THE EFFECT OF TEMPERATURE AND HEATING TIME ON THE FORMATION OF ALPHA, BETA UNSATURATED HYDROXYALDEHYDES IN VARIOUS VEGETABLE OILS AND FATS

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Abstract

A certain group of secondary lipid oxidation products, α, β-unsaturated-4-hydroxyaldehydes, is of special interest because of their high reactivity to various biologically important compounds like proteins and DNA. The formation of 4-hydroxy-2-trans-nonenal (HNE), 4-hydroxy-2-trans-hexenal (HHE), 4-hydroxy-2-trans-octenal (HOE) and 4-hydroxy-2-trans-decenal (HDE), as well as total polar and nonpolar aldehydes was investigated in commercial corn, soybean, peanut, canola oils, lard and beef tallow which were heat treated at different temperatures (145, 165 and 185 ℃) for different length of heating time. The formation of the four hydroxyaldehydes was monitored by the high-performance liquid chromatography (HPLC).

These oils and fats were selected based on their different degree of unsaturation and fatty acid distributions. Preliminary experiments were conducted using the thiobarbituric acid (TBA) assay to select the proper conditions of temperatures and heating time before the measurement of the aldehyde concentration by HPLC. These oils and fats were heat treated at 145, 165 and 185 ℃ for 1, 3, and 5 hours to measure the formation of the α, β-unsaturated-4-hydroxyaldehydes as 2,4-dinitrophenylhydrazone derivatives using HPLC system.

In general, the formation of HHE, HOE, HNE and HDE increased with higher temperatures (145, 165 and 185 ℃), longer heating time (in the range of 0 to 5 hours) and higher unsaturation in the samples. As was expected, the concentration of HNE was

much higher than that of the other three 4-hydroxyaldehydes in all the samples measured. The sum total of polar aldehydes increased with higher temperatures and longer heating time. The sum total of nonpolar aldehyde concentration increased slightly with increased temperatures and heating time in vegetable oils which contain longer chain fatty acids, while the concentration did not change or even decreased along with temperatures and heating time in animal fats which contain more short-chain fatty acids. The decrease of total nonpolar aldehydes formation seems to be related to the volatility of the short chain aldehydes at elevated temperatures.

It was found that the formation of all the four toxic α, β-unsaturated-4-hydroxyaldehydes was dependent on temperature, time of heat treatment and the level of linoleic acid in the oils.

Therefore, to minimize the formation of these toxic compounds in high linoleic acid containing oils, the lowest temperature and the shortest heating time should be used.

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Introduction

Lipid oxidation causes major chemical changes in oils and fats, especially at elevated temperatures. Degradation products include the primary oxidation product hydroperoxides and secondary lipid oxidation products, such as alkanals, alkenals, alkadienals and α, β- unsaturated-4-hydroxyaldehydes. The group of four α, β-unsaturated-4-hydroxyaldehydes is of particular interest because of their high reactivity to various compounds including biomolecules such as DNA and proteins (1-4). These four compounds are: 4-hydroxy-2-trans-nonenal (HNE), 4-hydroxy-2-trans-hexenal (HHE), 4-hydroxy-2-trans-octenal (HOE) and 4-hydroxy-2-trans-decenal (HDE). α, β-Unsaturated-4-hydroxyaldehydes are cytotoxic and mutagenic because they can react with the thiol (SH) and amino (NH_2) groups and this reaction results in DNA and protein modification. Recent experiments reported in the literature relate the toxicity of these compounds, especially HNE, to numerous diseases such as atherosclerosis, stroke, liver disease, Parkinson's disease, Alzheimer's disease and Huntington's disease (5-7). HNE can alter the structure and function of these disease related proteins. HNE has been shown to form in highly polyunsaturated fatty acids (PUFAS) oils and can be incorporated into food from the frying oil during the frying process (8, 9). Though this group of compounds is attracting more attention, there is still little study on HHE, HOE and HDE formation. In this laboratory, Seppanen and Csallany detected HHE, HOE,

HNE and HDE in heat-treated soybean oil (10, 11). Lang and Surh with their colleagues reported HHE in vegetable oils such as sunflower, olive, sesame, corn, soybean and perilla oils (12, 13).

The objective of this study was to determine the influence of temperature, heating time and unsaturation on toxic α, β-unsaturated-4-hydroxyaldehydes formation in frying oils and fats.

Part I: Literature Review

This literature review contains five parts. The first section introduces thermal oxidation and basic chemical reactions. The second section discusses the formation of secondary oxidation products. The third section reviews studies on oxidation measurement and the effects of temperature and time on oxidation in fats and oils. The fourth section contains the formation of HNE. Finally, the fifth section investigates the health aspects of thermal oxidized fats and oils.

