-oxidation products are also present in GC data, such as alkanes/ alkenes, including pentane/ 1-heptene/ octane/ 1,3-nonadiene/ nonene/ cyclopentane, butyl/ undecane but formation of non-oxidation products stays at lower rate. Although there are differences of ratio of different products between 180°C and 235°C, generally, 235°C will initiate a more complex system due to more complex products distribution at early stage

(5min) and these early products will lead to a more complicated system than lower temperature.

#### 5.4. Time sequence of degradation under air

##### 5.4.1. Oil heated at 100 °C, volatiles collected at 5/10/15/30/45/60/90/120 min

##### Summary of products function with collecting time at 100°C

At 100°C, major volatiles are alkanes/alkenes at early stage, after 60 minutes heating, hexanal becomes a major oxidation products. In addition, pentanal is also a major aldehyde after 90 minutes heating. Besides oxidation products, alkanes/alkenes are dominant volatiles at early stage, such as C-6/C-8/C-10. However, after 60 minutes heating, oxidation products are going to take over the major peak area. 2-Pentyl Furan is another marker products which forms at 15 minutes under air. The aldehyde are listed in Figure 38.

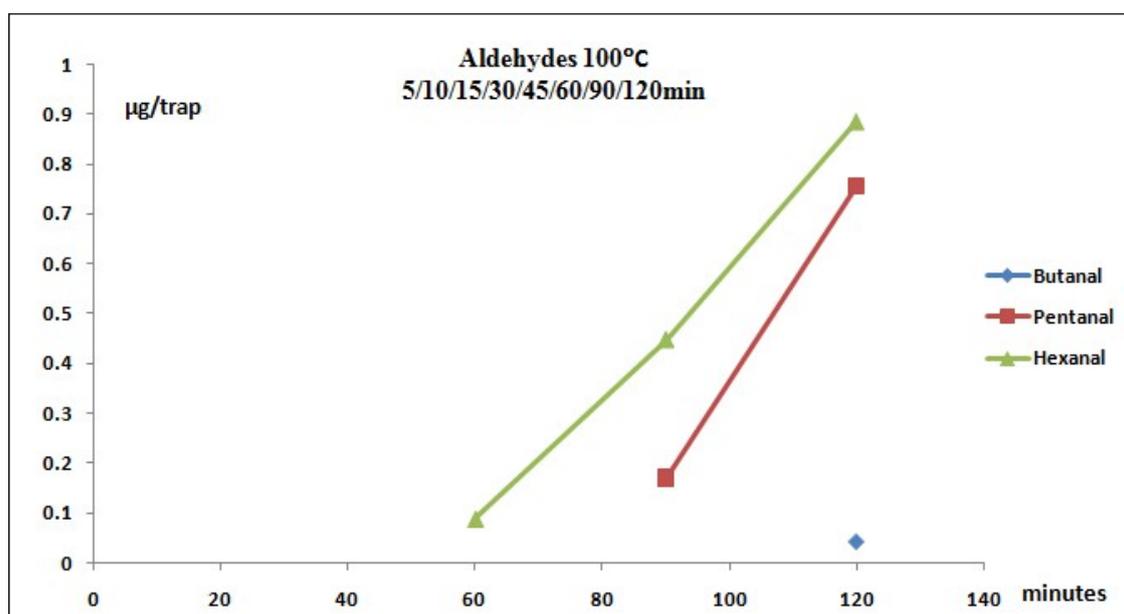
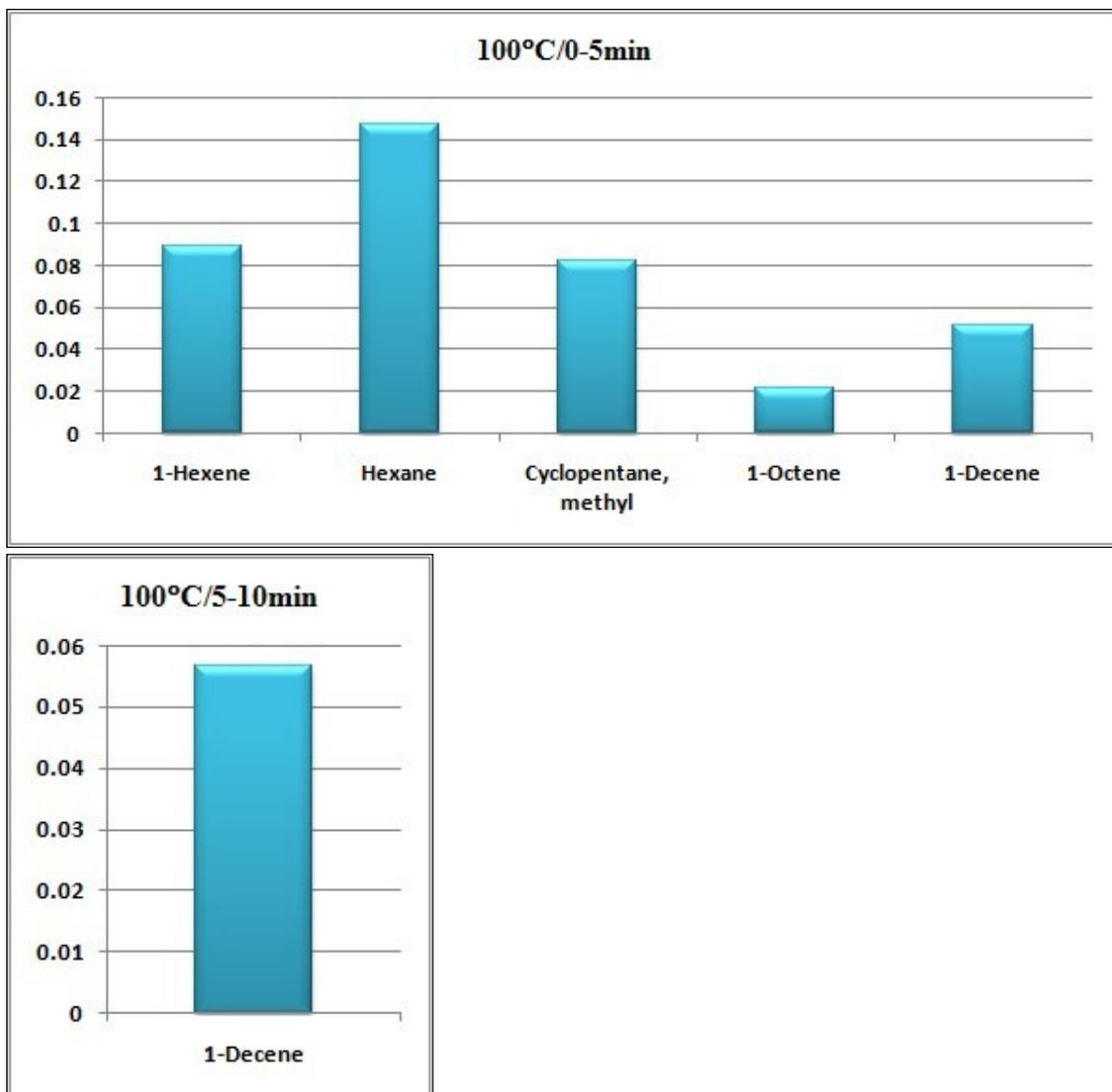
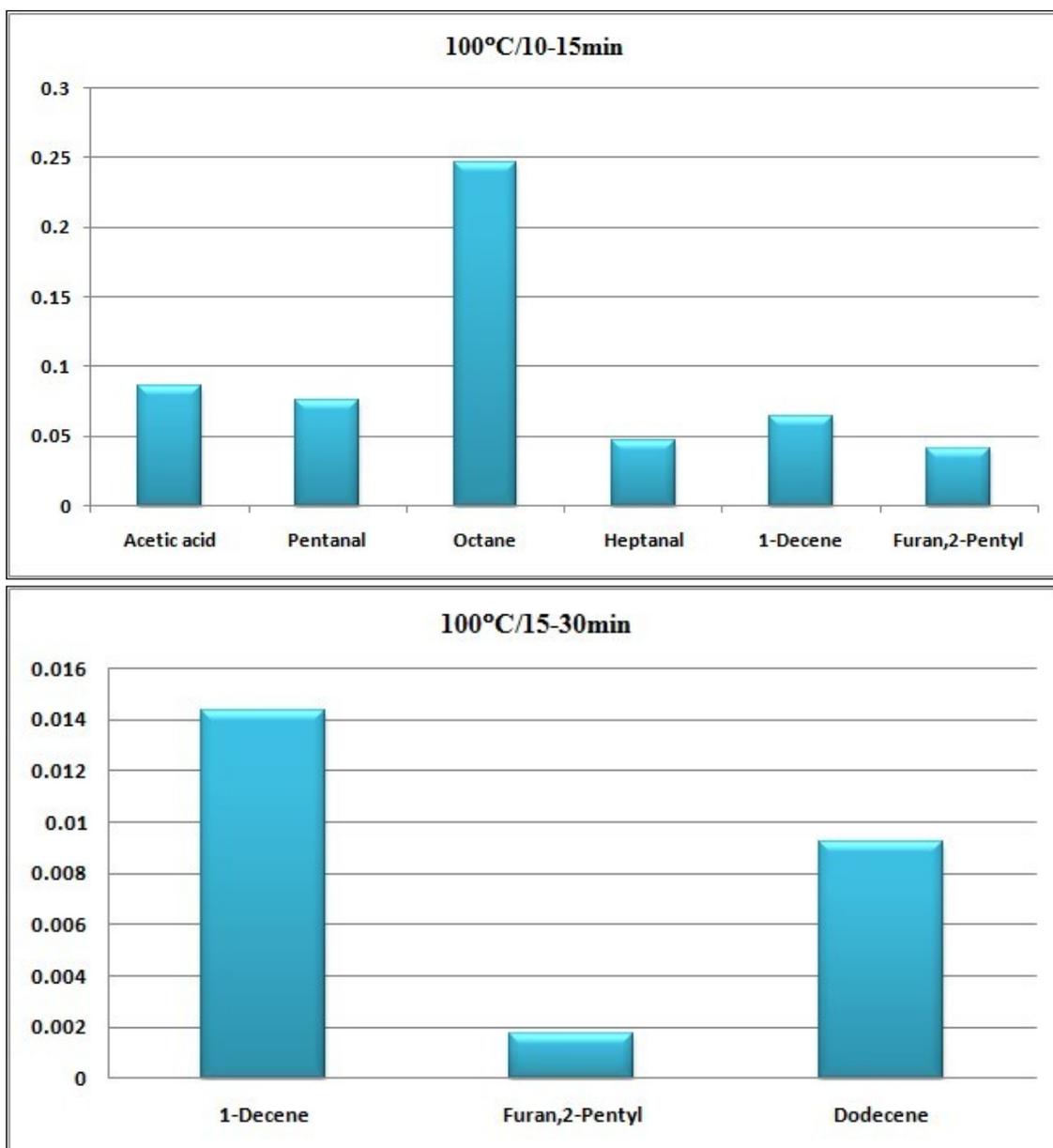


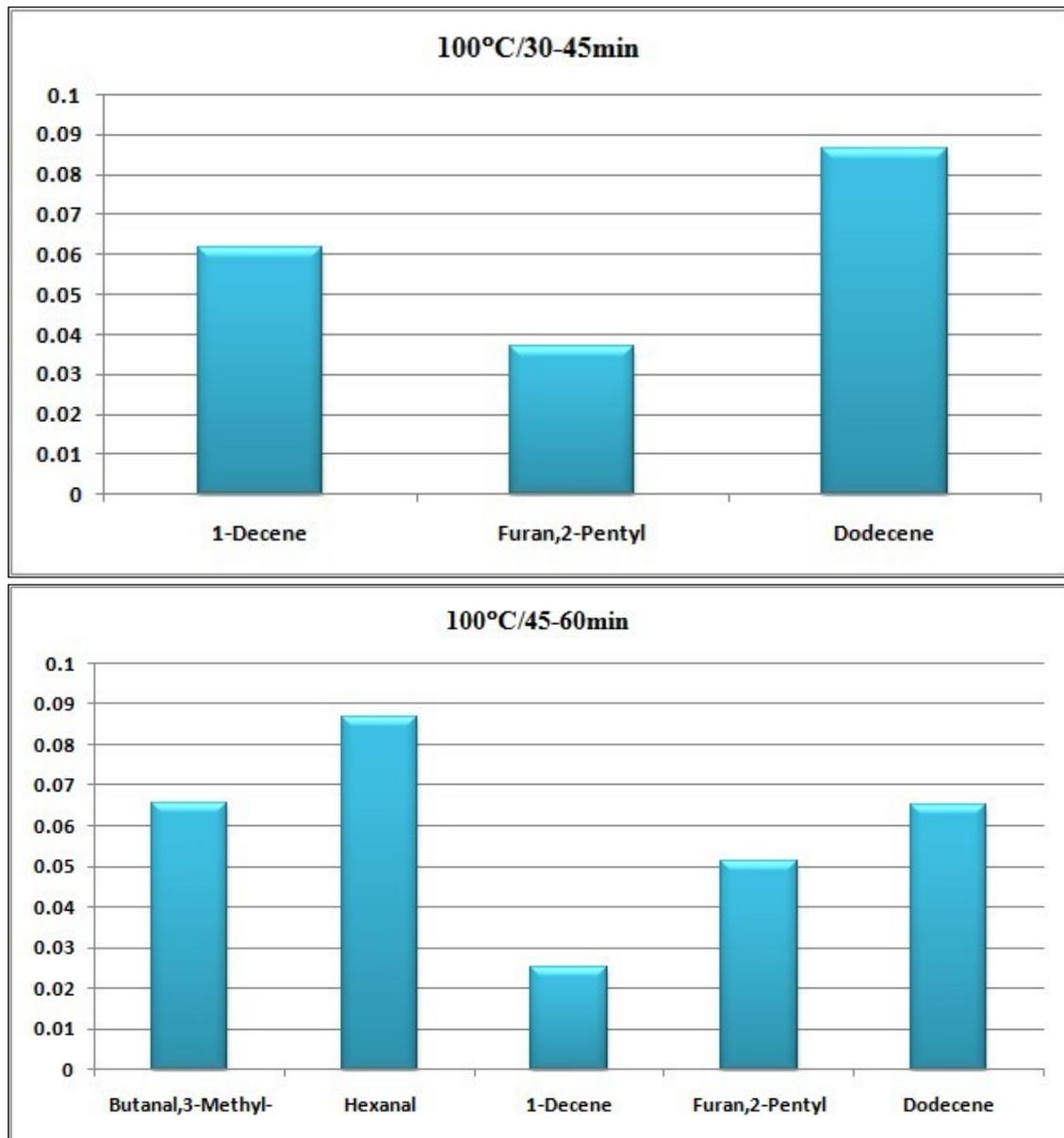
Figure 38. Aldehydes distribution under 100°C/air function with time.



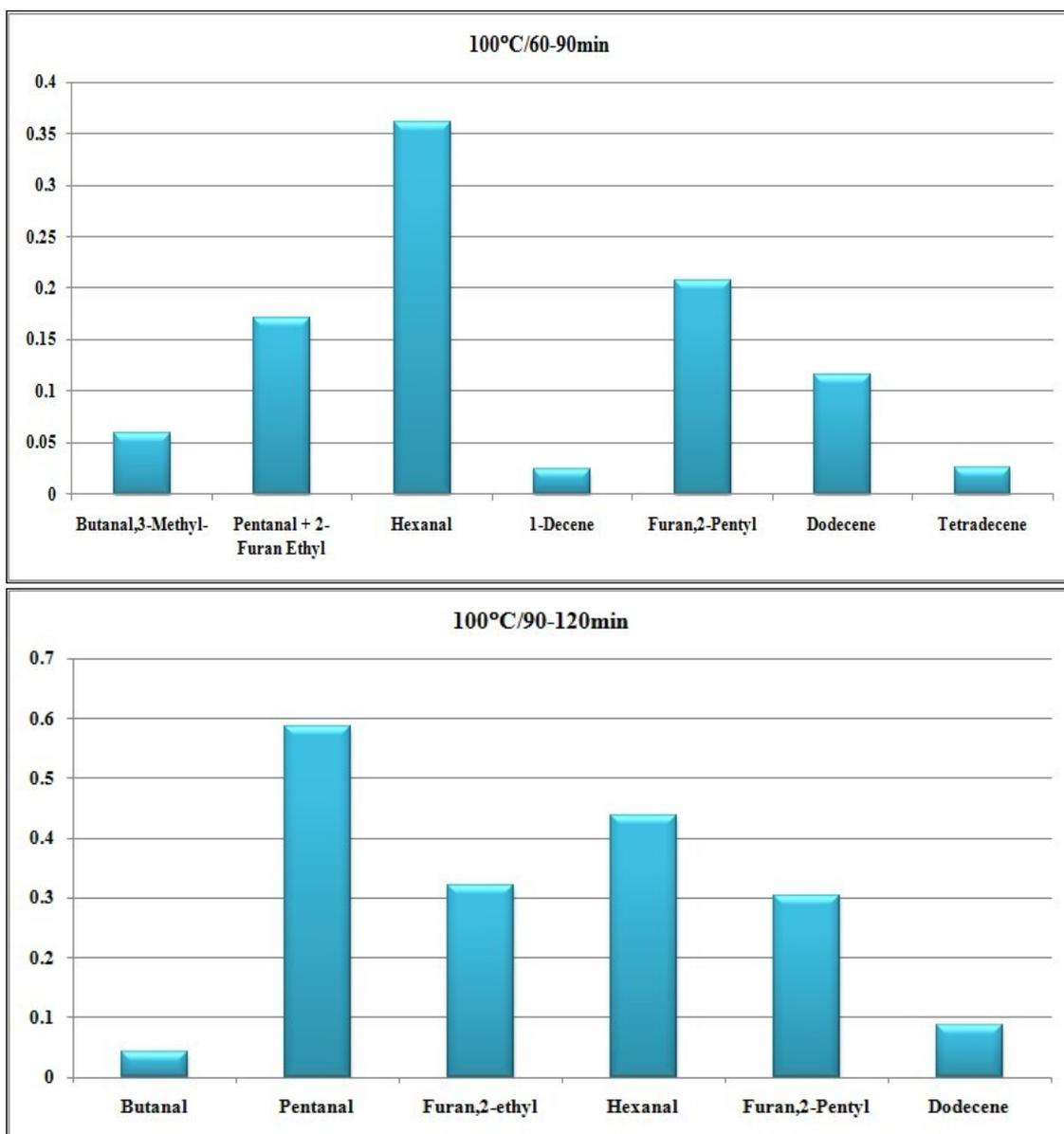
**Figure 39a.** Time sequence in development of products in corn oil heated at 100°C under air. Samples collected (0 to 5 minutes) (top) and (5 and 10 minutes) (bottom).



**Figure 39b.** Time sequence in development of products in corn oil heated at 100°C under air. Samples collected (10 to 15 minutes) (top) and (15 and 30 minutes) (bottom).



**Figure 39c.** Time sequence in development of products in corn oil heated at 100°C under air. Samples collected (30 to 45 minutes) (top) and (45 and 60 minutes) (bottom).



**Figure 39d.** Time sequence in development of products in corn oil heated at 100°C under air. Samples collected (60 to 90 minutes) (top) and (90 and 120 minutes) (bottom).

#### **5.4.2. Oil heated at 120 °C, volatiles collected at 5/10/15/30/45/60/90/120 min**

##### **Summary of products function with collecting time at 120°C:**

Early stage of frying at 120°C will generate alkanes/alkenes and small portion of oxidation products before 30 minutes. However, 2-pentyl furan is a major oxidation product up to 15 minutes collecting time. Alkanes/alkenes from early stage of frying are C-6/C-10/C-12. At 15 minutes, 2-pentyl furan firstly appeared in GC chromatograph and from 15 minutes to 30 minutes, both obvious amount of non-oxidation/oxidation products volatilize, such as pentane, octane, 1-octene, 1,3-octadiene, 1-decene, 1-dodecene/pentanal which is aldehyde prior to formation of hexanal. After 45 minutes of collection, hexanal/pentanal/2-pentyl furan dominate the oxidation products and major peak area. As collection time goes up to 90 minutes, hexanal peak is the highest one in chromatograph and pentanal/2-pentyl furan are other major oxidation products. At 90 minutes, from GC data, homologous series of oxidation products (aldehydes/alcohols) are present from C-4 to C-9: butanal, 1-butanol, pentanal, 2-pentenal (E)-, hexanal, hexanoic acid, heptanal, 2-heptenal (E)-, octanal, nonanal. When extend the collecting time to 120 minutes, the product distribution and concentration changes exponentially, hexanal increases from 45 µg/trap to 200 µg/trap. In addition, 1-pentanol from the trap collecting between 90 to 120 minutes is dominant product compared to other oxidation products, and more homologous series of oxidation products are generated than 60 to 90 minutes period such as 2-nonanal (E)-. Cyclization reaction occurs by generating large amount of cyclopentanol, 2-Methyl Cis-, 2-Ethylcyclohexanol. Generally, at 120 °C, the product distribution changes from 30 minutes with formation of major oxidation products to 90 minutes with formation homologous series of oxidation products. Up to 120 minutes, C-5 alcohol is a dominant

major compound and cyclization occurs in the reaction matrix, homologous series products are generating at exponential rate.