Thermal oxidation and its chemical reactions

In the food industry and culinary practice, the thermal treatment of edible fats and oils like frying is one of the main methods used for food processing. Fried foods have desirable flavor, color and textural attributes which make them very popular to consumers (14).The frying temperature is usually between 150 ℃ and 190 ℃ (15). During this common process, fatty acids react with oxygen in the air, and free radicals, hydroperoxides, aldehydes, related carbonyl compounds and polymers are produced. This reaction is called peroxidation, or thermal oxidation, when oils and fats are subjected to heat.

The mechanism of primary oxidation or autoxidation involves three stages: initiation, propagation and termination. Figure 1 shows the primary oxidation mechanism. In

initiation, the hydrogen with the weakest bond on the carbon will be removed and forms an alkyl free radical (R·); this hydrogen is usually on the methylene groups adjacent to the double bond, and it is very easily attached to the carbon between the two double bonds. A molecule of oxygen is then reacted with this carbon free radical forming a lipid peroxy radical (ROO·). This peroxy radical then attracts a hydrogen atom from another free fatty acid (RH) to form hydroperoxide (ROOH) in propagation and a new alkyl radical (R·) is formed on the other lipid acid. The new lipid free radical (R·) reacts with molecular oxygen and the sequence of reactions is repeated, forming a chain reaction. This chain reaction can greatly accelerate the thermal oxidation process. Propagation is followed by the reaction of two free radicals which form non radical compounds so that the oxidation is terminated.

Initiation $RH \rightarrow R\cdot + H\cdot$

Propagation $R\cdot + O_2 \rightarrow ROO\cdot$

$ROO\cdot + RH \rightarrow ROOH + R\cdot$

Termination $ROO\cdot + R\cdot \rightarrow ROOR$

$R\cdot + R\cdot \rightarrow RR2$

R: lipid alkyl

Figure 1. The initiation, propagation, and termination of free radical induced thermal oxidation of oils and fats

Many factors influence oxidative susceptibility in food systems. The main factors are fatty acid composition, temperature, time, oxygen concentration, catalysts and antioxidants (16). Unsaturated fatty acids, especially the highly unsaturated fatty acids, are more reactive than saturated fatty acids in thermal oxidation (17). High temperature leads to faster thermal oxidation, more secondary oxidation and polymerization products (18).

Secondary oxidation and the formation of aldehydes in thermal oxidation

Primary oxidation products, i.e. hydroperoxides, are easily decomposed to a wide variety of compounds such as aldehydes, ketones and short-chain hydrocarbons. Figure 2 shows the general scheme for lipid autoxidation.

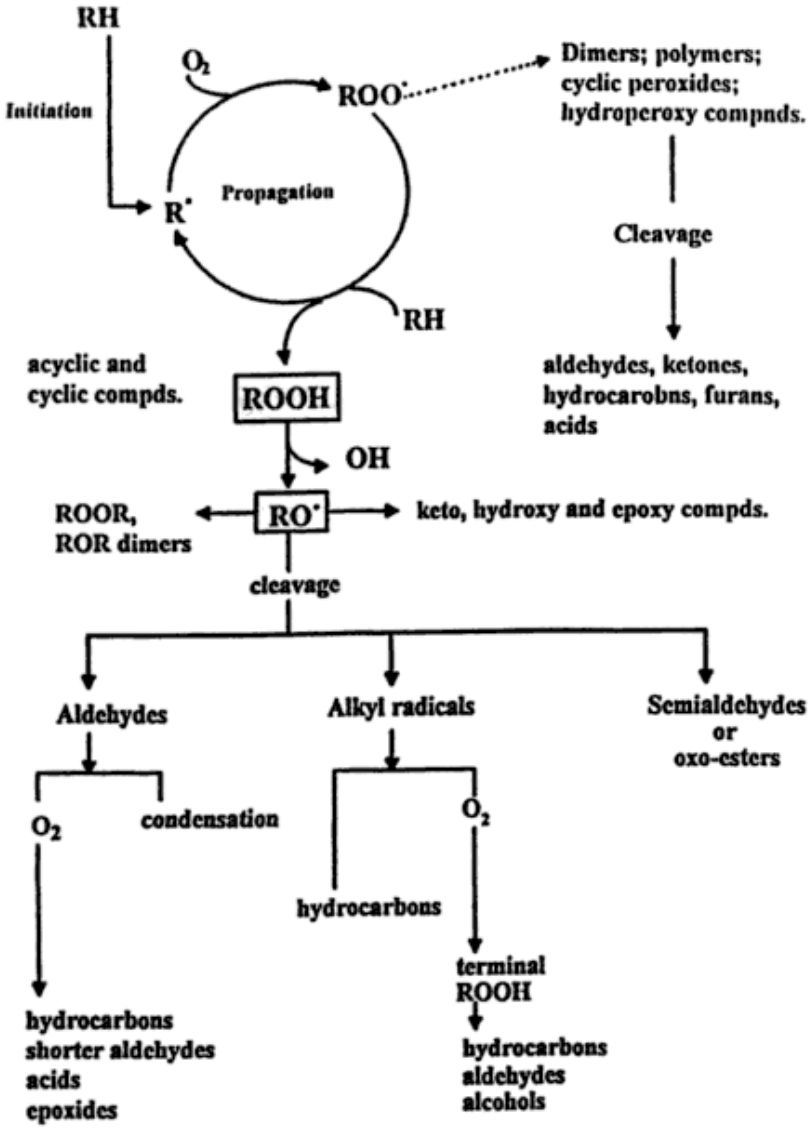


Figure 2. General scheme for lipid oxidation (From Ref.(19))

In hydroperoxide decomposition, first, homolytic cleavage between oxygen and a hydroxyl forms alkoxy and hydroxy radicals. The breakdown of alkoxy radicals produces oxo-compounds and alkyl radicals; this step is also called β-scission. After a number of rearrangements, secondary oxidation products such as aldehydes and ketones are produced (20, 21). Figure 3 shows the general mechanism of hydroperoxide decomposition which forms secondary oxidation products and Figure 4 shows the classes of substances formed by peroxidation of polyunsaturated fatty acid (PUFA).

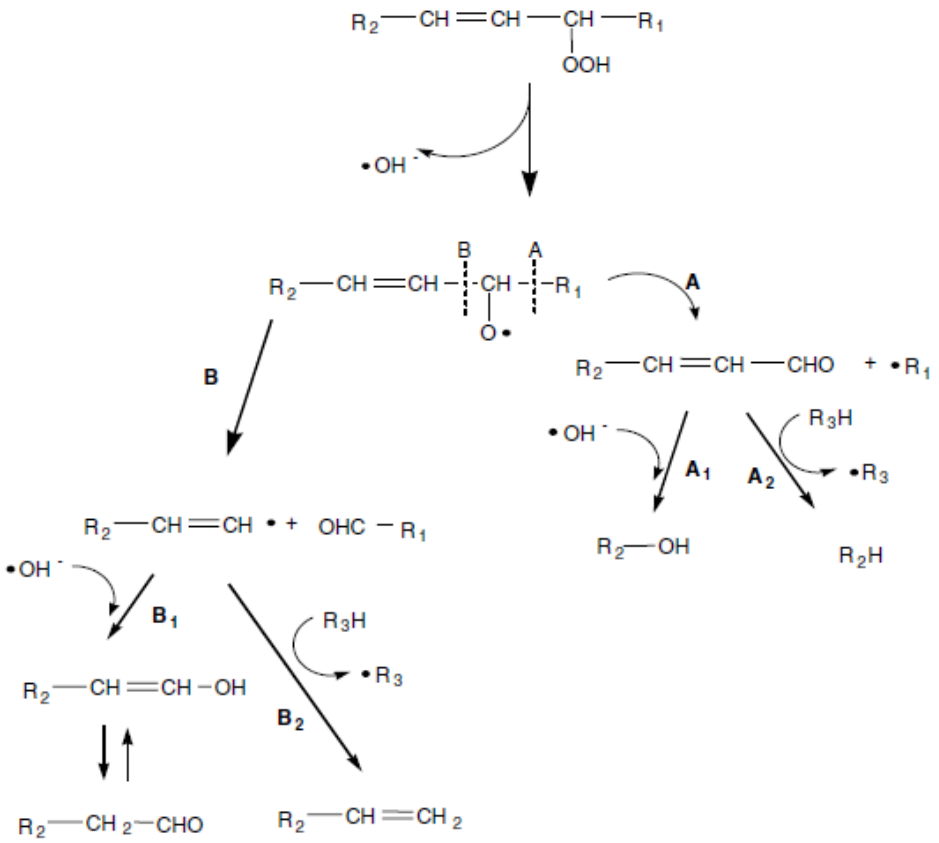


Figure 3. Mechanism of hydroperoxide decomposition to form secondary oxidation products (From Ref. (22))