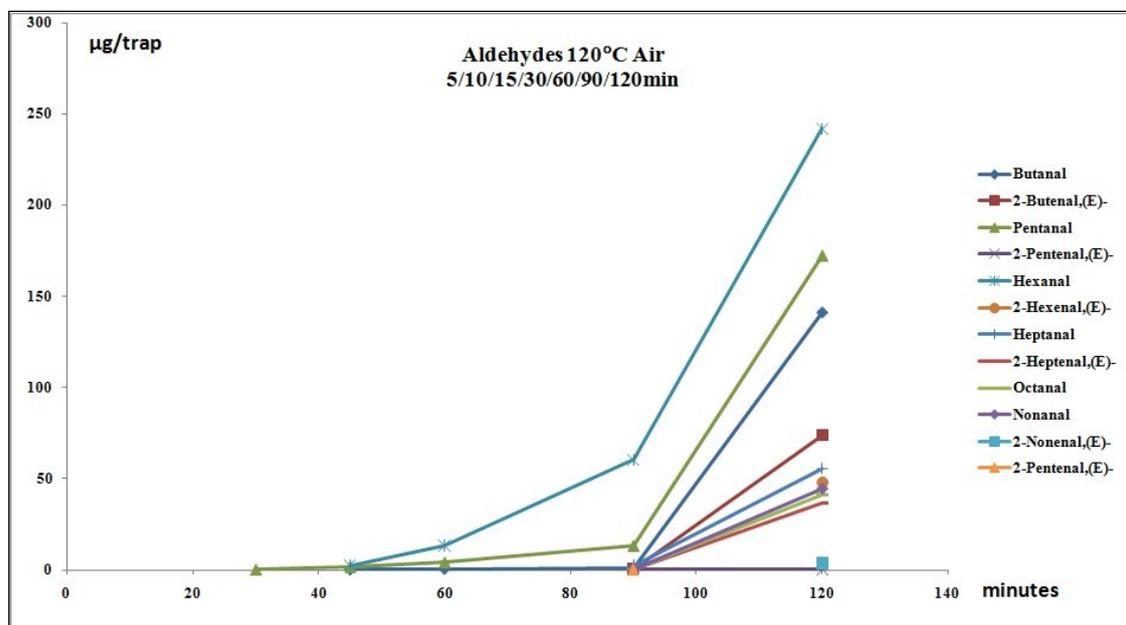
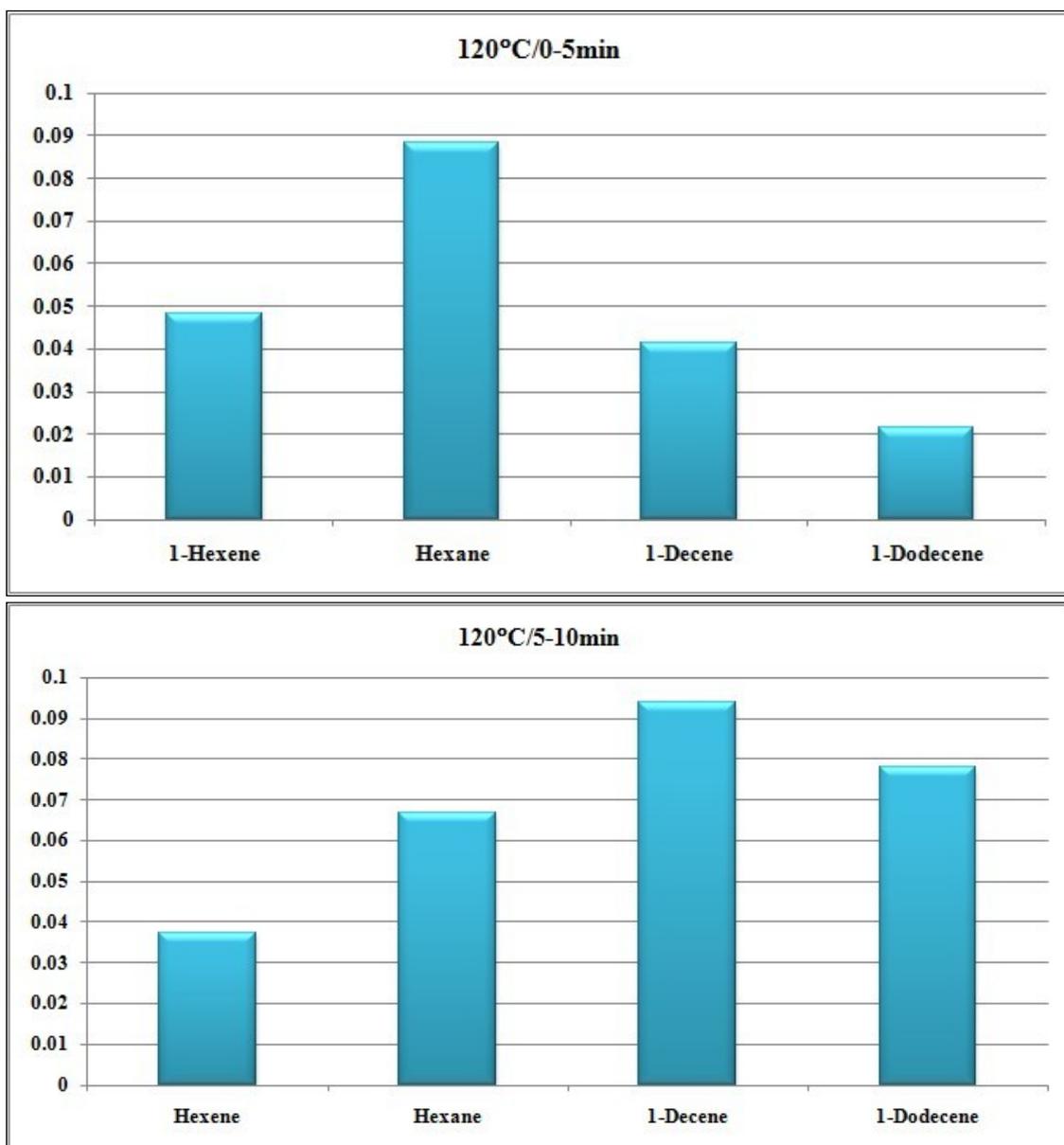
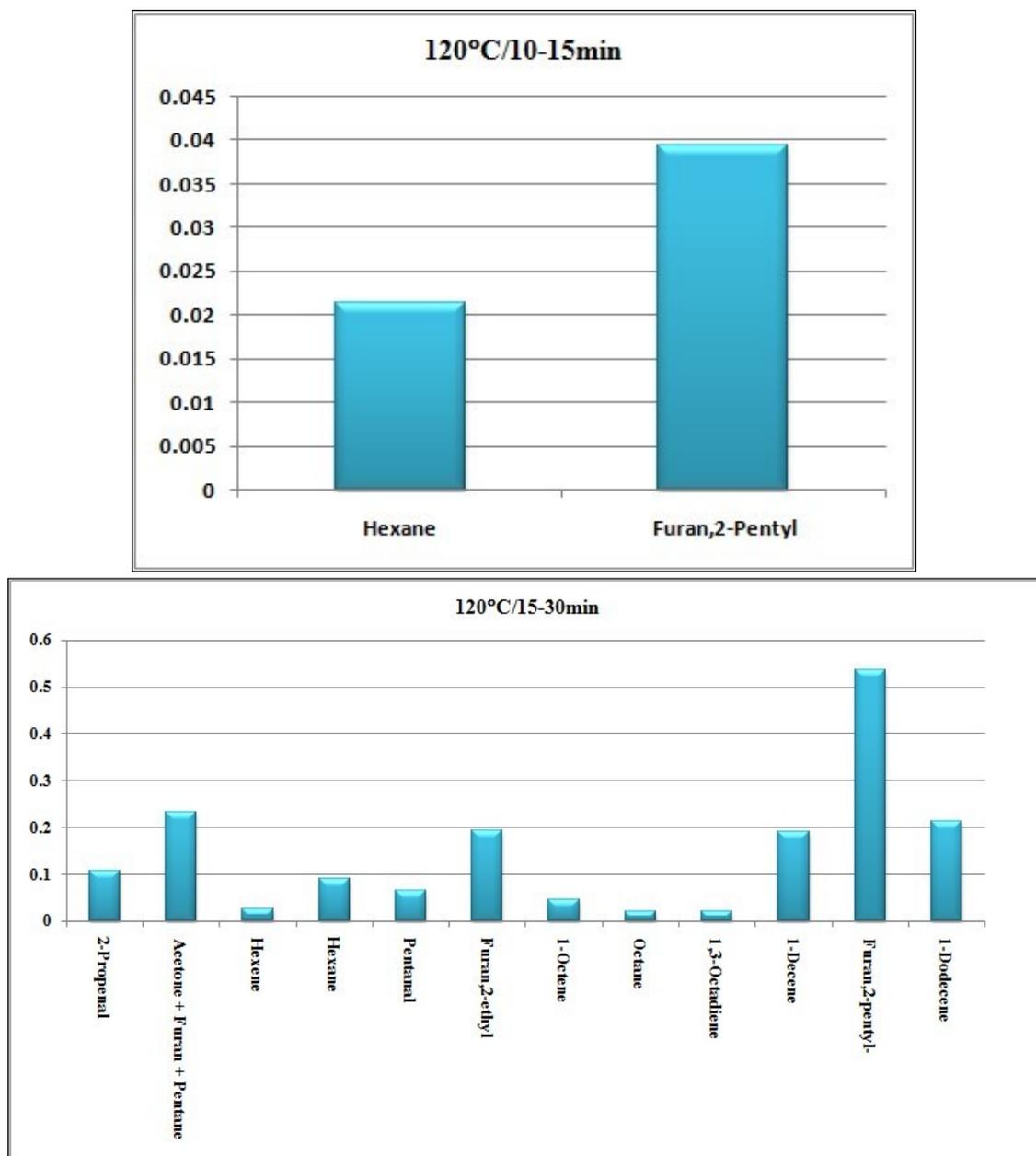


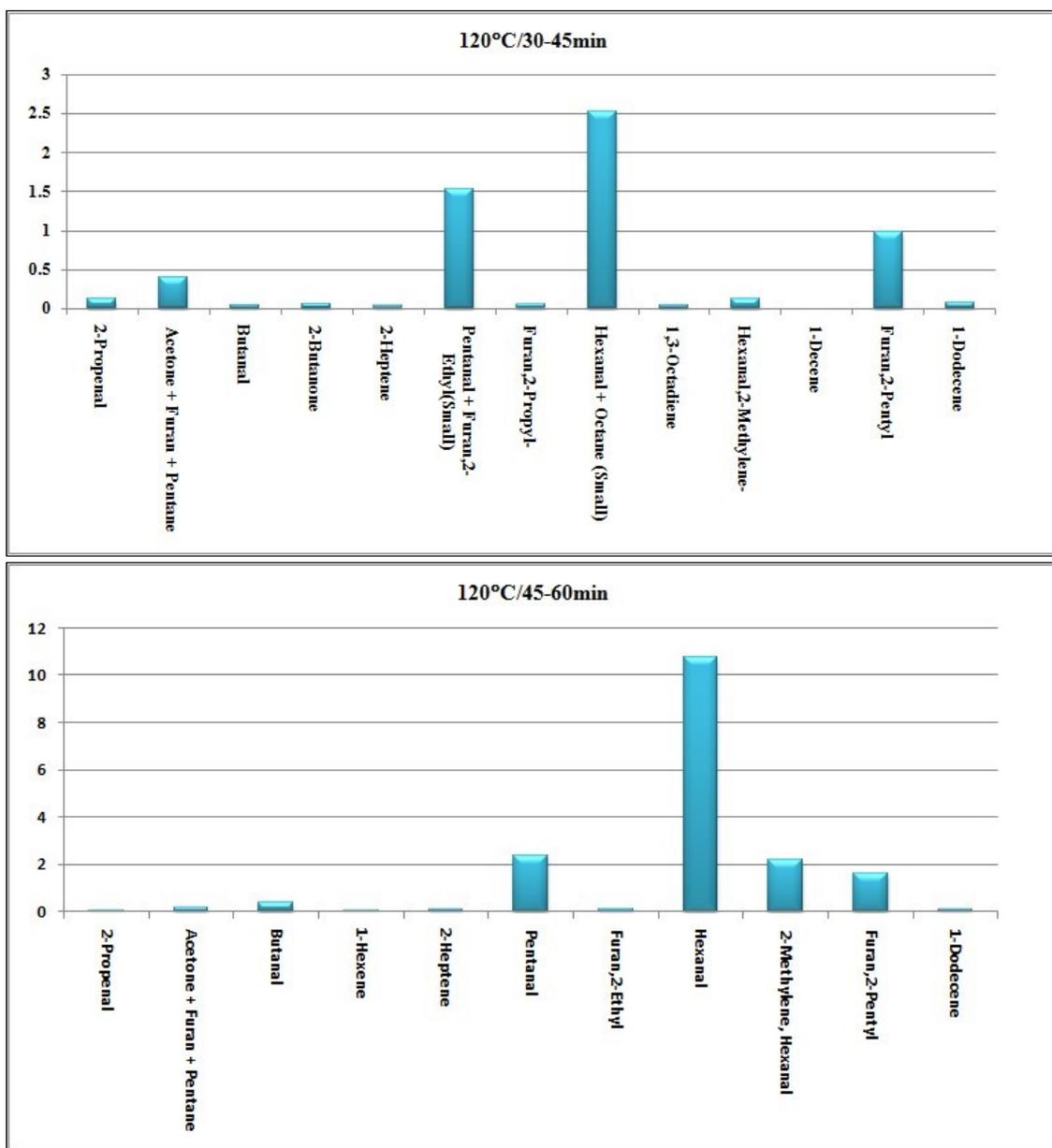
Figure 40. Aldehydes distribution under 120°C/air function with time.



**Figure 41a.** Time sequence in development of products in corn oil heated at 120°C under air. Samples collected (0 to 5 minutes) (top) and (5 and 10 minutes) (bottom).



**Figure 41b.** Time sequence in development of products in corn oil heated at 120°C under air. Samples collected (10 to 15 minutes) (top) and (15 and 30 minutes) (bottom).



**Figure 41c.** Time sequence in development of products in corn oil heated at 120°C under air. Samples collected (30 to 45 minutes) (top) and (45 and 60 minutes) (bottom).

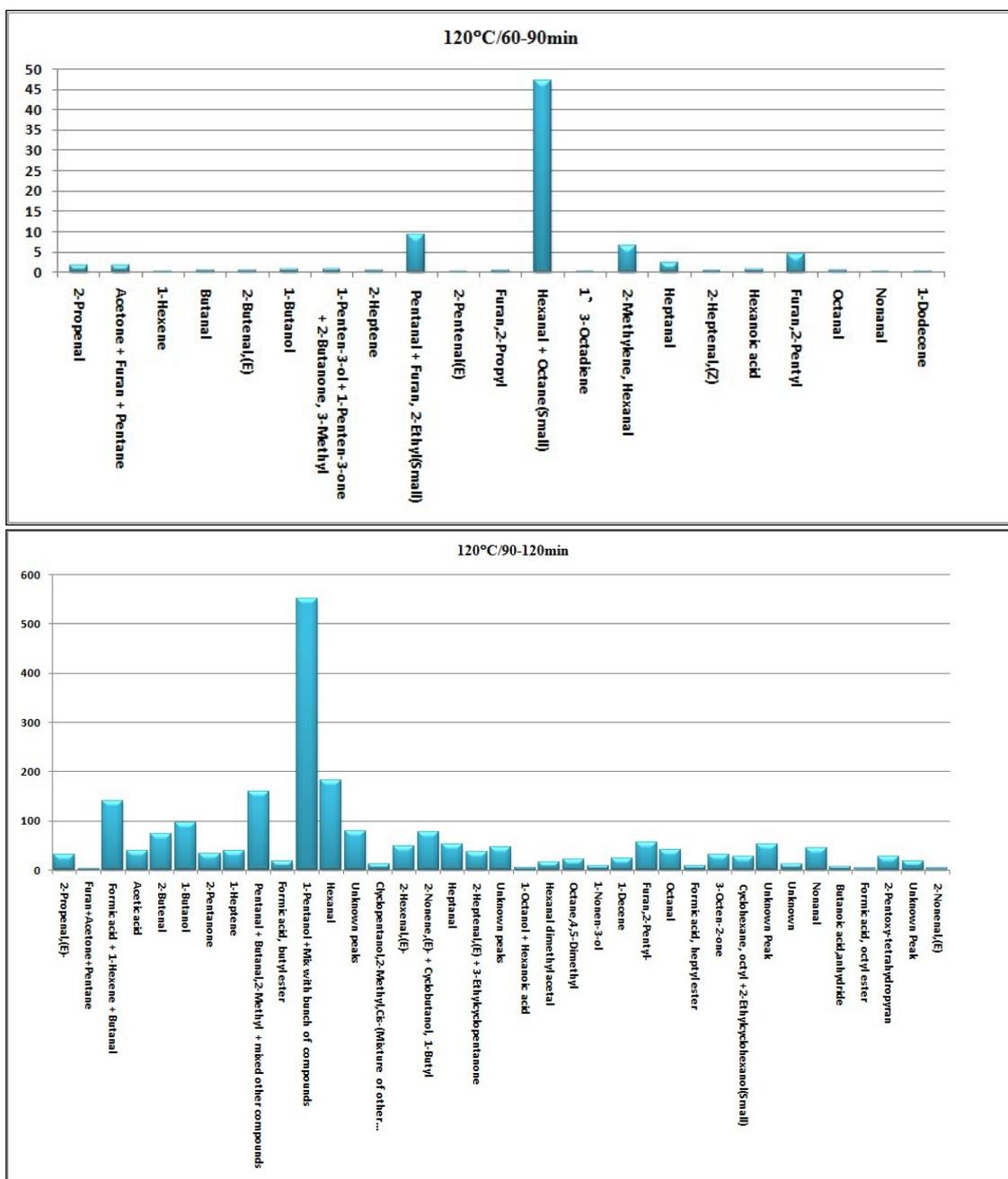


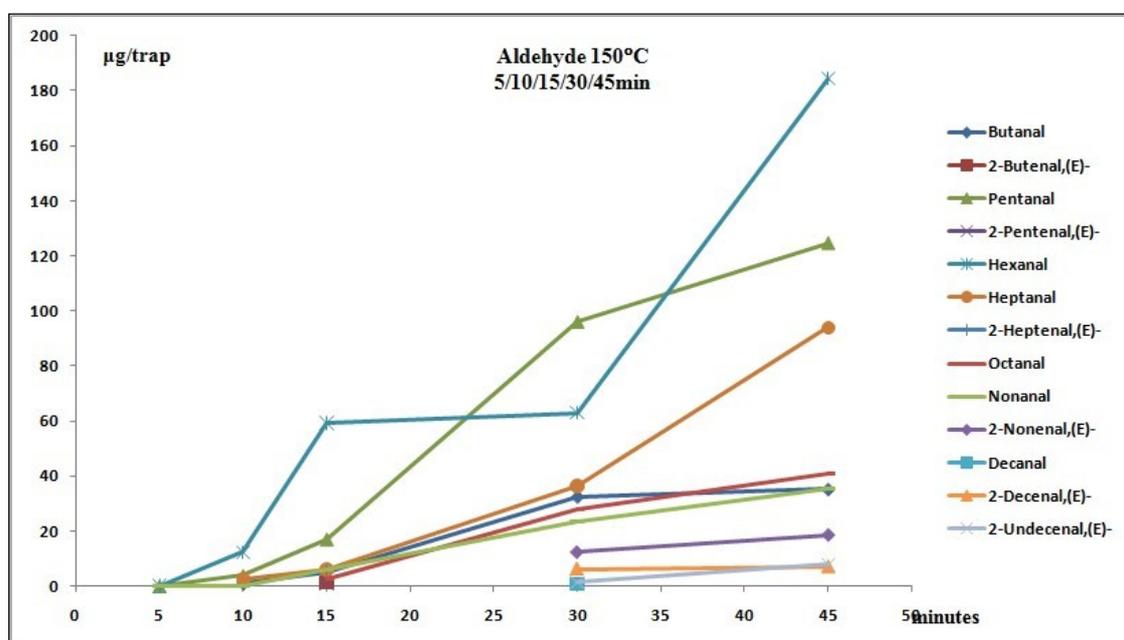
Figure 41d. Time sequence in development of products in corn oil heated at 120°C under air. Samples collected (60 to 90 minutes) (top) and (90 and 120 minutes) (bottom).

### 5.4.3. Oil heated at 150 °C, volatiles collected at 5/10/15/30/45 min

#### Summary of products function with collecting time at 150 °C

Under 150°C of heating, the distribution and concentration of volatiles are exponentially increased compared to 120°C. Even at early stage of frying (in 5min), volatiles collected in short path thermal desorption trap includes non-oxidation/oxidation products, such as alkanes/alkenes vs autoxidation products (hexanal). Both non-oxidation/oxidation products shared the equal total peak area. However, when heating time reaches up to 10 minutes, hexanal is dominant in products and pentanal/2-pentyl furan are major products, alkanes/alkenes stays at lower level and even formation of alkanes/alkenes are successfully competed by oxidation pathways. Volatiles in heating period of 10-15 minutes shows a significant homologous series of oxidation products as in lower temperature with longer heating time: butanal/ 2-butenal,(E)-/ 1-butanol/ 1-pentanol /pentanal/ hexanal/ 2-hexenal/ 2-heptanone/ heptanal/ 2-heptenal/ octanal/ nonanal. Cyclization occurs at 150°C, major cyclized products are cyclopentanone,2-methyl-/ cyclopentanol, 2-methyl-. In heating period of 15-30min, short chain oxidation products increased dramatically such as acetone, butanal, furan. Several products are co-eluted with each other, cyclization is more predominant and more cyclization reaction occurs and products such as cyclopentanone, 2-methyl-/ cyclohexanecarboxaldehyde/ 3-Ethylcyclopentanone. 2,4-Decadienal, (E,E)- is displayed in GC data with relative small amount which contributes important flavor to frying products. As heating time extends to 45 minutes, several co-eluted large peaks are displayed in GC chromatograph due to large amount of volatiles generated at 150°C, it is not easy to analyze the large-co-eluted peaks, however, from analysis of peaks, frying process and its product distribution are more

complex and relative small load of short path thermal desorption trap cannot meet the large amount of volatiles generated (especially in our Purge& Trap system). Large amount of alcohol (1-pentanol) and cyclization products are predominant after 45 minutes of heating. Thus, experiment at 150°C is carried out only at 45 minutes heating, in the future work, shorten the time gap of collecting is necessary to avoid the overloading effect of our system at higher temperature. Here is illustrated aldehydes function with collecting time Figure 42:



**Figure 42.** Aldehydes distribution under 150°C/air function with time.

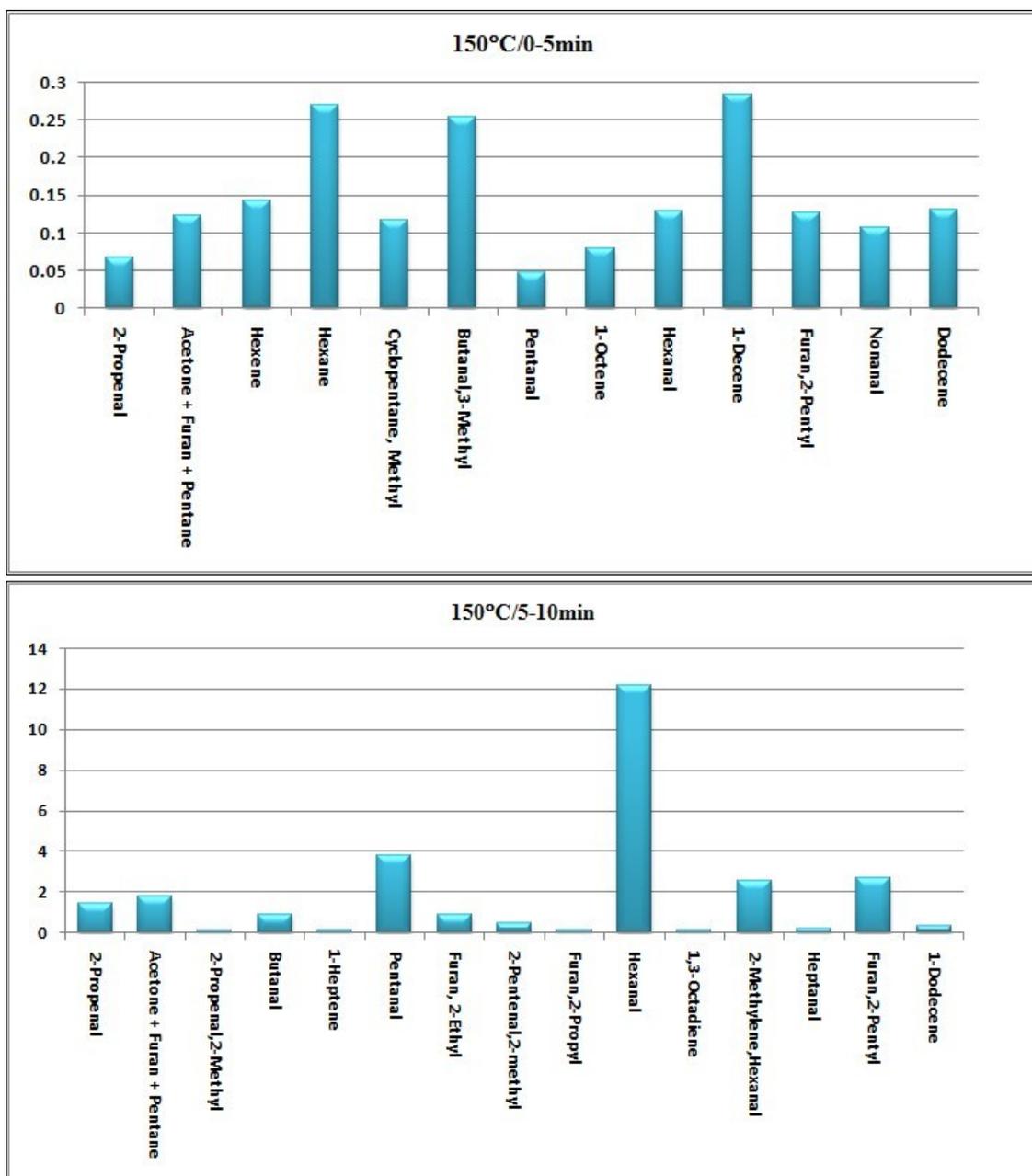


Figure 43a. Time sequence in development of products in corn oil heated at 150°C under air. Samples collected (0 to 5 minutes) (top) and (5 and 10 minutes) (bottom).