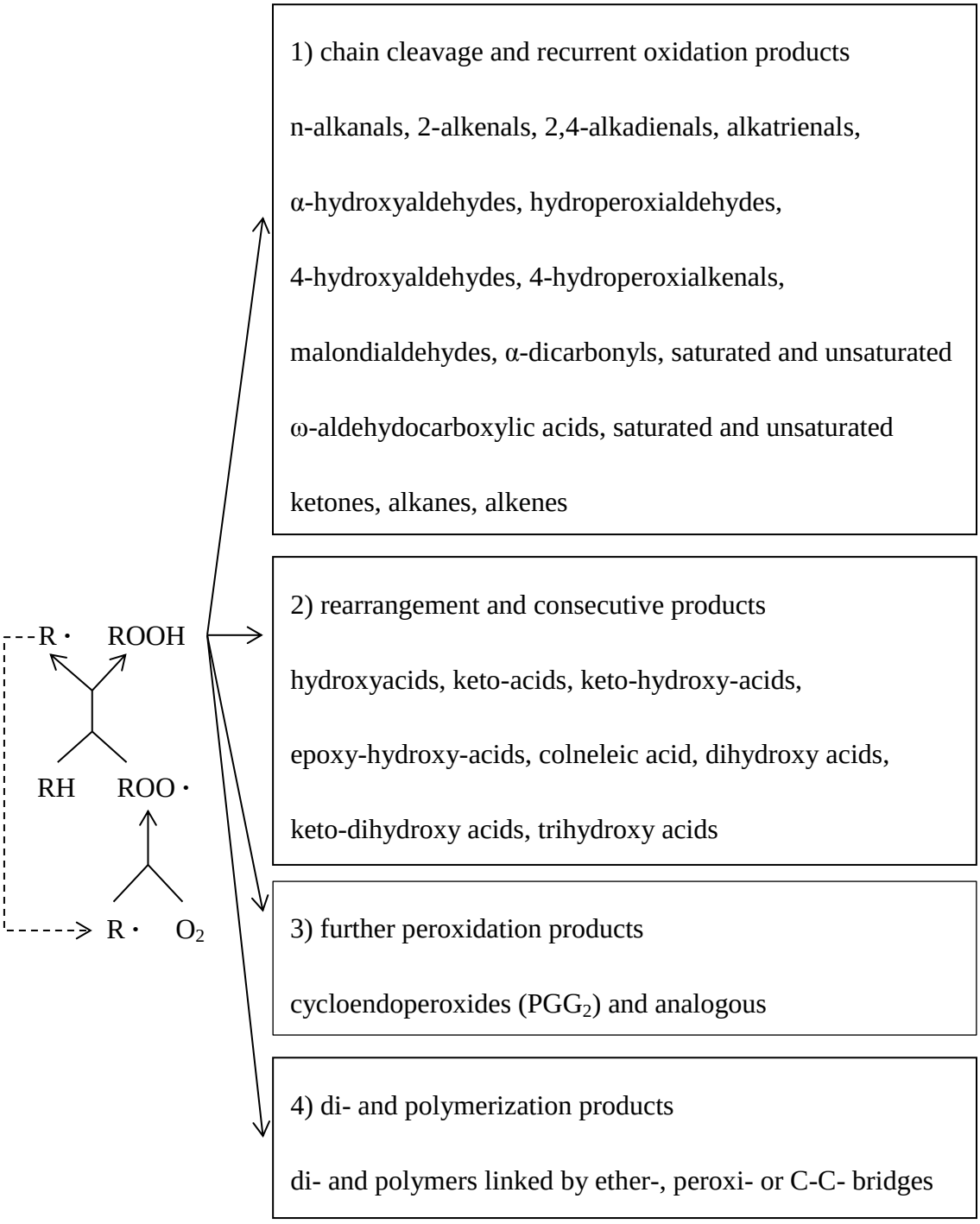


Figure 4. The classes of substances formed by enzymatic or non-enzymatic peroxidation of PUFA (from Ref (23))

Lipid oxidation at elevated temperatures is more complex than at lower temperatures. Both saturated and unsaturated fatty acids can react and decompose when exposed to heat and oxygen. A summary of these pathways is shown in Figure 5.