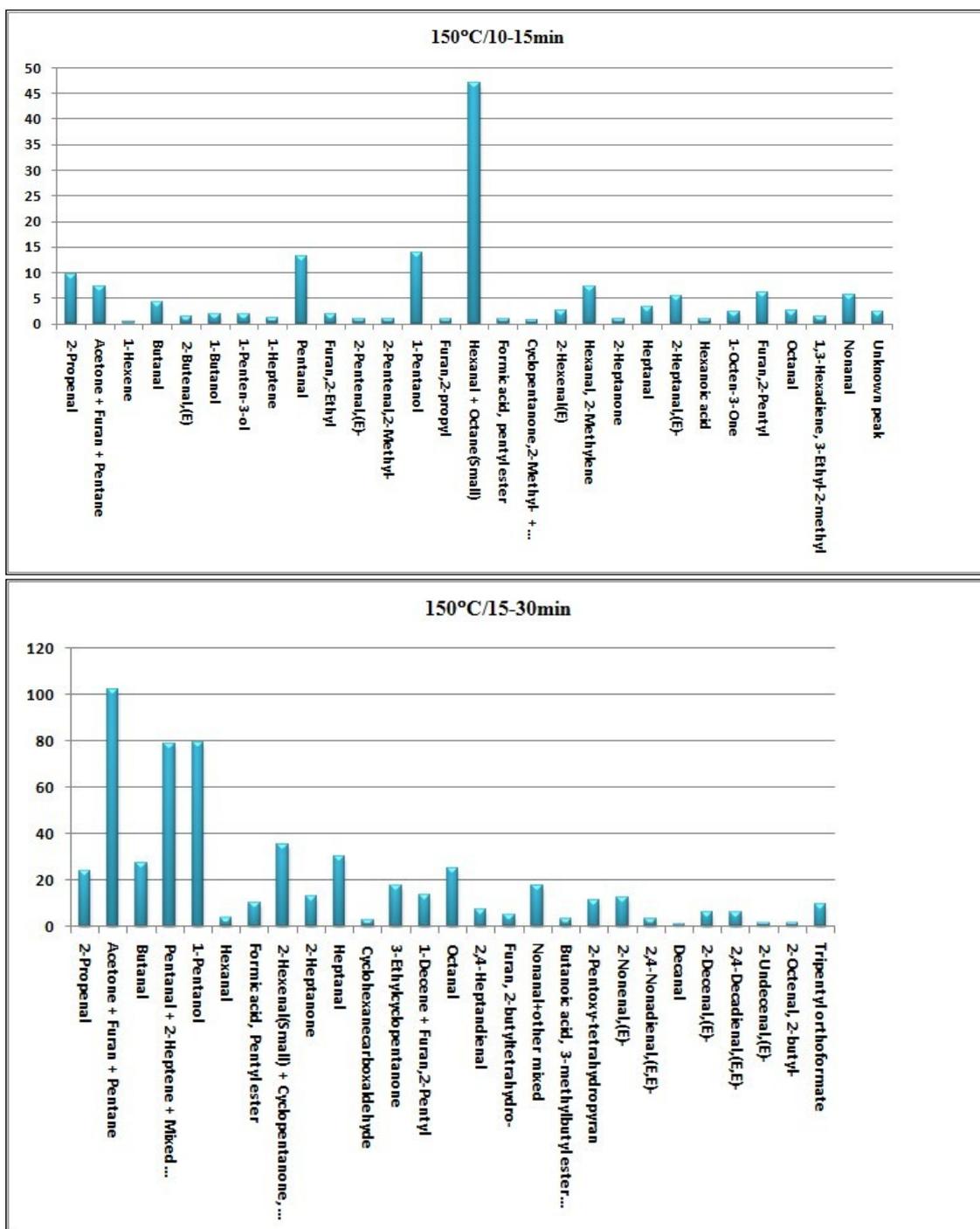


Figure 43b. Time sequence in development of products in corn oil heated at 150°C under air. Samples collected (10 to 15 minutes) (top) and (15 and 30 minutes) (bottom).

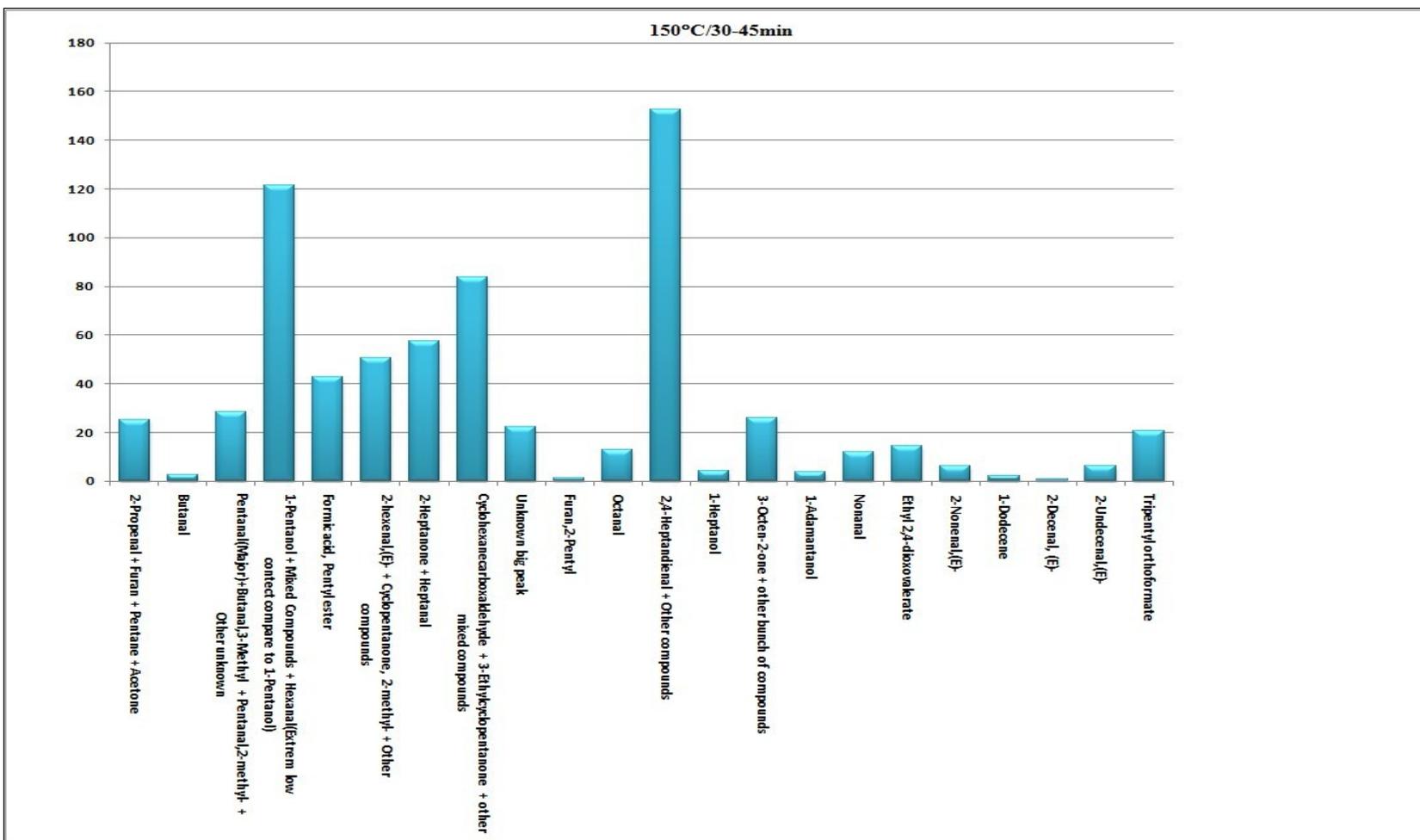


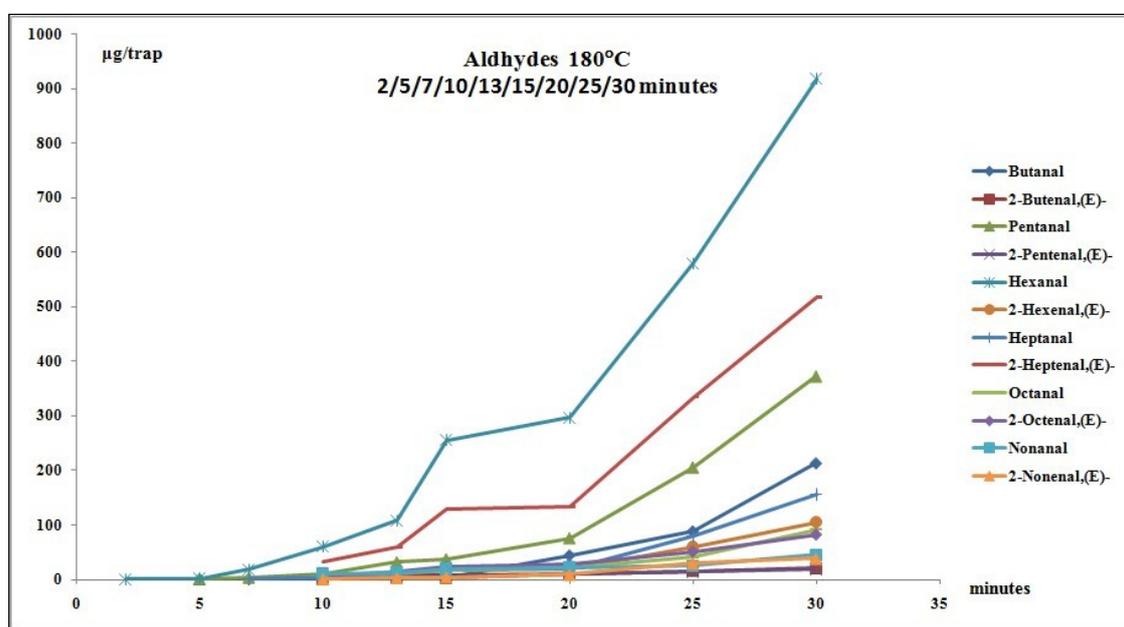
Figure 43c. Time sequence in development of products in corn oil heated at 150°C under air. Samples collected (30 to 45 minutes).

#### **5.4.4 Oil heated at 180 °C, volatiles collected at 2/5/7/10/13/15/20/25/30 min**

##### **Summary of products function with collecting time at 180 °C**

Under 180°C, shortened collecting time series are applied at 2/5/7/10/13/15/20/25/30 minutes to renew the short path thermal desorption trap at each time point. At 180°C, even at early stage 0-2 minutes, hexanal and 1-pentanol are major oxidation products and non-oxidation products does not exist in GC chromatograph. In heating period of 2-5 minutes and 5-7 minutes, butanal/ pentanal hexanal are volatilized to become major aldehydes at early stage, in addition, 1-pentanol/ heptanal/ 2-pentyl, furan/ pentanoic acid displays in GC data in 5-7 minutes period. However, trace amount of alkanes/alkenes can be found before 10 minutes, e.g 1-octene (5-7 minutes), heptanes (5-7/7-10 minutes), pentane in 7-10 minutes period. After 10 minutes, the distribution of aldehydes displays a homologous series which hexanal presents highest GC peak during 7-10 minutes. However, pentanal/1-pentanol/hexanal/2-heptenal, (E)- are predominant products during 10-13 minutes. Even at period of 13-15 minutes, C-5/C-6 are also presenting major peaks in GC chromatograph and homologous phenomenal is constant as longer collecting time. In period of 15-20 minutes, burst increase of short chain oxidation products occurs such as acetone/butanal/furan and short chain non-oxidation products such as pentane which is generated from beta-scission of C-13 hydroperoxide. Between 20-25 minutes and 25-30 minutes, homologous series of aldehydes and oxidation products with same carbon chain are constantly displayed in GC data, C-5/C-6 are major aldehydes compared to other aldehydes at different carbon chain which represents a homologous series. Among other aldehydes, 2-heptenal, (E)- is a major product with large peak area during late stage of frying (20-30 minutes). Cyclization occurs even at 7-10 minutes period,

such major cyclized compounds as 5-ethylcyclopent-1-enecarboxaldehyde/ furan, 2-(1-pen-tenyl),-E- which are contributing to important flavor of frying process. Other minor cyclized compounds include cyclopentanol, 2-methyl/ cyclopentanone, 2-methyl/ cyclo-hexene, 4-propyl/ cyclohexanal etc. Longer frying time can generate more complicated cyclization products, such as 1-Methylcyclohexanol/ 3-Ethylcyclopentanone/ 1,2-Cyclopentanediol,trans/ Cyclopentanecarboxaldehyde in heating period of 15-30 minutes. Aldehydes concentration and distribution are presented in Figure 44.



**Figure 44. Aldehydes distribution under 180°C /air function with time.**

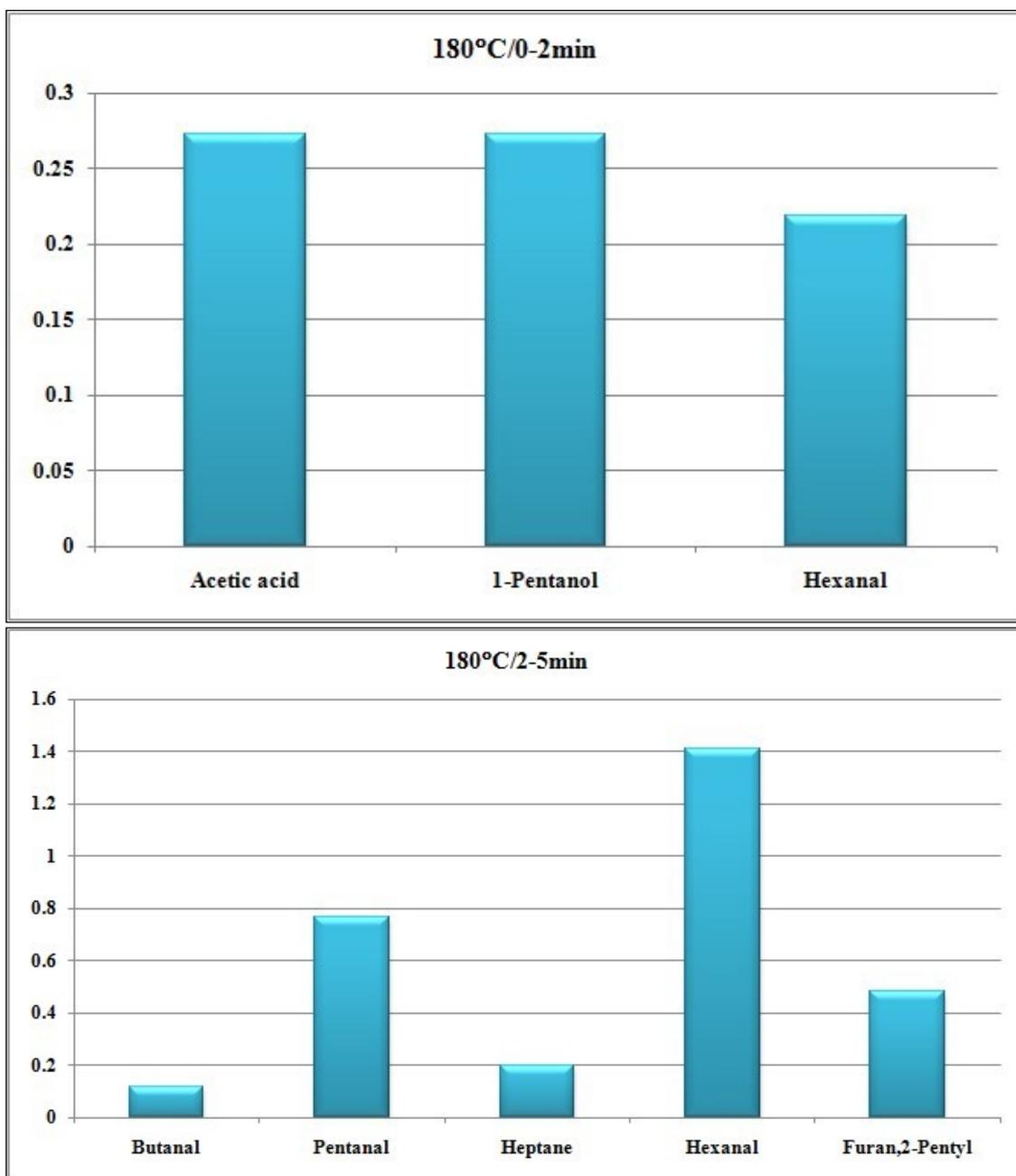


Figure 45a. Time sequence in development of products in corn oil heated at 180°C under air. Samples collected (0 to 2 minutes) (top) and (2 and 5 minutes) (bottom).

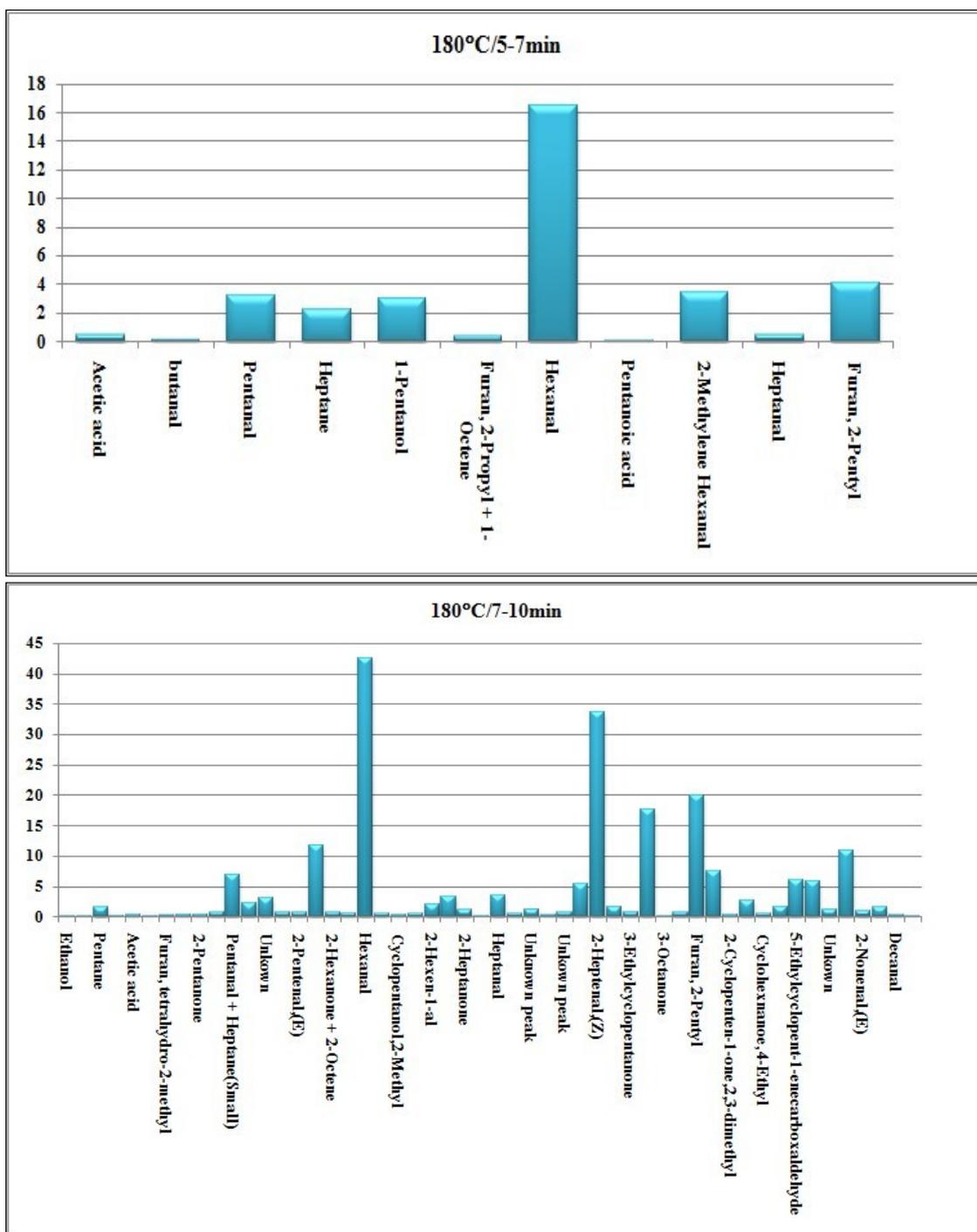


Figure 45b. Time sequence in development of products in corn oil heated at 180°C under air. Samples collected (5 to 7 minutes) (top) and (7 and 10 minutes) (bottom).

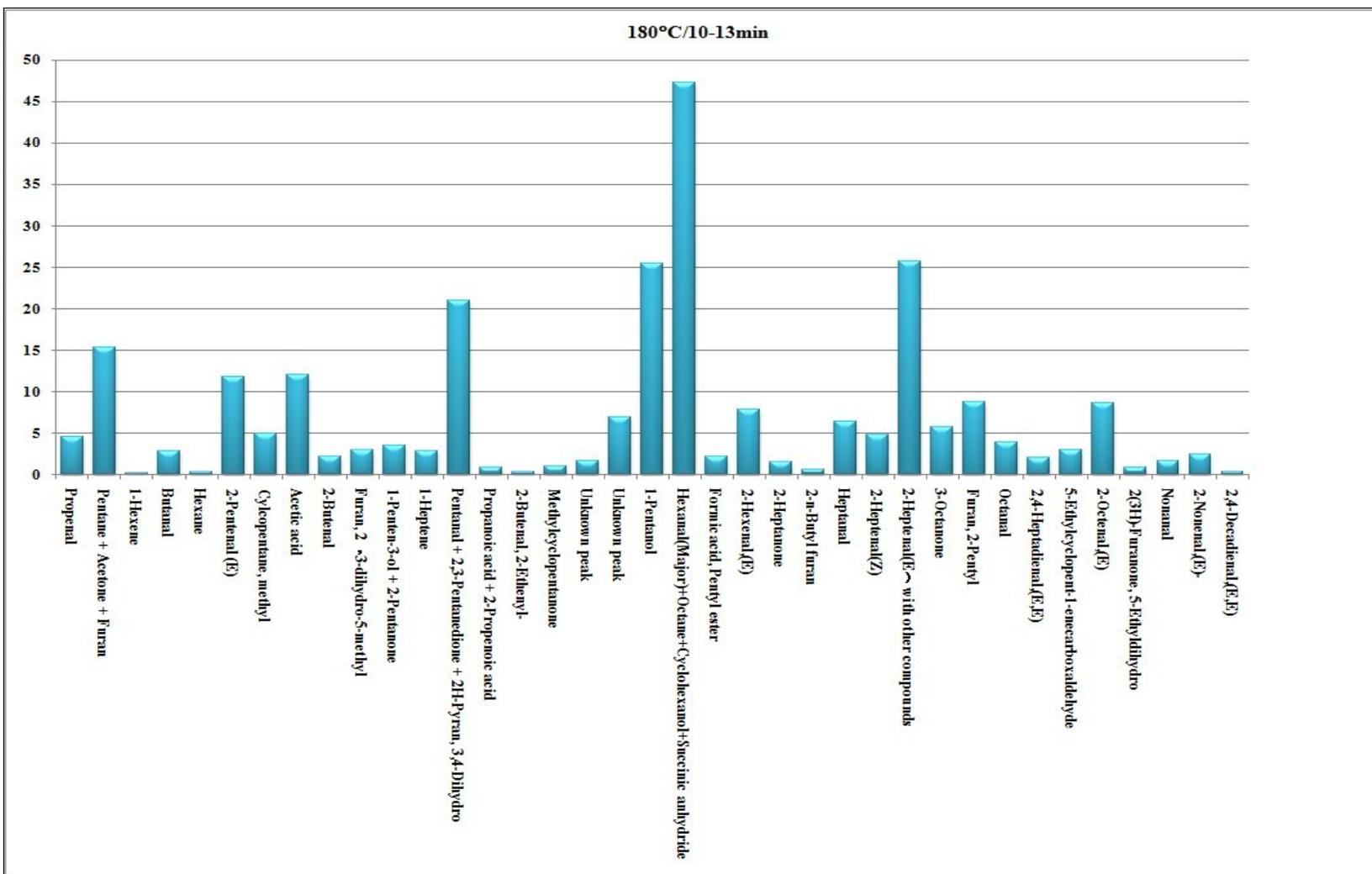


Figure 45c. Time sequence in development of products in corn oil heated at 180 °C under air. Samples collected (10 to 13 minutes).

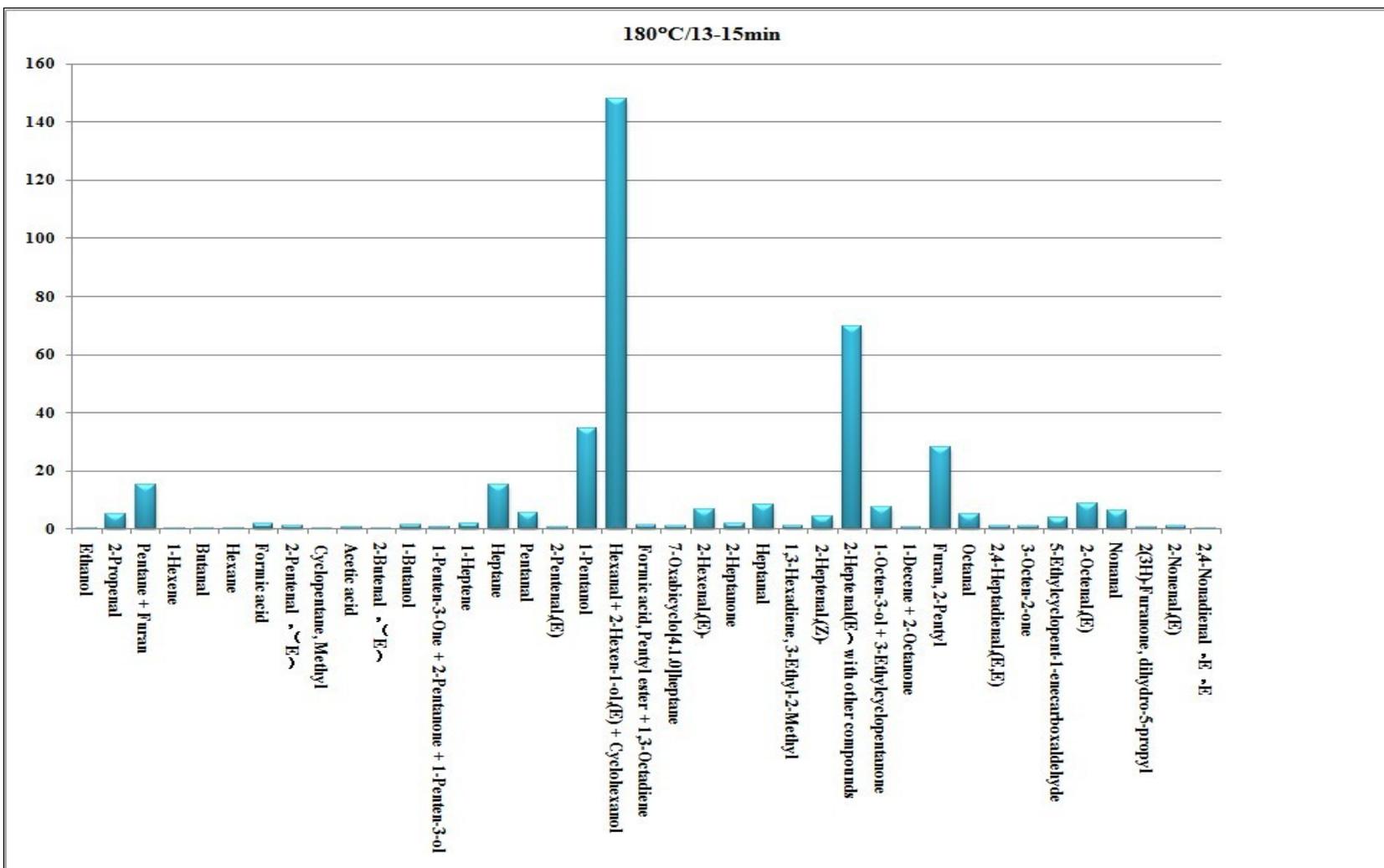


Figure 45d. Time sequence in development of products in corn oil heated at 180°C under air. Samples collected (13 to 15 minutes).

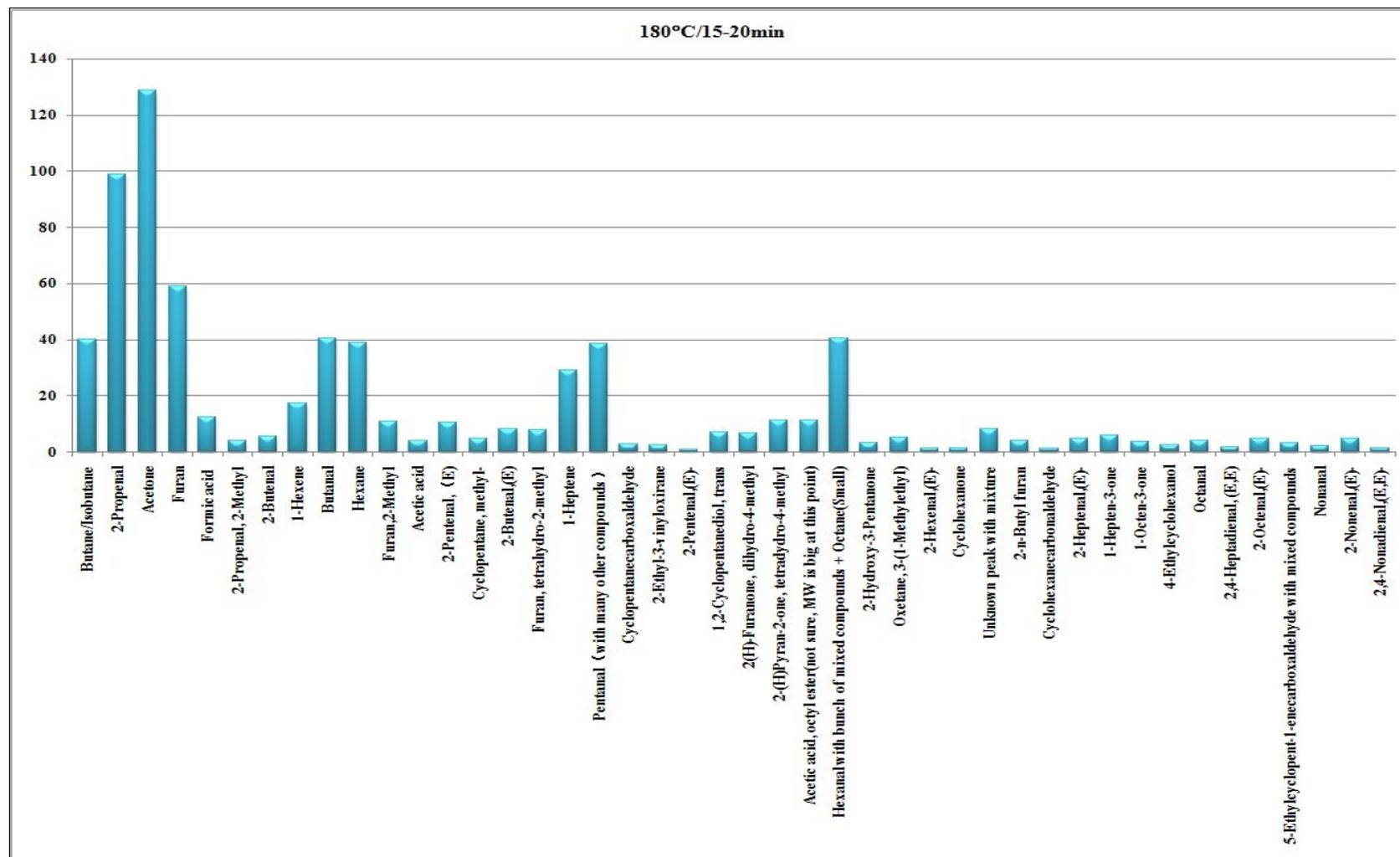


Figure 45e. Time sequence in development of products in corn oil heated at 180°C under air. Samples collected (15 to 20 minutes).

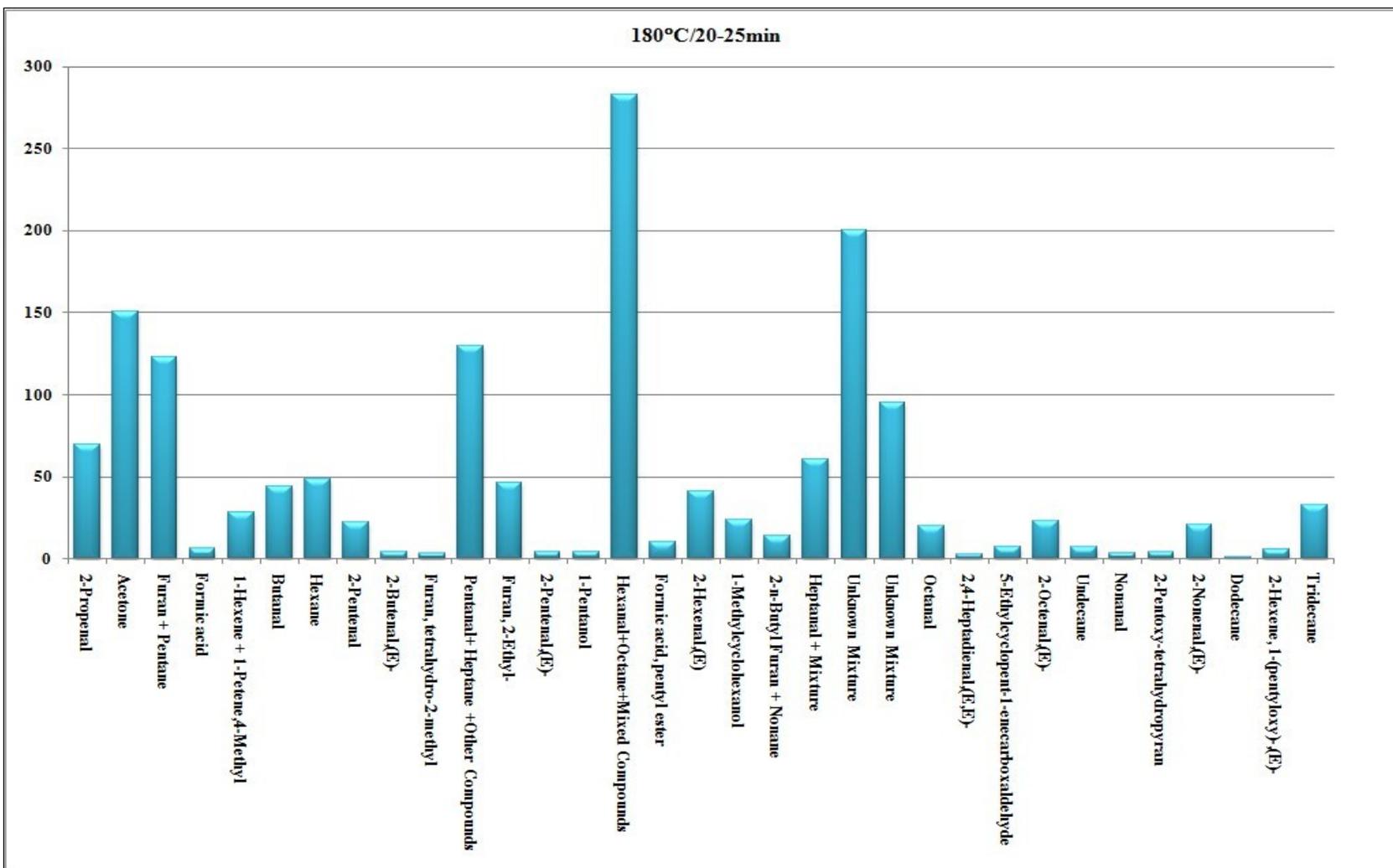


Figure 45f. Time sequence in development of products in corn oil heated at 180°C under air. Samples collected (20 to 25 minutes).

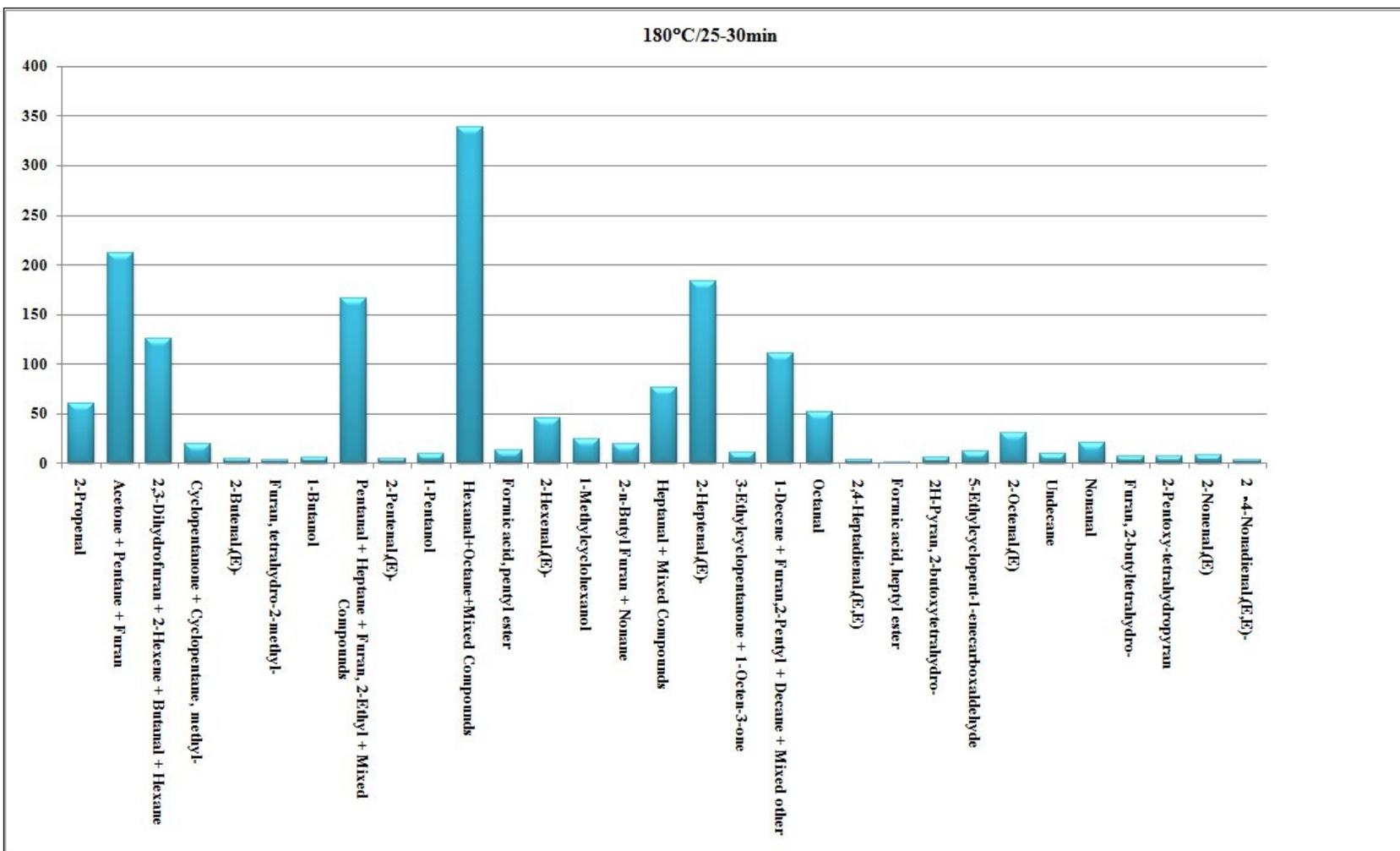


Figure 45g. Time sequence in development of products in corn oil heated at 180°C under air. Samples collected (25 to 30 minutes).

#### 5.4.5. Oil heated at 235 °C, volatiles collected at 2/5/7/10/13/15 min

##### Summary of products function with collecting time at 235°C

Extreme high temperature will cause oxidation at highest rate compared to lower temperature. During 0-2 minutes period, aldehydes are present in GC data which hexanal/ pentanal are dominant, homologous series of products are also present. As collecting time goes up to 15 minutes, pentanal/ 1-pentanol/ hexanal/ heptanal/ 2-heptenal, (E)- are major aldehydes, other oxidation products include homologous series of aldehydes with different carbon chain length and corresponding alcohols/acids/ketones. Cyclization occurs at 235°C with some same products pattern as lower temperature series: 5-Ethylcyclopent-1-enecarboxaldehyde/ cyclopentanone, 3-methyl/ cyclohexanecarboxaldehyde/ cyclohexanone. Here is an illustration about aldehydes under 235°C, Figure 46.

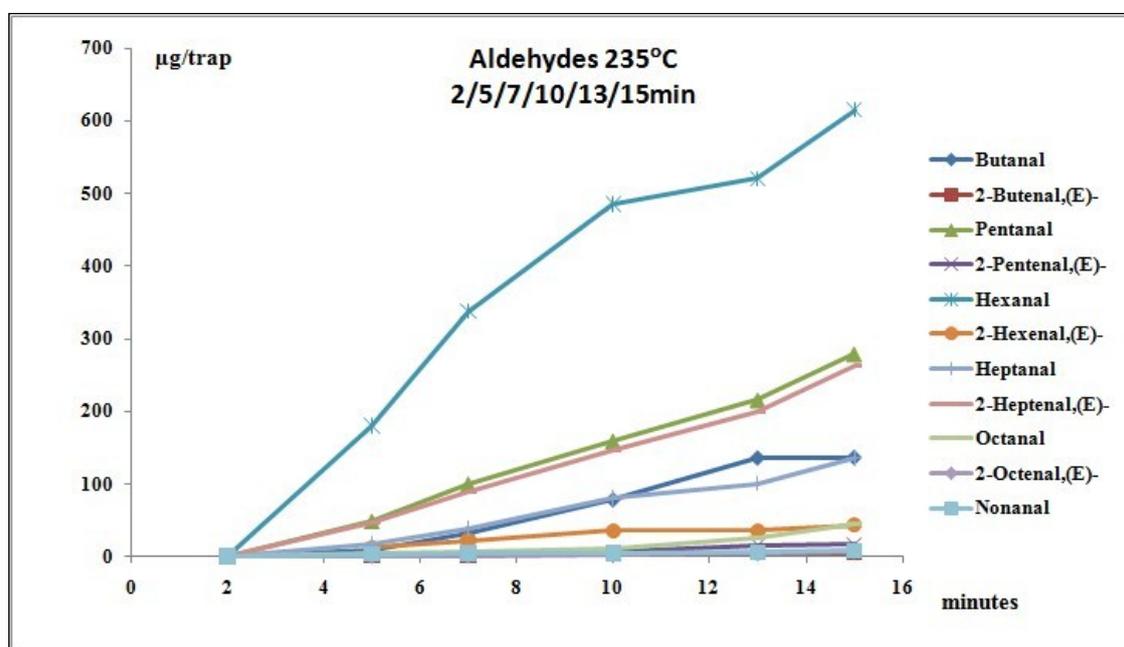


Figure 46. Aldehydes distribution under 235°C/air function with time.