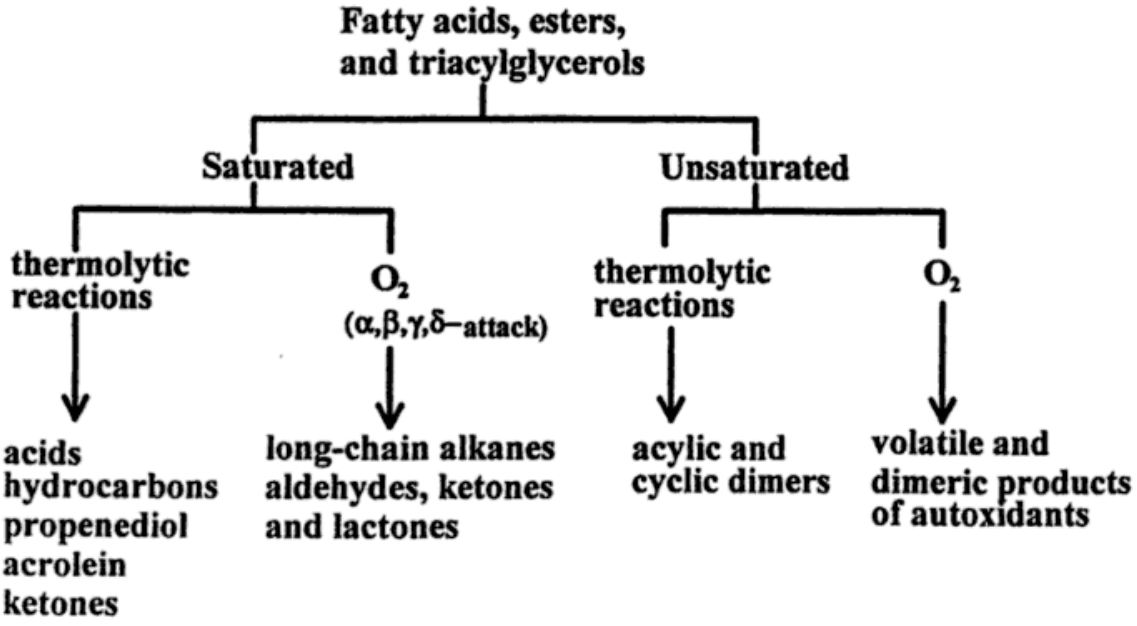


Figure 5. Generalized scheme for thermal decomposition of lipids (From Ref.(19))

Recent studies measuring oxidation in various oils and fats

Many studies have found that, in general, the higher the temperature and the longer heating time, the greater the oxidation. Researchers usually measure the change of color, peroxide values, iodine values, anisidine values, acid values, fatty acid content, total polar compounds and polymers to evaluate the oxidative status of oils. In recent years, some researchers have used HPLC and H NMR to monitor oil degradation processes. These methods can provide information on the nature and proportions of the aldehydes generated in these processes which are present in the oil liquid phase (24). Some methods are not feasible to directly monitor oxidation at high temperatures. Since hydroperoxides do not accumulate during frying as they are rapidly destroyed at temperatures near or above 100 ℃, peroxide values cannot be used to evaluate the oxidation at high temperatures. The thiobarbituric acid (TBA) test can measure secondary lipid oxidation product such as aldehydic degradation products and related carbonyl compounds including malonaldehydes (25).

Some studies measure several oxidation indexes to evaluate the oxidation of edible oils. In a study conducted by Coscione and Artz (26), the thermoxidation of partially hydrogenated soybean oil was investigated by heating oil samples continuously for 72 hours and sampling every 12 hours at 120, 160, 180 and 200 ℃. They examined the acid value, p-anisidine value, color, dielectric constant and the triacylglycerol polymer content

of oil samples. It was demonstrated that the value of each oxidation index increased with an increase in temperature and heating time.

Tyagi and Vasishtha (18) studied the changes in the characteristics and composition of soybean oil during deep-fat frying at 170, 180 and 190 ℃. The refractive index, specific gravity, color, viscosity, saponification value, and free fatty acids of soybean oil increased with frying temperature, whereas the iodine value decreased. Deep-fat frying for 70 hours at 170, 180, or 190 ℃ produced an increase in the conjugated fatty acids from initial values; the higher temperature of frying yielded somewhat higher quantities of conjugated dienes. Trans fatty acids, which were initially absent in both soybean oil samples, were present after 70 h of frying at 170, 180, and 190 ℃. No differences were found between trans isomer content of soybean oils at 170 and 180 ℃; however, at 190 ℃, trans isomers increased in soybean oils.

Aladedunye and Przybylski (27) investigated degradation and nutritional quality changes in regular canola oil heated at 185 ± 5 and 215 ± 5 ℃. Results showed that total polar components (TPC), anisidine value, color and trans fatty acid content increased significantly as a function of frying temperature and time. The extent of oxidative deterioration, as measured by TPC formation, was 2.6 times faster during frying at 215 ℃ compared to 185 ℃. For anisidine value, thermal degradation of the aldehydes occurred at higher temperature, which resulted in a lower accumulation in the oil at the

higher frying temperature. The PUFA in the oil decreased in direct proportion to frying temperature and time. After 7 days of frying at 215 ℃, the amount of PUFA was reduced by half and the trans isomer concentration increased 2.5 times.

Some studies measured compounds that were produced during the oxidation process at various temperatures. Houhoula, Oreopoulou, and Tzia (28) studied the thermoxidative alterations of cottonseed oil during frying in the temperature range between 155 and 195 ℃. They found that the content of polar compounds, conjugated dienes, conjugated trienes, and anisidine value increased linearly with the time of frying at a rate depending on temperature. The FA content, as a function of time during frying at 185 ℃, showed a significant increase in palmitic acid (C16:0) and a significant decrease in linoleic acid (C18:2). Oleic acid (C18:1) also showed a small but significant decrease. The same results were obtained for cottonseed oil heated at 185 ℃. Examination of anisidine value or conjugated dienes with polar compounds showed that both anisidine value and conjugated dienes had a linear relationship with increase of total polar compounds.