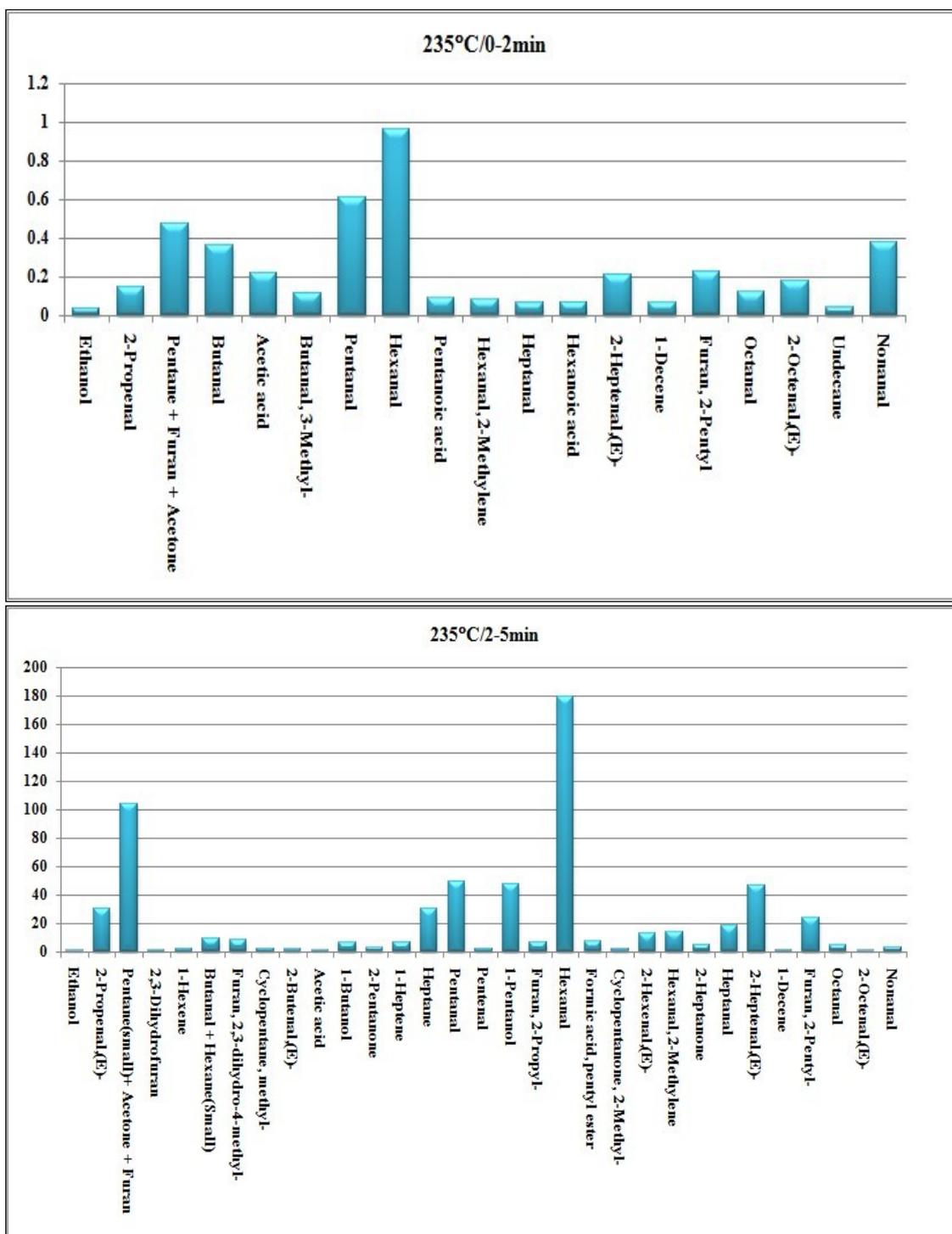


Figure 47a. Time sequence in development of products in corn oil heated at 235°C under air. Samples collected (0 to 2 minutes) (top) and (2 and 5 minutes) (bottom).

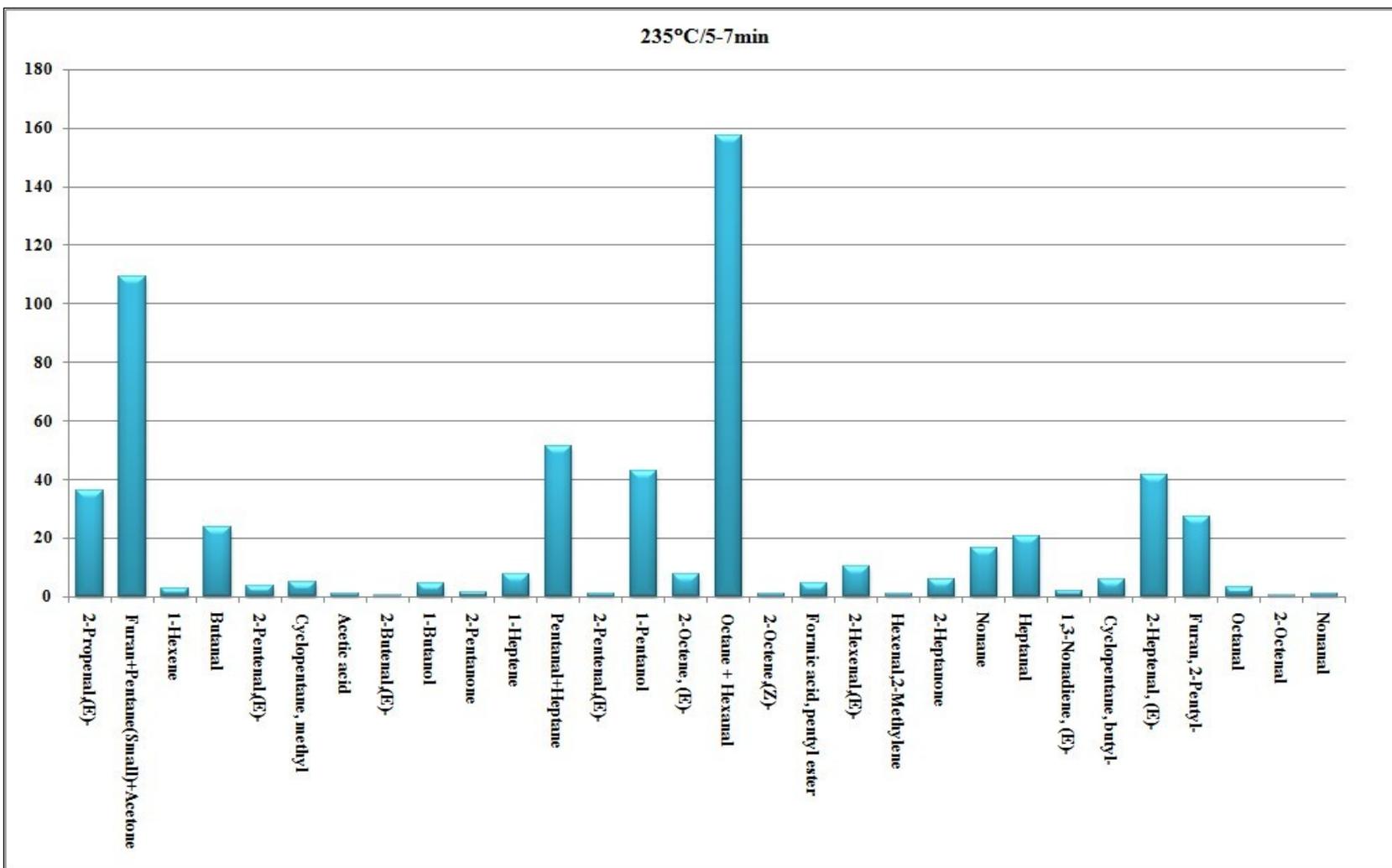


Figure 47b. Time sequence in development of products in corn oil heated at 235°C under air. Samples collected (5 to 7 minutes).

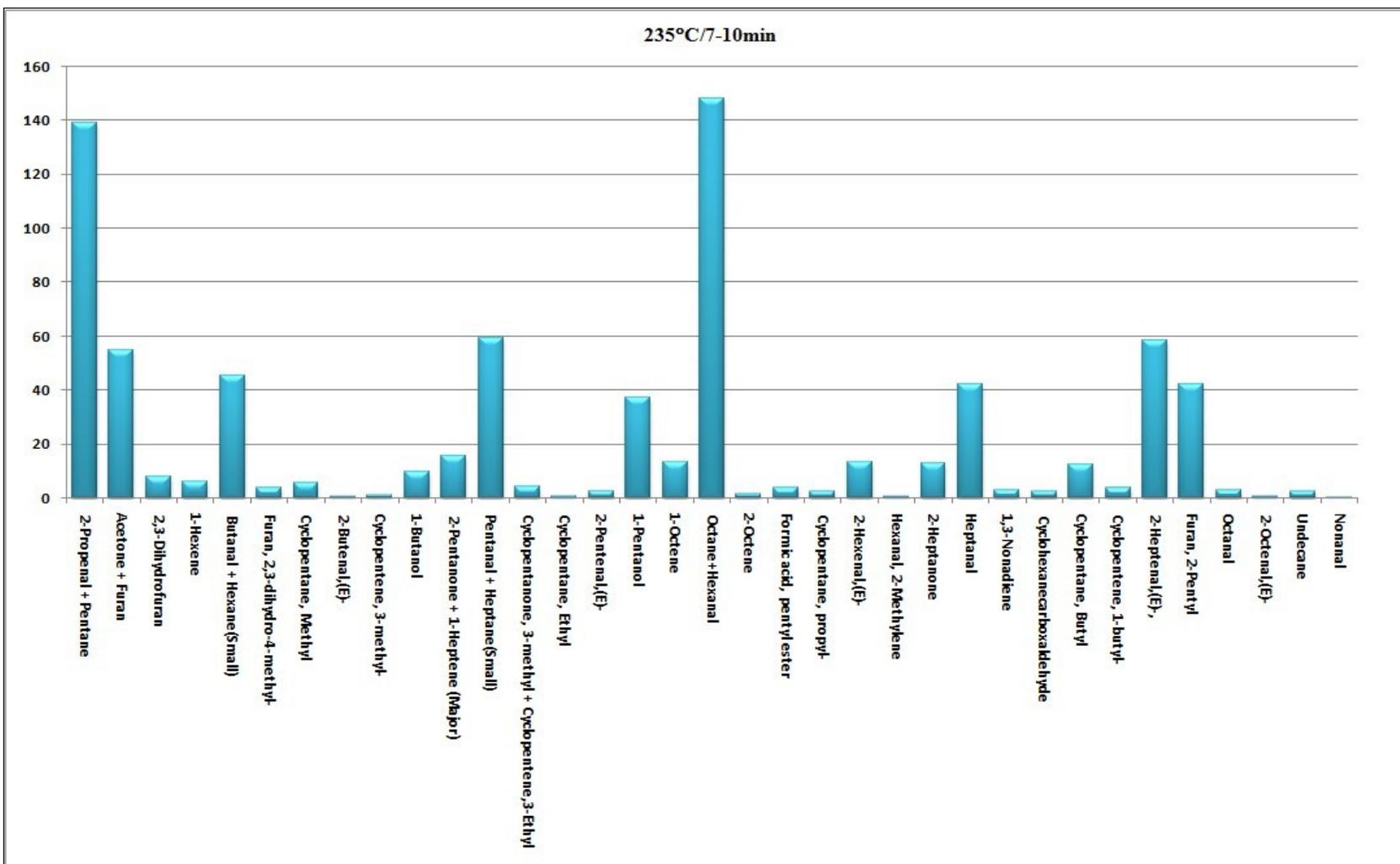


Figure 47c. Time sequence in development of products in corn oil heated at 235°C under air. Samples collected (7 to 10 minutes).

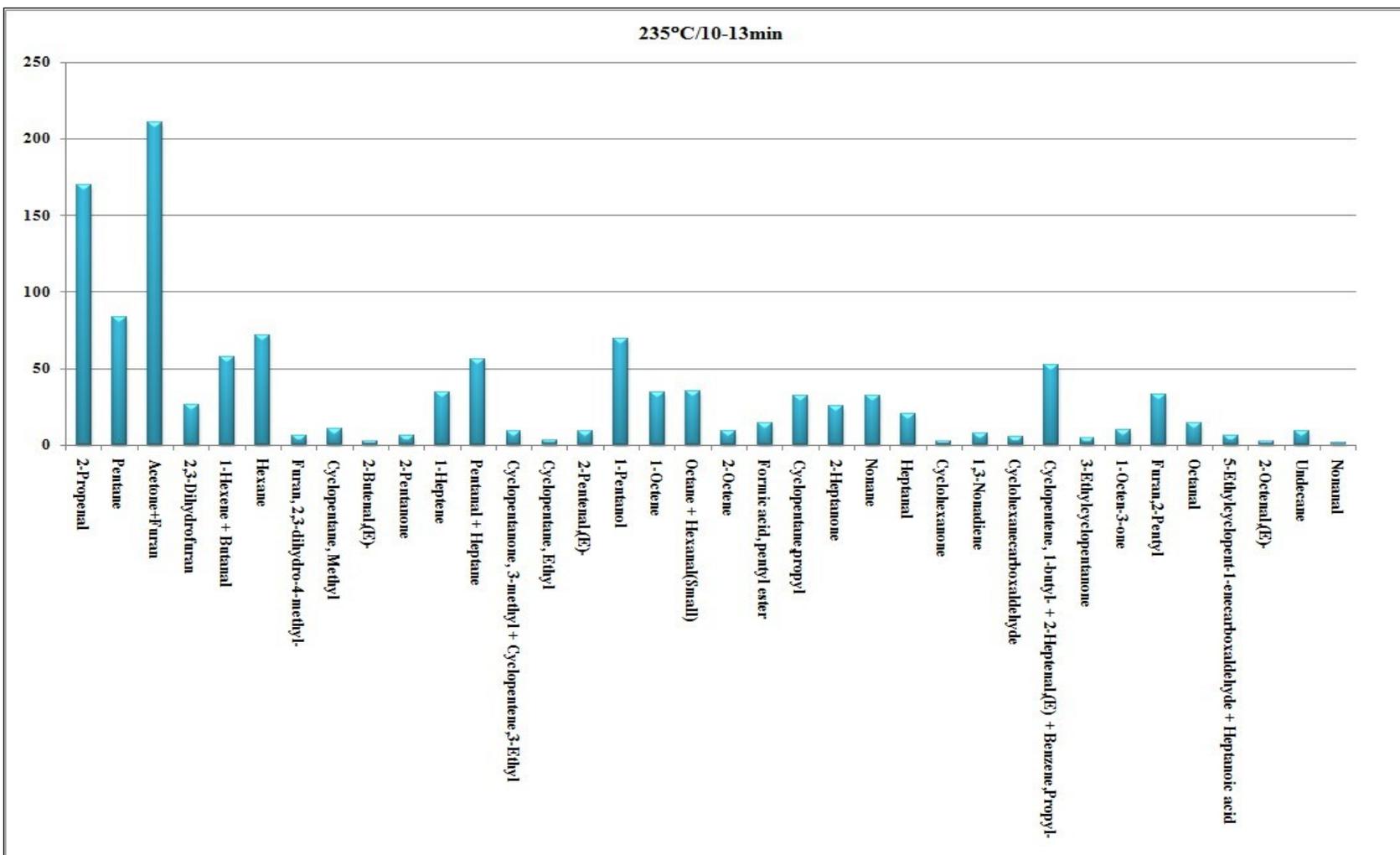
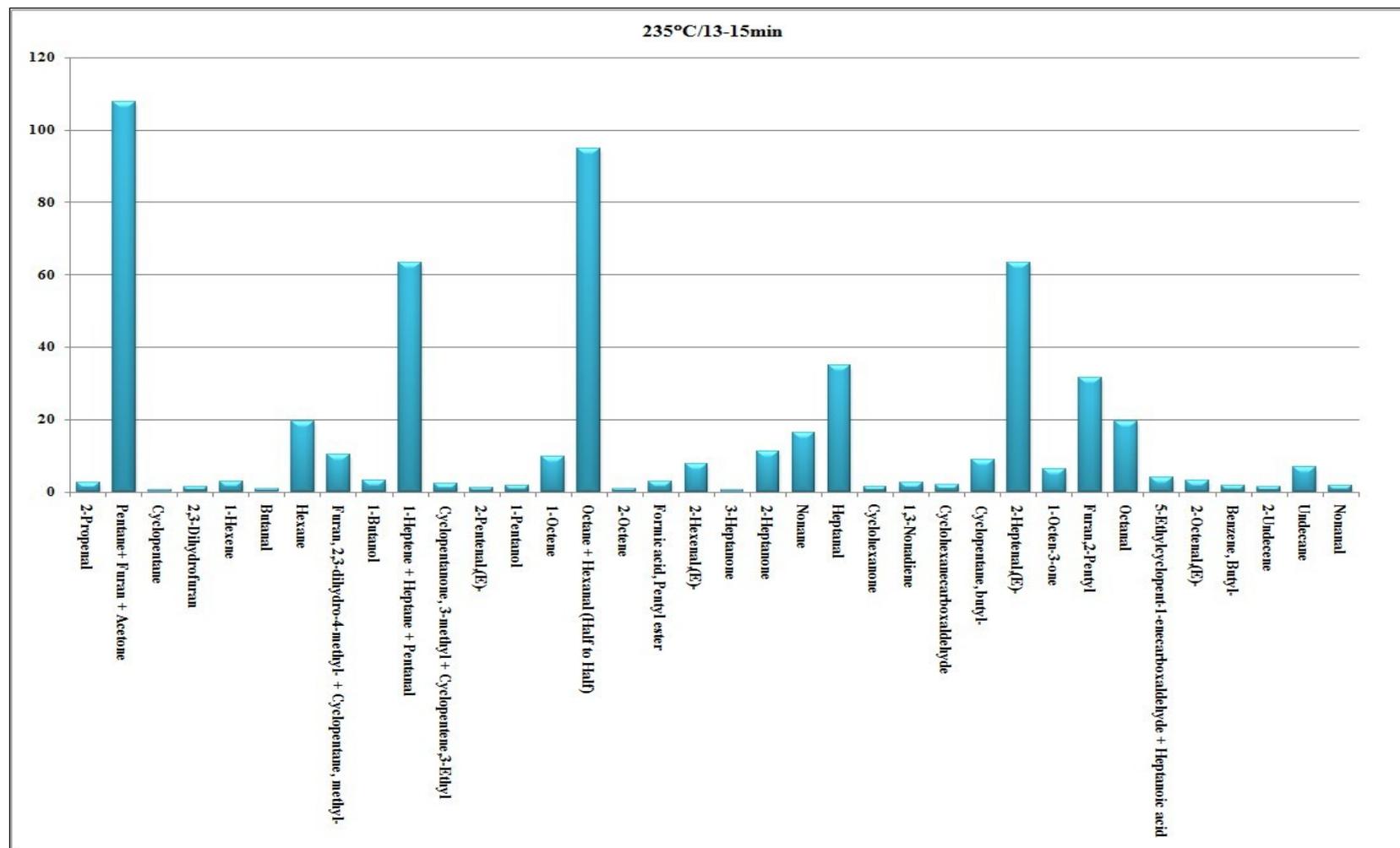


Figure 47d. Time sequence in development of products in corn oil heated at 235°C under air. Samples collected (10 to 13 minutes).



**Figure 47e.** Time sequence in development of products in corn oil heated at 235°C under air. Samples collected (13 to 15 minutes).

## 5.5. Discussion

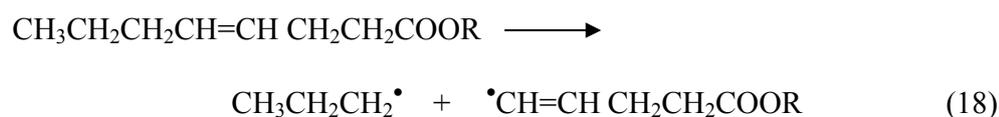
The gas chromatography data presented above clearly shows significant differences in oil degradation patterns when heating in air versus nitrogen. In contrast to most other frying studies, this thesis focused on early processes in degradation, from initial heating through two hours, and this may explain why our results differ from most of the scientific literature on frying.

The major clarification in this experiment is demonstration that thermal scissions are a source of degradation and radicals constantly present when oils are heated even to 100 °C; they play at least two critical roles in frying chemistry. First, these thermal scissions provide one independent degradation pathway that produces a homologous series of alkane and alkene products under inert atmospheres and corresponding oxidation products such as [terminal] hydroperoxides, aldehydes, carboxylic acids, ketones, and epoxides when air is present. These products contribute to the peroxide values, polar products, and free fatty acids normally measured to follow quality in frying oils, but in global analyses they are indistinguishable from and usually attributed to normal lipid autoxidation products. Second, traditional frying theory has rather loosely expected that radicals initiating lipid autoxidation at high temperatures arise from decomposition of preformed hydroperoxides. However, these hydroperoxides must come from somewhere, and it is difficult to accept that the very low starting peroxide levels allowed in industrial and commercial operations are sufficient to drive all the thermal degradations observed in frying chemistry. Given the levels of thermal scission products observed in this study, a much more reasonable explanation is that radicals generated by thermal scission of lipid acyl chains form terminal peroxy radicals in the presence of oxygen, and these peroxy radicals abstract hydrogens

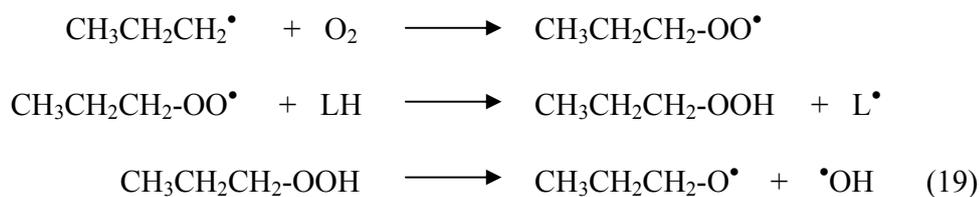
from sensitive sites on unsaturated fatty acids (e.g. C-9 and C-13 of linoleic acids and C8 and C-11 of oleic acid) to initiate autoxidation chains. The resulting hydroperoxides can be decomposed thermally to generate alkoxy and hydroxyl radicals, both of which are even more active radical initiators. Thus, in air radicals derived from thermal scissions provide the driving force to initiate and sustain conventional autoxidation chains that generate a separate and different line of degradation products.

Results from this study show that in air, three pathways are active:

- a) thermal scission of acyl chains to yield alkyl radicals that, by themselves, can add to double bonds or recombine to form dimers, but otherwise are relatively unreactive:



- b) oxidation of thermal scission radicals to form reactive terminal peroxy radicals that abstract hydrogens from specific sites on lipids to form hydroperoxides, which in turn decompose to generate even more reactive alkoxy and hydroxyl radicals:



- c) lipid radicals thus generated in this secondary process add oxygen to form lipid peroxy radicals, which then abstract hydrogens from other lipids to establish an autoxidation chain.