Chung, Eiserich, and Shibamoto (29) identified many volatile compounds in headspace samples of peanut oil heated under temperatures ranging from 50 to 200 ℃ using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). 42 Hydrocarbons, 22 aldehydes, 11 fatty acids, 8 alcohols, 8 ketones, 4 furans, 2 esters, and 2 lactones were identified. The total amount of volatile compounds increased

remarkably as the temperature of the oil was increased. Fatty, deep-fried, and rancid aromas were also found to be intensified as the temperature increased in heated peanut oil.

Some studies were conducted to measure the relationship between temperature and oxidation in relation to toxicity. Goicoechea and Guillen (30) investigated sunflower oil oxidation at two different temperatures (70 and 100 ℃) in the presence of air until total polymerization using ^{1}H nuclear magnetic resonance (^{1}H NMR). They monitored several functional groups. Two groups, which were identified as oxidized triacylglycerol structures with epoxy groups and oxygenated alpha, beta-unsaturated aldehydes, were given special attention because of their toxicity. Differences between the oxidation processes at two temperatures were tested. It was observed that the molar percentage of linoleic acyl group chains began to decrease after 120 hours of heating at 70 ℃ with the rate of 0.6% per hour and after 6 hours of heating at 100 ℃ with the rate of 1.6% per hour. In the same study, the evolution of the iodine value showed the same trend. It was also shown that at lower temperature`s, oxygenated alpha, beta-unsaturated aldehydes were generated at a higher rate in the sunflower oil, whereas toxic monoepoxy unsaturated acyl compounds were generated at a higher rate at higher temperatures in the sunflower oil degradation.

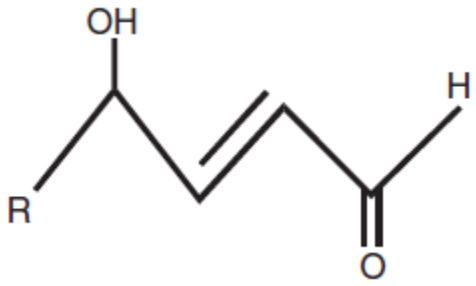
Guillén and Ruiz (24) observed that there were great differences between the nature and proportions of the various aldehydes generated at 70 and 190 ℃ in various oils. At 70 ℃, the formation of 4-hydroperoxy-trans-2-alkenals and of 4-hydroxytrans-2-alkenals was noteworthy, while the first were not detected in oils submitted to 190 ℃ and the second ones were detected in much smaller proportions. It showed that the reactions in which oxygen was involved were favored at intermediate temperatures whereas the formation of 2,4-alkadienals was favored at high temperatures. In addition, they found that oils rich in oleic acyl groups were more resistant to degradation and produced lower proportions of toxic aldehydes than oils rich in linoleic and linolenic acyl groups.

Some studies suggest that frying fats should not be heated to 180 ℃ (31). Fats and oils heated below 180 ℃ can reduce the formation of highly harmful cyclic fatty acids monomers, decomposition products, and polymers. Higher temperatures could accelerate the oxidative and thermal alterations, especially over 200 ℃ (32).

There are a lot of studies on the oxidation of oils, however they usually measure or monitor a group of compounds, therefore more detailed and specific compounds that are of interest need to be researched under various conditions.

The formation of 4-hydroxy-2-nonenal (HNE)

Among secondary oxidation products, aldehydes are a major class of compounds. These aldehydes include n-alkanals, 2-alkenals, 2,4-alkadienals, 4-hydroxyalkenals and malonaldehyde. Oils which are rich in PUFAS will generate high levels of aldehydes and ketones, whereas oils which are low in PUFAS will form low concentrations of selected aldehydes (33). Among the aldehydes, one important group is identified as α, β-unsaturated-4-hydroxyaldehydes. α, β-Unsaturated-4-hydroxyaldehydes includes 4-hydroxy-2-trans-hexenal (HHE), 4-hydroxy-2-trans-octenal (HOE), 4-hydroxy-2-trans-nonenal (HNE) and 4-hydroxy-2-trans-decenal (HDE). Figure 6 shows the general structures of these compounds. 4-Hydroxyalkenals include a carbonyl group, a trans double bond in position two and a hydroxyl group in position four. Only the chain length is different between the above four compounds. It has been found that linoleic acid is a precursor for HNE formation. HHE is from linoleic and linolenic acids with concentration higher in linolenic acid than in linoleic acid. HOE also comes from both linoleic and linolenic acids (34).