Evidence so far suggests that the rate of a) and b) are faster than c), so at least in early stages of frying (i.e short heating times), thermal scission processes dominate. Using neat corn oil in this study gave pentane as by far the dominant product at all temperatures and under both nitrogen and air. Short chain products such as 2-propenal, (E)-, acetone, furan were also major products, further supporting thermal scission processes.

Our data suggests that pentane is a key mediator of thermal degradation in corn oil. Pentane is the first product formed by thermal scissions under nitrogen and its derivatives remain in high concentrations relative to other alkanes. Pentane was generated at much lower levels under air than nitrogen, and this is an important observation differentiating the role of pentane here. As explained above, the pentyl radical generated by scission is poorly reactive, but it can be converted at diffusion controlled rates to reactive peroxy radicals. The ultimate product mix then is determined by the relative rates at which the pentyl-OO• is converted to other oxidation products such as pentanal and 1-pentanol in competition with hydrogen abstraction from C13 of intact linoleic acids to initiate autoxidation chains. This generates pentyl-OOH and eventually C<sub>13</sub>-OOH. C<sub>13</sub>-OOH decomposes by  $\alpha$ -scission to yield the hexanal that is the second major product in air, and by  $\beta$ -scission to regenerate pentane. Pentyl-OOH decomposes to pentanol and pentanal, providing second pathways to these products.

It is thus interesting that pentane is the major product of both primary thermal scission and secondary autoxidation of linoleic acid. Corresponding thermal scissions at C-8, C-11 and C-13 nearby positions on oleic acid give major alkane/alkene products but at lower concentrations. In air, these are converted to corresponding oxidation products, but

levels of secondary abstraction products from lipid autoxidation chains remain low. Thus, pentane is clearly the dominant mediator in extending oxidation.

Data presented here is in general agreement with publications of Nawar (Nawar 1967; Nawar 1969; Nawar 1984; Nawar 1996), whose fundamental research on thermal degradation mechanisms remains the standard for the field, even if it has not been applied practically. Nawar's work and this study both indicate that thermal scission involves "physically" (by thermal energy deposition) chopping the carbon chains with preferential scission points at  $\alpha > \beta > \gamma$  to double bonds. Our data suggests that the scissions occur preferentially on CH<sub>3</sub> side of double bonds in both linoleate and oleate, and products from thermal scissions at these positions rather than conventional hydrogen abstraction positions dominated all conditions of this study.

It is also important to note that it is accepted in lipid oxidation that H abstraction from linoleic acid occurs equally at C-9 and C-13 (Schaich 2005). In contrast, in this study, H abstraction occurred almost exclusively from C-13, at least during short time heating. 2,4-(E,E)-decadienal, a common product of C9-O•  $\alpha$ -scission in autoxidation, was not observed after heating under air for 30 minutes. However, it was present after heating under nitrogen at the higher temperatures (150, 180, and 235 °C) for longer times: 60-90 minutes at 150 °C, 20-25 minutes period at 180 °C, and 13-15 minutes at 235 °C. Other 2,4-dienals such as 2,4-heptadienal and 2,4-nonadienal,(E,E)- were also present under these conditions. That heat alone without oxygen was sufficient to form these diene products suggests a third effect of heat – changing effective bond energies and reactivity of individual groups within fatty acids and triacylglycerols.

Generally, autoxidation can generate limited variety of free radicals and corresponding secondary products, while thermal scission can generate many different kinds of free radicals and much more extensive product mixes. Our results show that frying (heating oil) under air gives much more complex products than if autoxidation is the only reaction. Faster oxidation with higher levels of more products during heating of oils has traditionally been attributed to Arrhenius kinetics which doubles the rate of oxidation with every 10 °C increase in temperature. However, H abstraction in autoxidation is site-specific and the decomposition products associated with these sites are well-known. Given this established background, it is not possible to reconcile the products observed in this study with autoxidation mechanisms either alone or as a primary action in heated oils occurring in competition with thermal scissions.

Rather, our results suggest that lipid autoxidation is a process that occurs secondary to or as a result of oxidation of thermal scission radicals and their subsequent reactions. It is *oxidation of the thermal scission radicals* that so rapidly increases product yields under oxygen. Supporting this are the initial short chain alkanes and alkenes formed and the transformation under oxygen to aldehydes, alcohols, and carboxylic acids of the same chain length during early heating. As temperature and heating time increases, more products from radical recombination (e.g. ketones) appear, but the structure of these shows they arise from scission products rather than autoxidation products. Except for hexanal, arising from  $\alpha$ -scission of a C13-O<sup>•</sup> on linoleic acid, products expected from lipid autoxidation showed up only after long heating times at the highest temperatures.

Several unique reaction characteristics are worth nothing. At 25-40 °C, alkoxy radicals have the option to abstract hydrogens to propagate radical chains, add to external

double bonds to yield dimers, add to neighboring internal double bonds to form epoxides, or undergo  $\alpha$  or  $\beta$  scission to generate alkanes, alkenes, and aldehydes. The latter requires a proton source for stabilization so normally occurs most rapidly in the presence of water or a protic solvent. These product patterns suggest that at high temperatures in dry oils, aldehydes may be formed by mechanisms other than  $\alpha$  or  $\beta$  scission of alkoxy radicals, and that H abstraction by alkoxy radicals is facilitated (Schaich 2005).

A second unique characteristic is the unexpected appearance of products arising from internal hydroperoxides of linoleic acid. For example, 2-(E)-heptenal probably originated from the 12-hydroperoxide of linoleic acid. This was not expected because internal hydroperoxides are normally associated with photosensitized oxidation (Frankel, Neff et al. 1982; Frankel 1984), but this was a purified oil and the heating was conducted in the dark so little if any singlet oxygen should have been present. This observation raises questions about whether thermal energy changes electron distributions within unsaturated fatty acids and activates sites other than the allylic carbons.

A third unique characteristic was the number of hydrocarbon rearrangement products (e.g. iso-alkanes) and cyclization products (e.g. 1, 3-cyclohexadiene, 5-butyl 1, 3-cyclohexadiene, 5-butylcycloheptane) that were formed at high temperatures ( $>120^{\circ}\text{C}$ ), even under nitrogen. Furans are formed in autoxidation of linoleic and linolenic acids (Chang, Smouse et al. 1966; Ho 1978), but the number and variety of cyclization products is much greater at high temperatures, and the structures suggest they arise from reactions of thermal scission radicals. To be sure, they all stayed at low concentrations, but these are clearly thermal rather than autoxidation products. Cyclized compounds usually volatilized after longer heating times.

Concentrations of products (peak intensities) increased considerably under air and with higher temperature (150/180/235°C) and longer frying time (120 minutes at 150 °C, 30 minutes at 180/235 °C). When extremely large amounts of volatiles tended to overload the short path thermal desorption trap, some peaks in the chromatograms became relatively huge and co-eluted groups of compounds. This was shown when two products were listed together in tables. The Purge & Trap system offers great advantages in being able to trap and concentrate volatiles under controlled conditions, but it has the disadvantage of being a microsystem relative to laboratory, commercial, or industrial frying vats. Consequently, thermal degradation in the Purge & Trap tubes occurs exponentially faster than in real-life frying. Overloading and co-elution problems were reduced but shortening the collection time, e.g. to 5 minutes at 235 °C, but co-elution still complicated interpretation of data in some cases. Modification of the system and volatiles collecting methods will be necessary for future work at high temperatures..

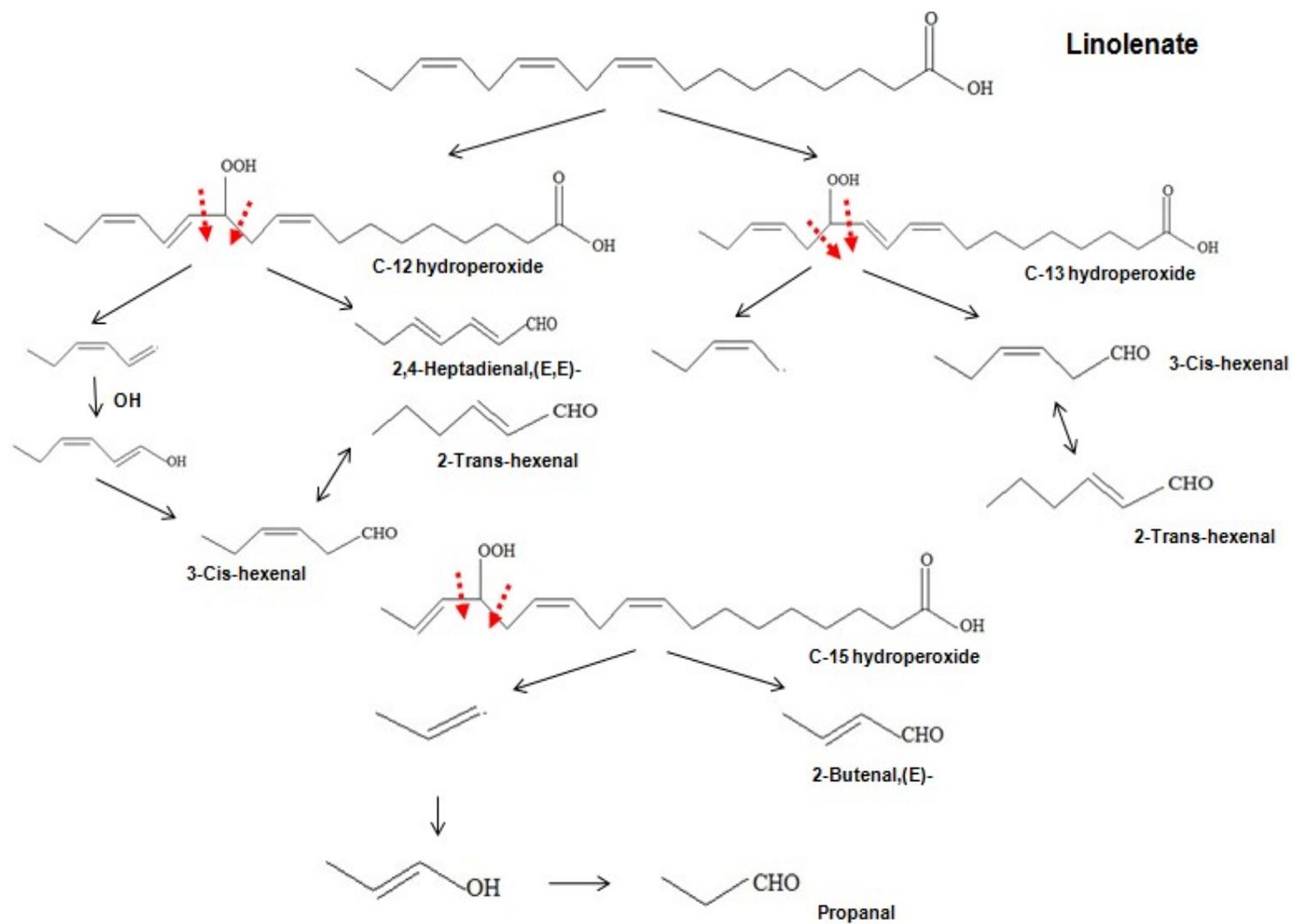
Many volatiles observed in this study are important flavor compounds in heated oils, such as hexanal, 2,4-decadienal, (E, E)-, 2-pentyl furan, 1-octen-3-ol, 1-octen-3-one, benzaldehyde, 1-penten-3-one, 1-penten-3-ol. 1-Octen-3-one and 1-penten-3-one have some metallic flavored homologues (Stark 1966). Researchers found that 1-penten-3-one and pentanal mixed in deodorized vegetable oil which has a similar flavor to autoxidized soya bean oil (Hill 1965). In our study(air atmosphere), 1-penten-3-one is firstly present at 120 °C at period of 60-90 minutes with low concentration and at 180 °C at period of 13-15 minutes; 1-octen-3-one is present at 180 °C at period of 15-20 minutes. 1-Octen-3-ol and 1-penten-3-ol are mushroom-flavored homologue compounds (Stark 1966). In our data, 1-octen-3-ol is initially present at 180 °C at the period of 7-10 minutes which displayed as

a major peak approximately  $\sim 17\mu\text{g}/\text{trap}$ ; 1-penten-3-ol firstly volatilize under  $120\text{ }^\circ\text{C}$  at period of 60-90 minutes. Benzaldehyde contributes characteristic pleasant almond flavor (Gérald 1997). In our data, benzaldehyde volatilize only under nitrogen and stay at extremely low concentration (first appearance occur at  $100\text{ }^\circ\text{C}$  45-60 minutes) and co-elute with 1-octen-3-ol and 1-octen-3-one at later stage. Hexanal is an important compounds in flavor chemistry to produce fruity flavor (Frankel 1982),

As has been noted several times, the degradation chemistry of food oils under frying conditions is very complex. We have attempted to integrate known radical and lipid oxidation chemistry with product distributions observed in this study to outline plausible reactions that can account for individual products. These reaction schemes are shown for products derived from linoleic acid (Figure 49), oleic acid (Figure 50), and linolenic acid (Figure 51). The reaction schemes show how initial thermal scissions can be combined with secondary oxidations and hydrogen abstractions from intact lipids to generate three streams of products. The oxidation and autoxidation products probably control the quality of commercial and home frying, while thermal scissions and trace oxidations are more likely the critical reactions in industrial processes run under inert atmosphere. The differences between the pathways raise interesting questions about approaches for stabilizing frying oils. Exploring this can be a productive next step in research.







**Figure 50. Proposed thermal and initiated autoxidation reactions generating products from linolenic acid in this study.**

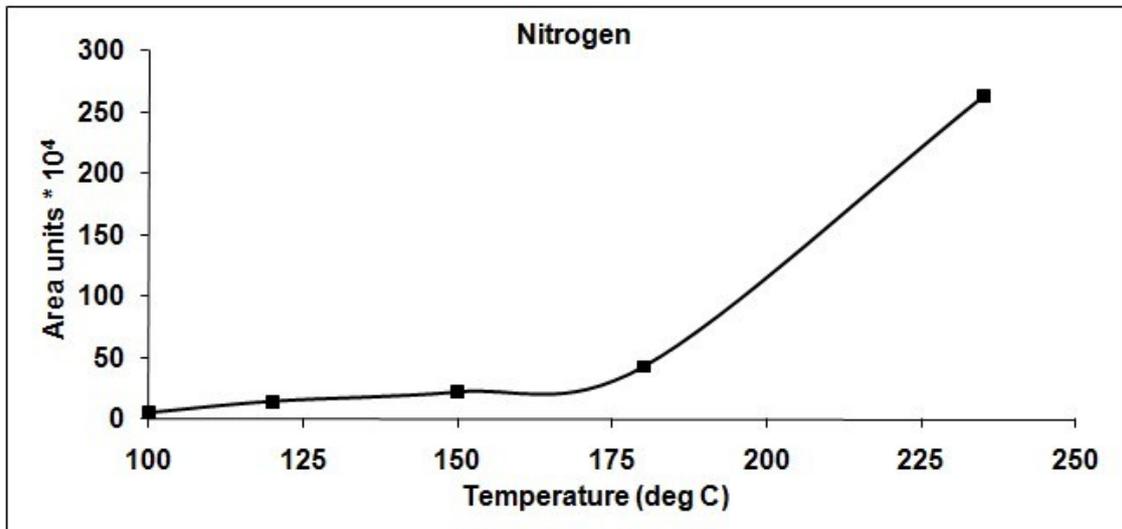


Figure 51. Total volatiles generated in temperature sequence run under nitrogen.

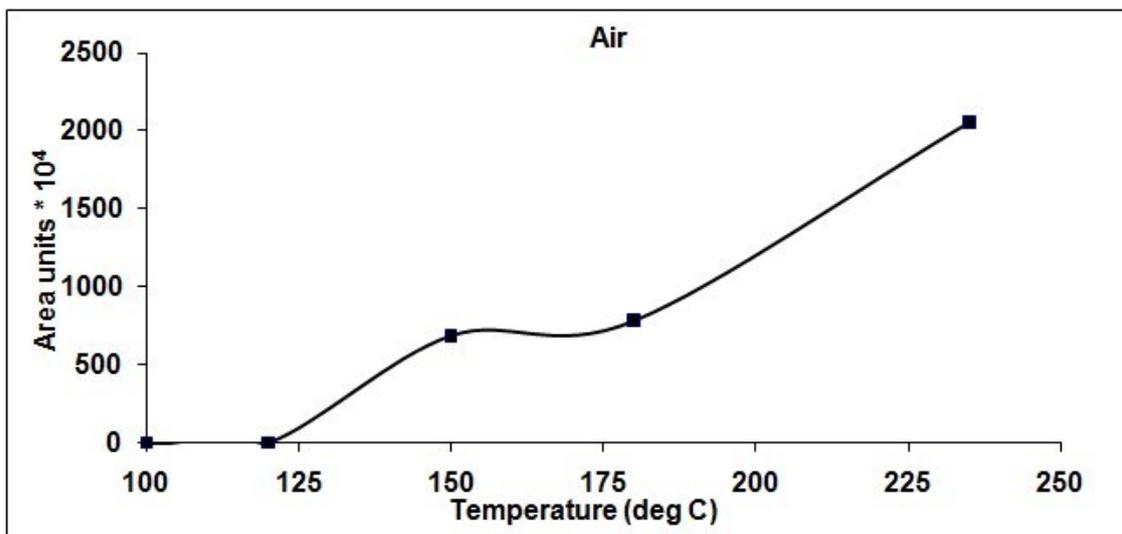
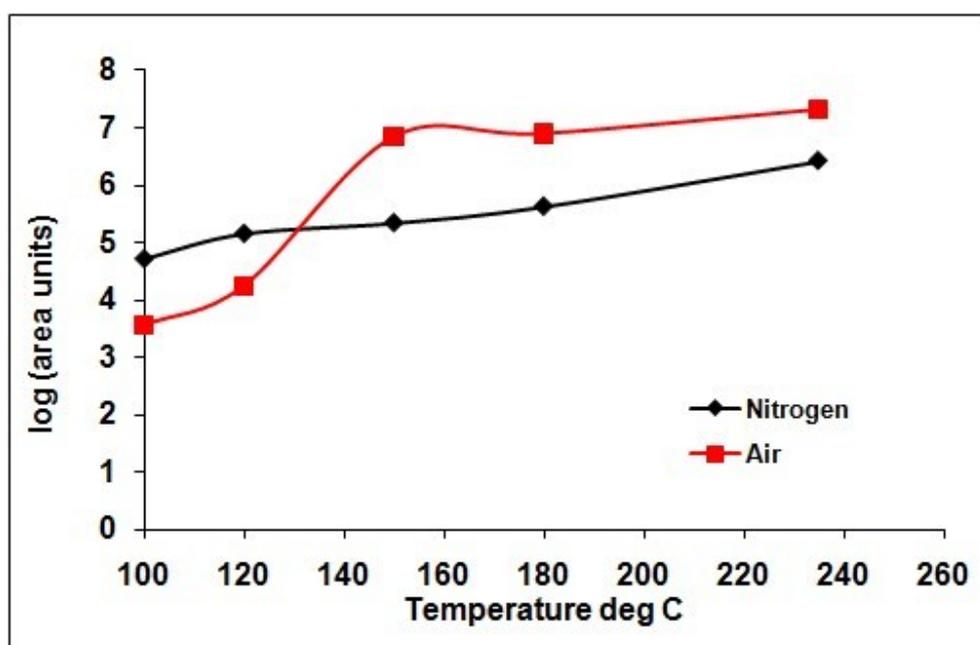


Figure 52. Total volatiles generated in temperature sequence run under air.

As we can obtain the total volatiles from GC-data, under nitrogen (Figure 51), total volatiles are almost linear from 100°C to 180°C. After 180°C, there is a sharp increase of total concentration of volatiles which may result from reaction mechanism shift due to extremely high temperature from 180°C to 235°C. From Figure 52, two major concentration increase of volatile occurred between 120°C to 150°C and another shift between 180°C and 235°C which is identical to observation from GC-chromatography.



**Figure 53. Logarithm data of total volatiles concentration between air and nitrogen.**

Logarithm data of total volatiles are illustrated in Figure 53, under nitrogen,  $\log(\text{concentration})$  versus temperature is almost linear which can conclude that thermal scission is dominant under limited oxygen and remain constant rate from 100°C to 235°C. In contrast, air data display an exponential increase between 120°C and 150°C and remain linear from 180°C to 235°C.

Due to limited oxygen present in nitrogen temperature sequence run, alkenes were decreasing while aldehydes were increasing (Figure 54, 55), however, initially, alkenes are

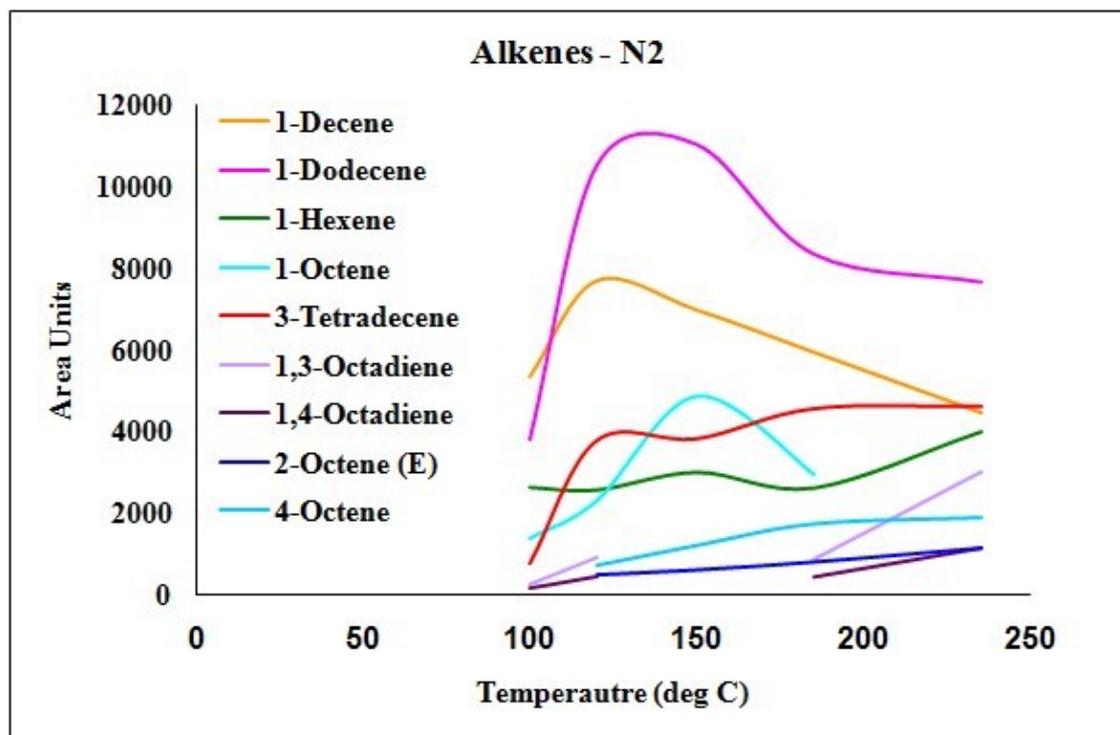


Figure 54. Alkenes generated under nitrogen versus temperature.

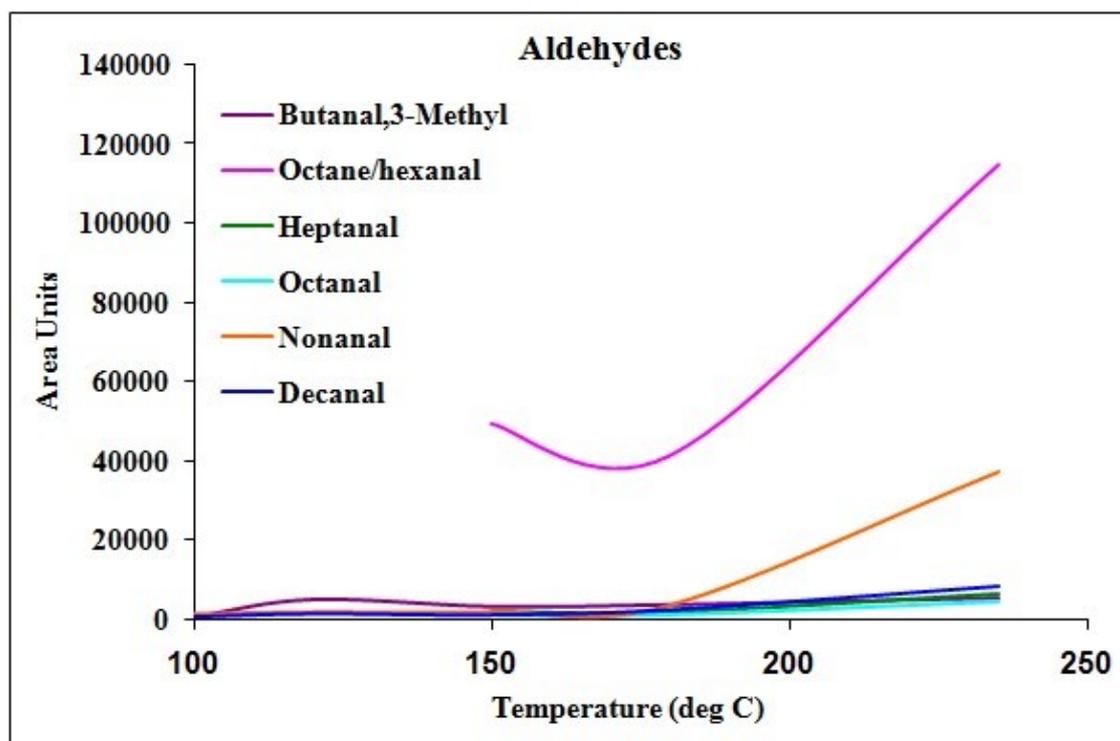


Figure 55. Aldehydes generated nitrogen versus temperature.

increasing from 100°C to 150°C and dropped above 150°C. Assumption can be made that under nitrogen, high temperature (> 150°C) can transform O<sub>2</sub> to O·, which can attack 1-alkene radicals from thermal scission directly to form aldehydes.

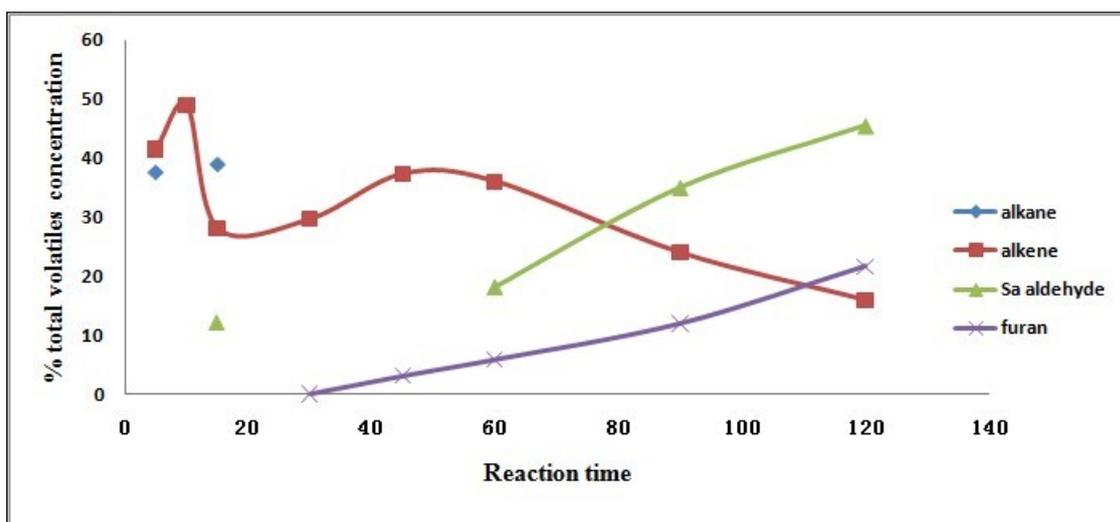
### Types of volatiles released

	% of Total Volatiles				
	100	120	150	180	235 °C
<b>N<sub>2</sub></b>					
alkane	57.19	61.61	51.72	59.17	61.62
alkene	27.29	20.38	14.20	19.84	3.97
branched alkane	7.90	7.88	4.58	5.12	1.19
cyclic alkane/alkene		2.19	1.65	1.78	1.66
aldehyde	4.89	2.51	6.22	10.34	14.85
alcohol	1.93	2.16	2.41	0.00	3.18
acid	0.48	2.61	17.76	2.44	4.23
furan	2.26	2.44	2.14	4.27	5.03
ester		0.27	1.45	0.00	0.30
ketone			0.45	0.00	3.36
epoxy			0.22	0.55	0.00
<b>Air</b>					
alkane	18.82	9.25	9.15	9.55	6.16
alkene	53.65	38.82	1.32	1.11	3.87
branched alkane	10.09	3.38		0.70	1.23
cyclic alkane/alkene	10.09	3.38		0.00	2.32
aldehyde - unsat	13.69	13.35	34.05	10.36	19.71
aldehyde - sat	3.75	0.00	24.06	18.59	30.39
alcohol		0.00	13.72	5.59	15.47
acid		35.19	3.21	17.75	1.05
furan			8.18	13.46	10.06
ester			0.80	6.23	2.74
ketone			3.38	9.49	8.38
epoxy				2.36	0.00

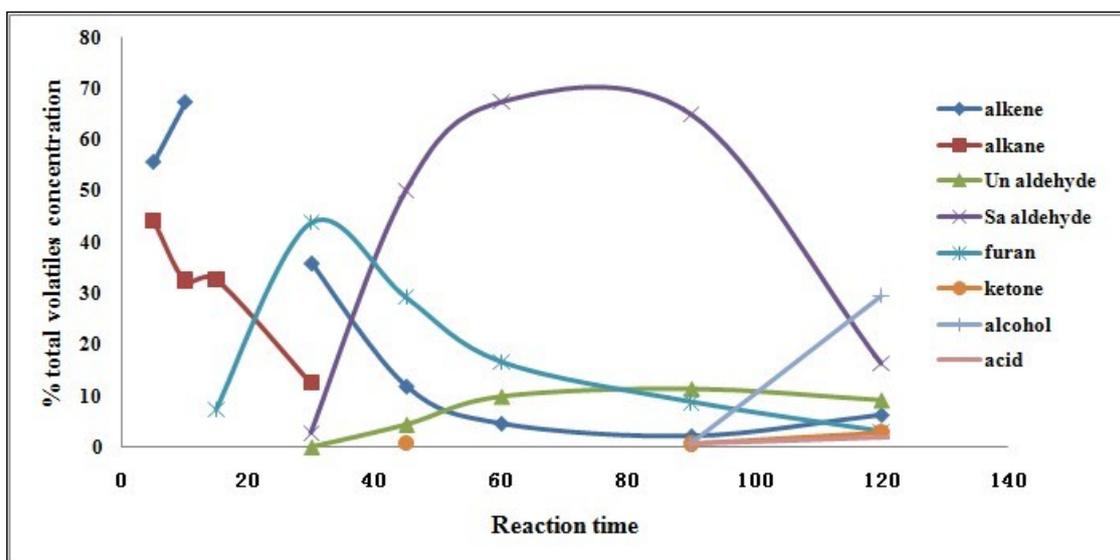
cyclic ald/ketone	3.18	4.66	0.88
other		0.63	0.00

**Table 26. Products released from nitrogen and air by different types.**

From time sequence GC data under air and nitrogen, we plotted type of products as percentage of total volatiles versus collecting time (Figure 56-63).



**Figure 56. Ratio of volatile as function of reaction time under 100°C/Air.**



**Figure 57. Ratio of volatile as function of reaction time under 120°C/Air.**

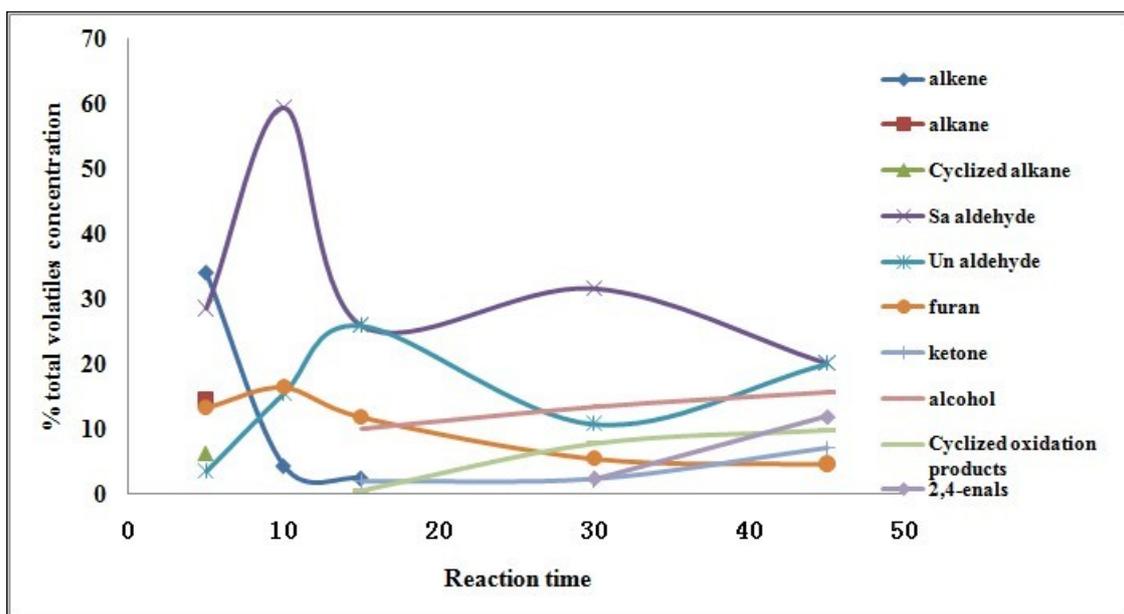


Figure 58. Ratio of volatile as function of reaction time under 150°C/Air.

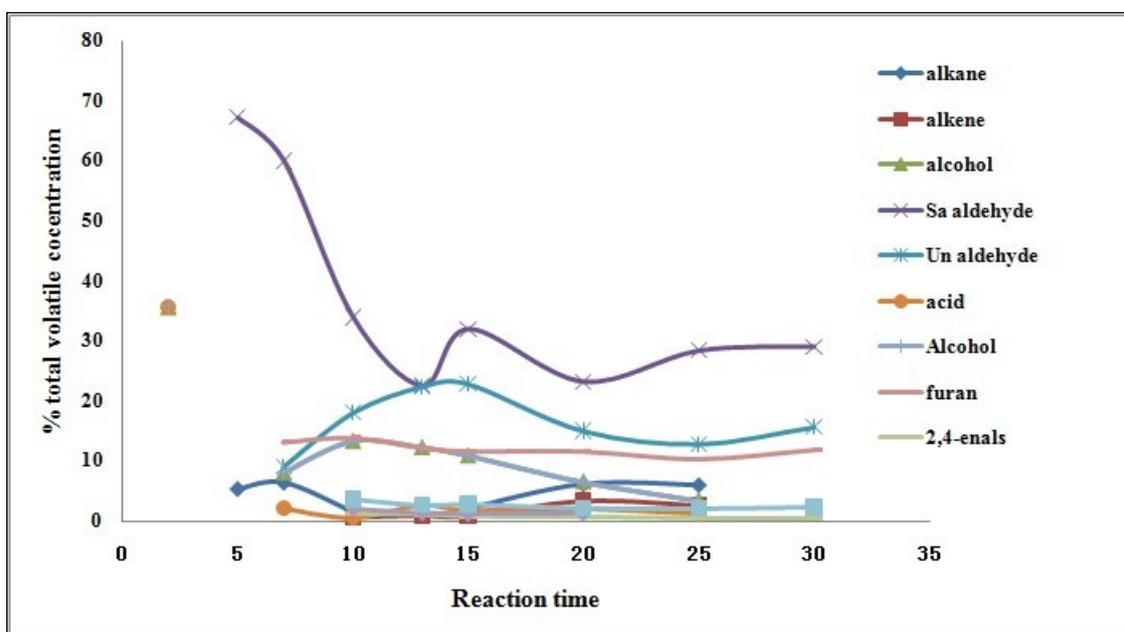


Figure 59. Ratio of volatile as function of reaction time under 180°C/Air.

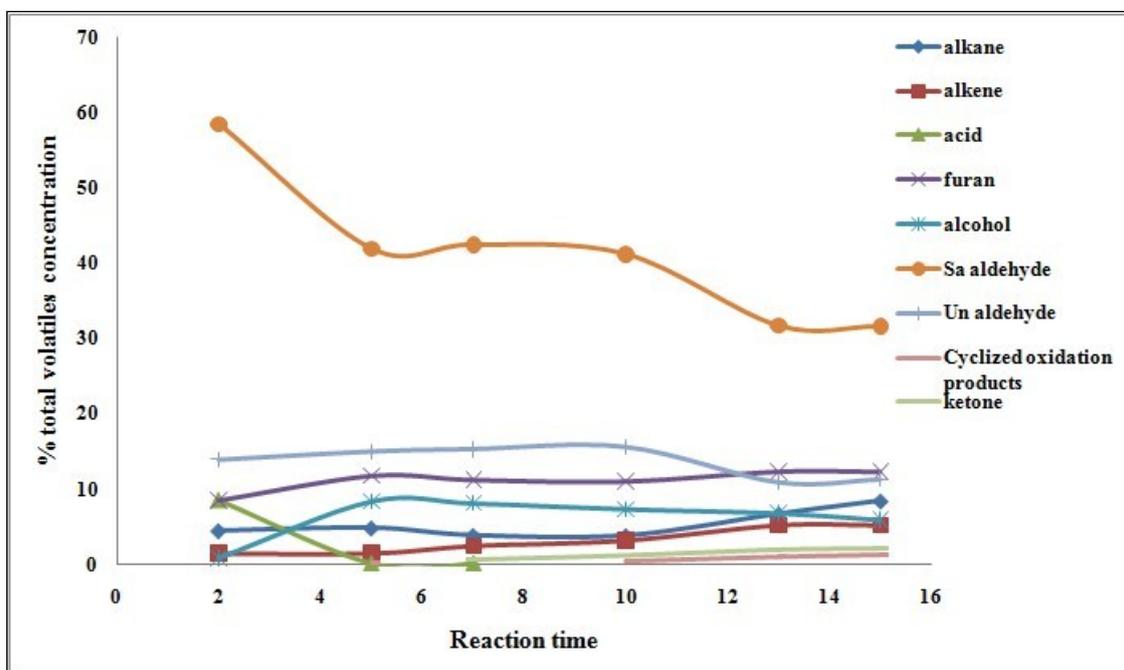


Figure 60. Ratio of volatile as function of reaction time under 235°C/Air.

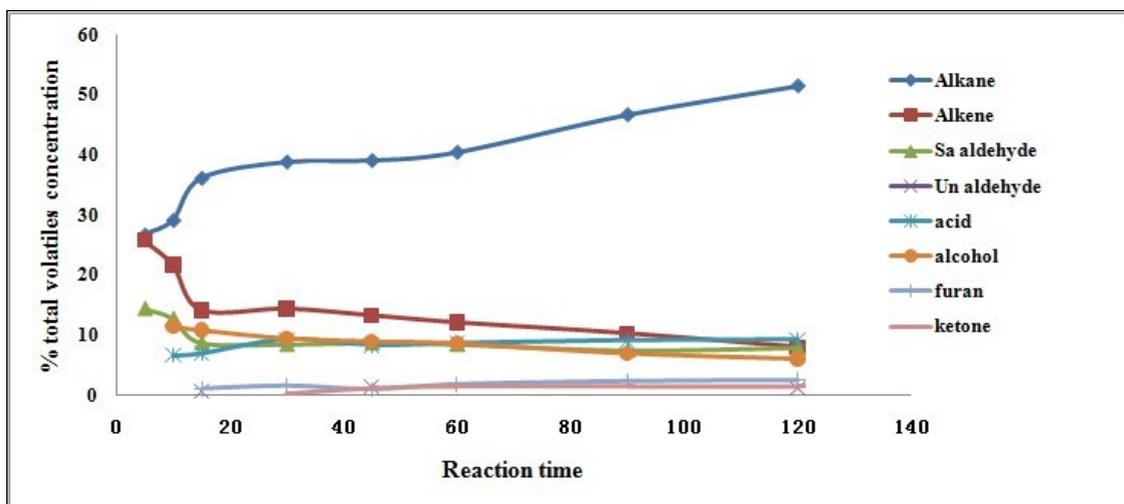


Figure 61. Ratio of volatile as function of reaction time under 100°C/N<sub>2</sub>.

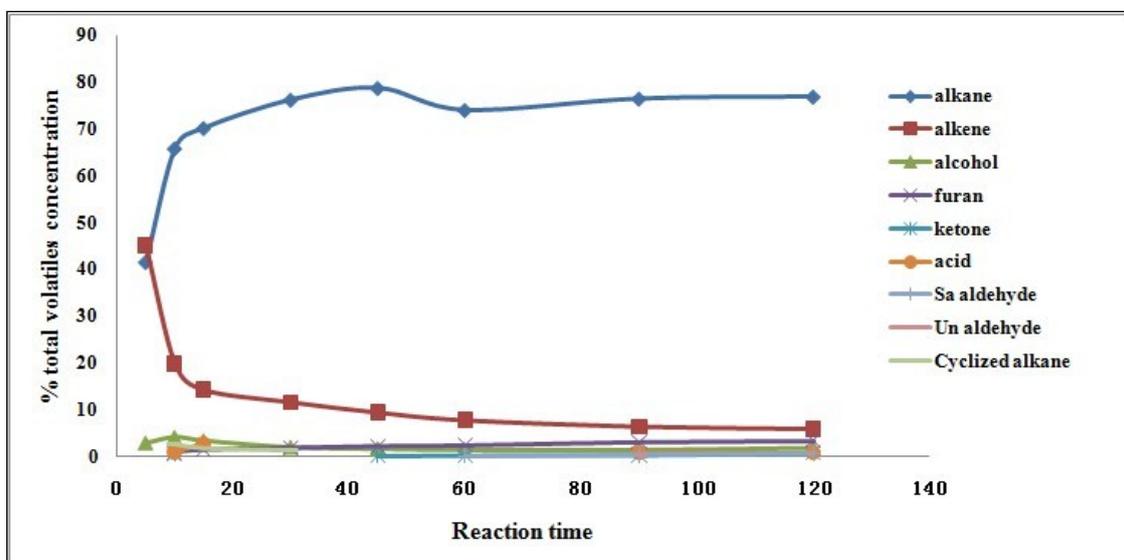


Figure 62. Ratio of volatile as function of reaction time under 120°C/N<sub>2</sub>.

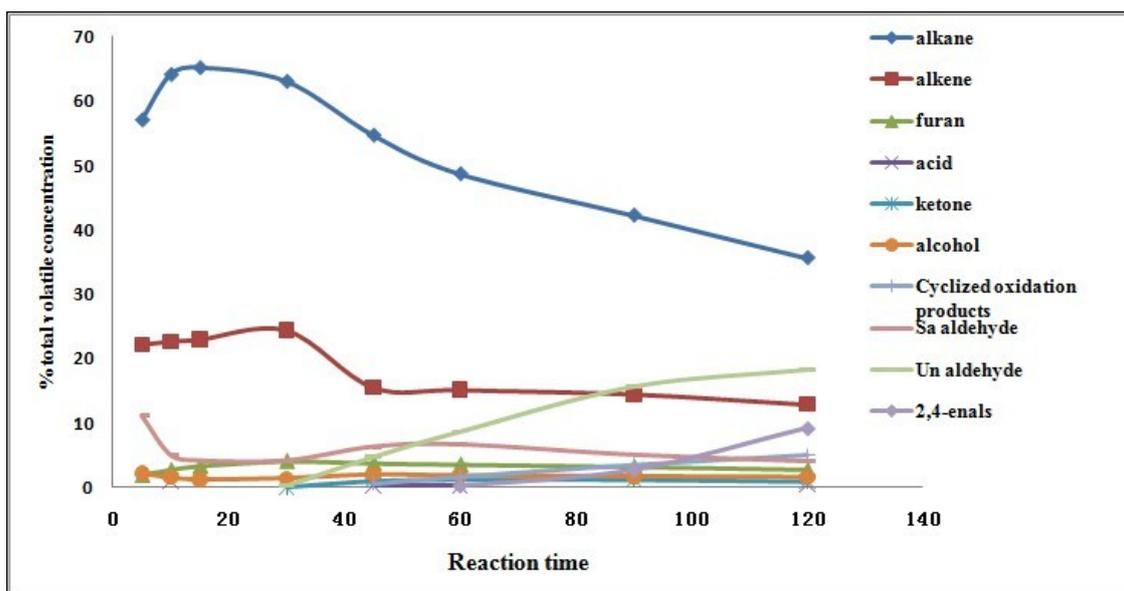


Figure 63. Ratio of volatile as function of reaction time under 150°C/N<sub>2</sub>.