R = C_2H_5: 4-hydroxy-2-hexenal, HHE

R = C_4H_9: 4-hydroxy-2-octenal, HOE

R = C_5H_{11}: 4-hydroxy-2-nonenal, HNE

R = C_6H_{13}: 4-hydroxy-2-decenal, HNE

Figure 6. Chemical structures of the four α, β-unsaturated-4-hydroxyaldehydes

Among these aldehydes, HNE is the most important because it has been found to be the most toxic compound and it is abundant in high PUFA oils after heat treatment. In 1993, it was suggested by Grein et al. (35) that HNE was originating from linoleic acid through the formation and decomposition of 2,4-decadienal. Han and Csallany in this laboratory did not find decadienal to be a precursor for HNE (34). The presently acknowledged pathways for the formation of HNE were published by Schneider et al. (36). In their study, 9- and 13-hydroperoxides of linoleic acid were used as starting materials. Figure 7 shows the formation of 9- and 13-hydroperoxides from linoleic acid. Two different pathways lead to the formation of 4-hydroperoxy-2E-nonenal (4-HPNE)

from the hydroperoxides of linoleic acid (Figure 8). One pathway is C-8 of 13S-hydroperoxyoctadecenoic acid (HPODE) attracts a hydrogen and then forms 10,13-dihydroperoxide. The cleavage between C-9 and C-10 of 10, 13-dihydroperoxide yields 4S-HPNE. The other pathway is 9S-HPODE cleaves directly to form 3Z-nonenal. 3Z-nonenal can be converted to 4-HPNE by 3Z-alkenal oxygenase. Then 4-HPNE can be subsequently converted to HNE (37).