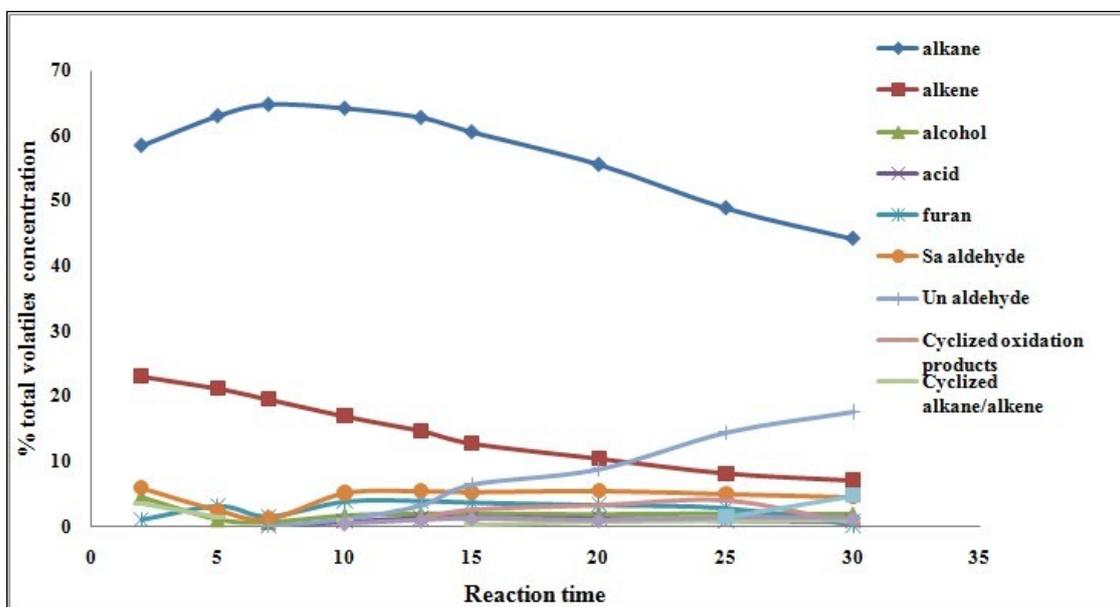


Figure 64. Ratio of volatile as function of reaction time under 180°C/N₂.

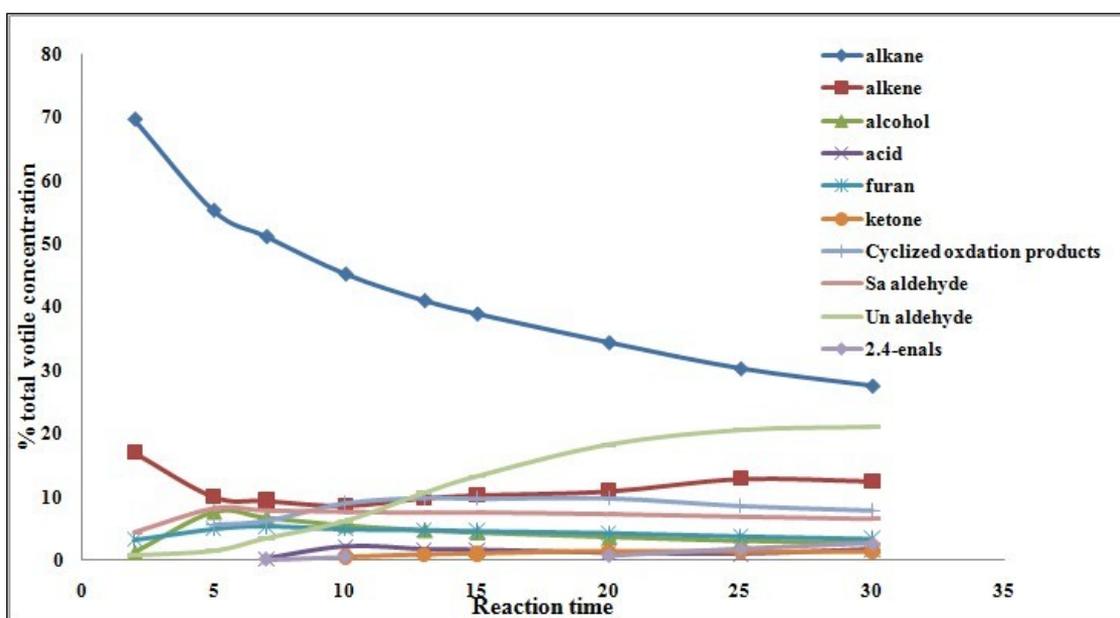


Figure 65. Ratio of volatile as function of reaction time under 235°C/N₂.

## 6. SUMMARY AND CONCLUTIONS

Corn oil was heated to temperatures ranging from 100 to 235 °C under nitrogen and air in a Purge&Trap system to assess contributions of thermal scissions and conventional lipid autoxidation to overall degradation of oils during frying. Products were separated by gas chromatography, identified by mass spectrometry, and compared for heating temperature, heating time, and atmosphere.

Under nitrogen, products were a homologous series of short chain alkanes and alkenes generated from thermal scission of lipid acyl chains. Based on product yields, preferred scission points were, in order, at carbons  $\alpha$ ,  $\beta$ , and  $\gamma$  to double bonds. In linoleic acid, thermal scission occurred preferentially at the  $\text{CH}_3$  end of the acyl chain, yielding pentane which was the dominant product under all conditions. Little thermal scission near C-9 was observed until after long heating times. In oleic acid, in contrast, scission occurred on both sides of the single double bond, although preferentially on the acid end of the acyl chain. Main products were present from the beginning of heating and increased in concentration during the entire heating period. Some secondary products were present from the beginning and remained at very low levels during heating; other secondary products did not appear until after long heating times and/or only at the higher temperatures (180 and 235°C).

Under air, products were oxidation compounds of the same thermal scission alkanes/alkenes. Initially, products were primarily aldehydes and alcohols, but increased levels of ketones and furans grew in with longer heating times and higher heating temperatures. Few of the main classical products expected from lipid autoxidation were observed except hexanal. Interestingly, because C13 of linoleic acid is the major scission

point, pentane can be generated by both thermal scission and lipid autoxidation. Contributions from both pathways may account for its complete dominance as a product.

Perhaps most importantly, this study showed clearly that thermal scissions are a major process underlying degradation in frying oils, generating distinctive products directly, whether in air or under nitrogen. Radicals from thermal scission add oxygen to form terminal peroxy radicals that initiate a cascade of reactions and products. Our results suggest that thermal scission radicals are the key initiators of lipid autoxidation that occurs secondary to thermal processes in frying oils. Thermal scission and autoxidation then run simultaneously in heated oils. In early stages of heating and under low oxygen conditions, thermal scissions clearly dominate. As heating progresses, thermal scission and lipid oxidation co-exist, with the balance determined by temperature, heating time, and atmosphere.

The underlying body of thermal scission radicals is a constant with frying. It has been recognized but ignored in the past. However, to stabilize polyunsaturated oils in frying operations, thermal scission processes must be accounted for in developing any strategies for protecting quality of frying oils or fried products.

## 7. REFERENCES

- Alla, H. F., Yinon Rudich (2001). "Analysis of Semivolatile Organic Compounds in Atmospheric Aerosols by Direct Sample Introduction Thermal Desorption GC/MS." Enviro. Sci. Techol. **35**(11): 2326-2333.
- Avila, D. V., C. E. Brown, et al. (1993). "Solvent effects on the competitive  $\beta$ -scission and hydrogen atom abstraction reactions of the cumyloxyl radical. Resolution of a long-standing problem." J. Am. Chem. Soc. **115**: 466-470.
- Beltrán, A. R., M. Grané, N. Martín, M.L. Garrigós, M.C. (2010). "Monitoring the oxidation of almond oils by HS-SPME-GC-MS and ATR-FTIR: Application of volatile compounds determination to cultivar authenticity." Food Chem. **126**(2): 603-609.
- Blumenthal, M. M. (1991). "The chemistry and technology of deep-fat frying." Food Technology **45**: 68-71.
- Bors, W., Erben-Russ, M., Saran, M. (1987). "Fatty acid peroxy radicals: their generation and reactivities." J. Electroanal. Chem. **232**: 37-49.
- Bors, W., D. Tait, et al. (1984). "Reactions of alkoxy radicals in aqueous solutions." Isr. J. Chem. **24**: 17-24.
- Bosset, J. O. G., R. (1993). "Comparison of the volatile flavour compounds of six european 'AOC' cheeses by using a new dynamic headspace GC-MS method." International Dairy Journal **3**(4-6): 359-377.
- Brodnitz, M. H. (1968). "Autoxidation of saturated fatty acids. A review." Agric. Food Chem. **16**(6): 994-999.
- Cédric, G. A. M., Berg. Stephan, R. Müller, Urs. Dommann, René P. Schwarzenbach (1998). "Determination of Organotin Compounds in Water, Sediments, and Sewage Sludge Using Perdeuterated Internal Standards, Accelerated Solvent Extraction, and Large-Volume-Injection GC/MS." Analytical Chem. **70**(14): 3094-3101.
- Chan, H. W.-S. and D. T. Coxon (1987). Lipid hydroperoxides. Autoxidation of Unsaturated Lipids. H. W.-S. Chan. London, Academic Press: 17-50.
- Chang, S. S., T. H. Smouse, et al. (1966). "Isolation and identification of 2-pentyl-furan as contributing to the flavor reversion of soyabean oil." Chem. Ind.: 1926-1927.
- Claxson, A. H., G. Richardson, D. Naughton, D. Haywood R. Chander, C. Atherton, M. Lynch, E. (1994). "Generation of lipid peroxidation products in culinary oils and fats during episodes of thermal stressing: a high field  $^1\text{H}$  NMR study." Federation of European Biochemical Societies **335**: 81-90.

- Coni, E., E. Podesta, et al. (2004). "Oxidizability of different vegetable oils evaluated by thermogravimetric analysis." Thermochimica Acta.
- De Maria, B. S., M. Dias, U, Jose. Marana, M. (2000). "Stabilization of soybean oil with heated quercetin and 5-caffeoylquinic acid in the presence of ferric ion." J. Agr. Food Chem. **48**(9): 3935-3938.
- Dobson, G. C., W. W. Sebedio, J. L. (1997). "Saturated bicyclic fatty acids formed in heated sunflower oils." Chemistry and Physics of Lipids **87**: 137-147.
- Ellis, R., A. M. Gaddis, et al. (1966). "Carbonyls in oxidizing fat. IX. Aldehydes isolated from autoxidized methyl arachidonate." J. Food Sci. **31**(2): 191-195.
- Ellis, R., A. M. Gaddis, et al. (1968). "Carbonyls in oxidizing fat: XII. The isolation of free aldehydes from autoxidized triolein, trilinolein and trilinolenin." J. Amer. Oil Chem. Soc. **45**(8): 553-559.
- Esterbauer, H. and H. Zollner (1989). "Methods for determination of aldehydic lipid peroxidation products." Free Radical Biology & Medicine **7**: 197-203.
- Frankel, E. N. (1962). Hydroperoxides. Symposium on Foods: Lipids and Their Oxidation. H. Schultz, E. D. Day and W. O. Sinnhuber. Westport, CT, AVI Publishing: 51-76.
- Frankel, E. N. (1982). "Volatile lipid oxidation products." Prog. Lipid Res. **22**: 1-33.
- Frankel, E. N. (1984). "Lipid oxidation: mechanisms, products, and biological significance." J Amer. Oil Chem. Soc. **61**(12): 1908-1917.
- Frankel, E. N. (1985). "Chemistry of free radical and singlet oxidation of lipids." Prog. Lipid Res. **23**: 197-221.
- Frankel, E. N. (1987). "Secondary products of lipid oxidation." Chem. Phys. Lipids **44**: 73-85.
- Frankel, E. N. (1991). "Recent advances in lipid oxidation." J. Sci. Food Agric. **54**: 495-511.
- Frankel, E. N., W. E. Neff, et al. (1979). "Analysis of autoxidized fats by gas chromatography-mass spectrometry: V. Photosensitized oxidation." Lipids **14**(12): 961-967.
- Frankel, E. N., W. E. Neff, et al. (1982). "Photosensitized oxidation of methyl linoleate: Secondary and volatile thermal decomposition products." Lipids **17**(1): 11-18.

Frankhauser-Noti, A. F., K.; Biedermann-Brem, S.; Grob, K (2006). "Assessment of epoxidized soy bean oil (ESBO) migrating into foods: Comparison with ESBO-like epoxy fatty acids in our normal diet." Food and Chemical Toxicology **44**(8): 1279-1286.

Fritsch, C. W. (1981). "Measurement of fryin fat deterioration: a brief review." J Amer. Oil Chem. Soc. **58**(3): 272-274.

Gérald, R. A., A.D.Yves-loïc, M., Gilles G. M. Gérard, J. M. (1997). "Authentication of Bitter Almond Oil and Cinnamon Oil: "Application of the SNIF-NMR Method to Benzaldehyde." J. Agr. Food Chem. **45**(10): 4042-4048.

Gillat, P. (2001). Flavour and aroma development in frying and fried foods. In: Frying. Improving quality. J. B. Rossell, CRC press: 266-335.

Guillen, M. D. (2009). "Oxidation of corn oil at room temperature: Primary and secondary oxidation products and determination of their concentration in the oil liquid matrix from H nuclear magnetic resonance data." Food Chem. **116**(1): 183-192.

Hartman, T. G. K. K. S. J., P. Ruiz, Reginald. Lech, Joseph. Rose, Rober T. (1994). Effect of packaging on the lipid oxidation storage stability of dehydrated pinto beans. Lipids in food flavors, American Chemical Society. **558**: 158-167.

Hill, F. D. H., E. G. (1965). "The oxidized-metallic and grassy flavor components of autoxidized milk fat." J. Dairy Res. **27**: 381.

Ho, C. T. S., M.S. Chang, S.S. (1978). "The synthesis of 2-(1-pentenyl) furan and its relationship to the reversion flavor of soybean oil." J Amer. Oil Chem. Soc. **55**(2): 233-237.

Howard, J. A. and K. U. Ingold (1967). "Absolute rate constants for hydrocarbon autoxidation. VI. Alkyl aromatic and olefinic hydrocarbons." Can. J. Chem. **45**: 793-802.

Howard, J. A. and K. U. Ingold (1968). "The self-reaction of *sec*-butylperoxy radicals: confirmation of the Russell mechanism." J. Am. Chem. Soc. **90**: 1056-1058.

Kamal-Eldin, A. G. M.-R., Carmen Dobarganes and Lars-Ake Appelqvist (1997). "Characterisation of aldehydic acids in used and unused frying oils." Journal of Chromatography A **776**(2): 245-254.

Kaufmann, A. S., B. (2001). "Comparison of different methods to determin polar compounds in frying oils." Eur. Food Res. Technol. **213**: 377-380.

Kerwin, J. L. T., J. J. (1996). "Identification of monohydroxy fatty acids by electrospray mass spectrometry and tandem mass spectrometry." Anal. Bioanal. Chem. **237**: 56-64.

Kochi, J. K. (1973). Free Radicals, Vol. I. New York, John Wiley and Sons.

- Labuza, T. P. (1971). "Kinetics of lipid oxidation in foods." CRC Crit. Rev. Food Sci. Nutr. **2**(3): 355-405.
- Lea, C. H. (1962). Symposium on Foods: Lipids and Their Oxidation. H. W. Schultz, E. A. Day and R. O. Sinnhuber. Westport, CT, AVI Publishing: Ch 1.
- Lindsay, D. A., J. A. Howard, et al. (1973). "The bimolecular self-reaction of secondary peroxy radicals, Product studies." Can. J. Chem. **51**(6): 870-880.
- Makinen, M. P., V. Hopia, A. (1996). "Postcolumn chemiluminescence, Ultraviolet and evaporative light-scattering detectors in high-performance liquid chromatography determination of triacylglycerol oxidation products." Journal of Chromatography **734**: 221-229.
- Marmesat, S. V., J. Dobarganes, M. C. (2008). "Quantitative determination of epoxy acids, keto acids and hydroxy acids formed in fats and oils at frying temperatures." J. Chromatog. A. **1211**(1-2): 129-134.
- Matezto, L. S., C. Andueza, S. Paz De Pena, M. Bello, Jose. CID, Concepcion (2001). "Characterization of Espresso Coffee Aroma by Static Headspace GC-MS and Sensory Flavor Profile." J. Agr. Food Chem. **49**(11): 5437-5444.
- Moreno, M. (1999). "Study of the formation of carbonyl compounds in edible oils and fats by H-NMR and FTIR." Journal of Molecular Structure **482-483**: 557-561.
- Nawar, W. W. (1967). "Thermal decomposition of methyl oleate." J Amer. Oil Chem. Soc. **45**(2): 100.
- Nawar, W. W. (1969). "Thermal degradation of lipids." J. Agr. Food Chem. **17**(1): 18-21.
- Nawar, W. W. (1984). "Chemical changes in lipid produced by thermal processing." Journal of chemical education. Journal of chemical education **61**(4): 269-302.
- Nawar, W. W. (1996). Lipids. Food Chemistry. O. R. Fennema. New York, Academic: 225-320.
- Naz, S., R. Siddiqi, et al. (2005). "Deterioration of olive, corn, and soybean oils due to air, light, heat, and deep-frying." Food Research International **38**: 127-134.
- Neff, W. E., K. Warner, et al. (2000). "Odor significance of undesirable degradation compounds in heated triolein and trilinolein." Journal of the American Oil Chemists' Society **77**(12): 1303-1313.

- Nobuo, O. K., S. Masahiko, T. Satoru, Y. Shigeki, D. Arnd, H. Andreas, H. (2001). "Determination of trace amounts of off-flavor compounds in drinking water by stir bar sorptive extraction and thermal desorption GC-MS." Analyst **126**: 1652-1657.
- Palmer, D. A., M. Jolly, R. (1984). "Separation of some neutral lipids by normal-phase high-performance liquid chromatography on a cyanopropyl column: Ubiquinone, dolichol, and cholesterol levels in sheep liver." Analytical Biochemistry **140**(2): 315-319.
- Pavia, D. L. G. M. L., George S. Kriz, Randall G. Engel (2006). Introduction to organic laboratory techniques, Thomson brooks/Cole.
- Pawliszyn, J. (1999). Application of solid phase microextraction, Royal Society of Chemistry.
- Pellegrini, N. V., F. Buratti, S. Brighenti, F. (2001). "Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating." J. Agr. Food Chem. **49**(5): 2532-2538.
- Pokorny, J. (1987). Major factors affecting the autoxidation of lipids. Autoxidation of Unsaturated Lipids. H. W.-S. Chan. London, Academic Press: 141-206.
- Porter, N. A. (1986). "Mechanisms for the autoxidation of polyunsaturated lipids." Acct. Chem. Res. **19**: 262-268.
- Porter, N. A., M. O. Funk, et al. (1976). "The formation of cyclic peroxides from unsaturated hydroperoxides: Models for prostaglandin biosynthesis." J. Am. Chem. Soc. **98**(19): 6000-6005.
- Porter, N. A., L. S. Lehman, et al. (1981). "Unified mechanism for polyunsaturated fatty acid autoxidation. Competition of peroxy radical hydrogen atom abstraction, B-scission, and cyclization." J. Am. Chem. Soc. **103**: 6447-6455.
- Reblova, Z. (2006). "The effect of temperature on the antioxidant activity of tocopherols." Eur. J. Lipid Sci. Technol. **108**(10): 858-863.
- Saguy, I. S. D. D. (2003). "Integrated approach to deep fat frying: engineering, nutrition, health and consumer aspects." Journal of Food Engineering **56**(2-3): 143-152.
- Schaich, K. M. (2005). Lipid oxidation in fats and oils: An integrated view. Bailey's Industrial Fats and Oils, 6th Edition. F. Shahidi. New York, John Wiley: 2681-2767.
- Schaich, K. M. (2008). Co-oxidations of oxidizing lipids: Reactions with proteins. Lipid Oxidation Pathways. Volume 2. A. Kamal-Eldin and D. B. Min. Boca Raton, FL, CRC Press: in press.

- Schneider, C. S., P. Herderich, M. (1997). "Analysis of lipoxygenase-derived fatty acid hydroperoxides by electro-spray ionization tandem mass spectrometry." Lipids **32**(3): 331-336.
- Schulte, E. (2002). "Determination of higher carbonyl compounds in used frying fats by HPLC of DNPH derivatives." Analytical and Bioanalytical Chemistry **372**(5-6): 644-648.
- Scott, G. (1965). Atmospheric Oxidation and Antioxidants. New York, Elsevier.
- Shen, N., W. Fehr, et al. (1997). "High-temperature stabilities of oils from soybeans that lack lipoxygenases." Journal of the American Oil Chemists' Society **74**(4): 381-385.
- Sjovall, O. K., A. Maria, L. Muher, J. J. (1997). "Elution factors of synthetic oxotriacylglycerols as an aid in identification of peroxidized natural triacylglycerols by reverse-phase high performance liquid chromatography with electro-spray mass spectrometry." Lipids **32**(1211-1218).
- Smith, S. A., R. E. King, et al. (2007). "Oxidative and thermal stabilities of genetically modified high oleic sunflower oil." Food Chemistry **102**: 1208-1213.
- Stark, W. S., J. F. Forss, D. A. (1966). "n-Pent-1-en-3-ol and n-pent-1-en-3-one in oxidized dairy products." J. Dairy Res. **34**: 123.
- Summo, C. C., F. (2008). "Assessment of the oxidative and hydrolytic degradation of the lipid fraction of mortadella by means of HPSEC analyses of polar compound." Meat Sci. **79**: 722-726.
- Tomaino, A. C., F. Zimbalatti, V. Venuti, V. Sulfaro, V. De Pasquale, A. Saija, A. (2005). "Influence of heating on antioxidant activity and the chemical composition of some spice essential oils." Food Chem. **89**(4): 549-554.
- Uran, S. L., A. Jacobsen, P. Skotland, T. (2001). "Analysis of phospholipid species in human blood using normal-phase liquid chromatography coupled with electrospray ionization ion-trap tandem mass spectrometry." J. Chromatography B: Biomedical Sciences and Applications **758**(2): 265-275.
- Uri, N. (1961). Physico-chemical aspects of autoxidation. Vol. 1. Autoxidation and Antioxidants. W. O. Lundberg. New York, Interscience: 55-106.
- Velasco, J., M. L. Andersen, et al. (2004). "Evaluation of oxidative stability of vegetable oils by monitoring the tendency to radical formation. A comparison of electron spin resonance spectroscopy with the Rancimat method and differential scanning calorimetry." Food Chem. **85**: 623-632.

Warner, K. and M. Gupta (2003). "Frying quality and stability of low-and ultra-low-linolenic acid soybean oils." Journal of the American Oil Chemists' Society **80**(3): 275-280.

Yu, J. (1997). "Thermal Decomposition of C10-C14 Normal Alkanes in Near-Critical and Supercritical Regions: Products Distribution and Reaction Mechanism." Ind. Eng. Chem. **36**: 574-584.