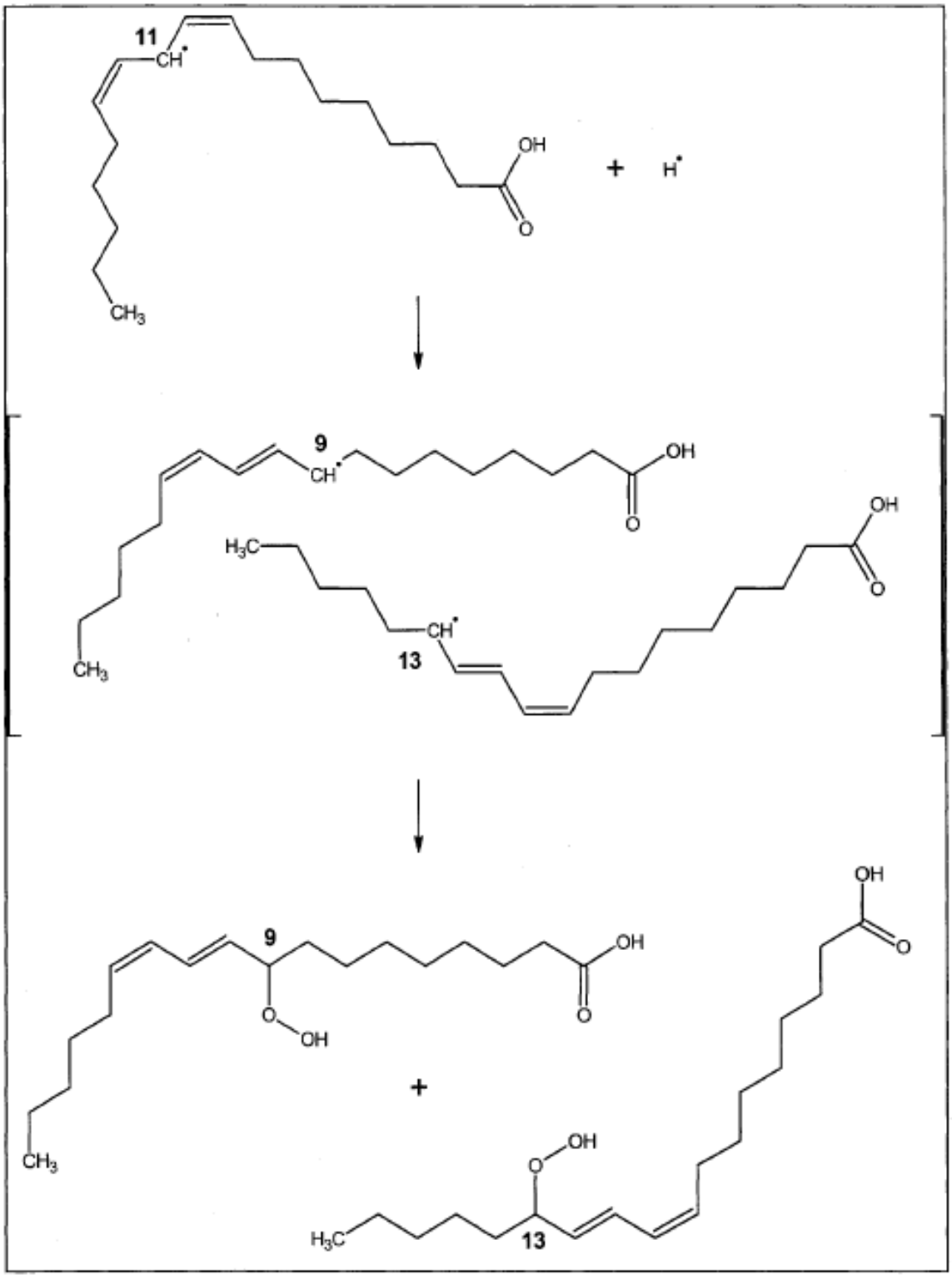


Figure 7. Formation of 9- and 13- hydroperoxides from linoleic acid

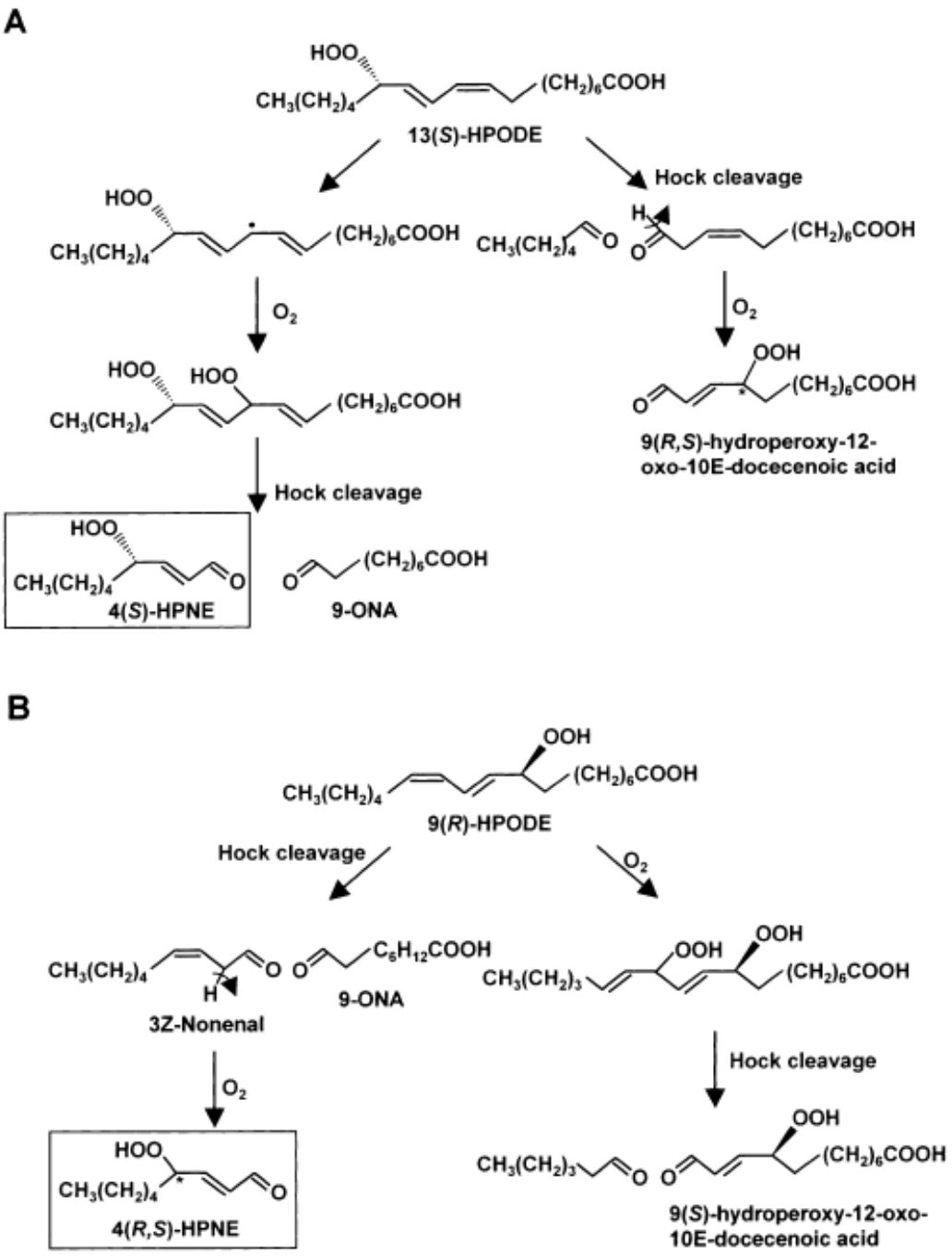


Figure 8. Mechanism of formation of HNE from 13(S)-HPODE (A) and 9(R)-HPODE (B)

Health aspects of thermoxidized oils and fats and recent studies on HNE in food systems

When oils and fats are subjected to high temperatures, their physical and chemical characteristics can be changed by oxidation (18, 38). The oxidation reaction can produce undesirable flavors and odors which lower the quality of food. Furthermore, it can result in the loss of nutrients, even forming toxic compounds which affect human health (39). Liposoluble vitamins, essential fatty acids and proteins decompose during the thermal oxidation process (40). Animal and biochemical studies show that lipid oxidation products represent health risks. Though the acute toxicity is very low, chronic uptake of large amounts of oxidized lipids have shown to give rise to a higher incidence of tumor formation and atherosclerosis in animals (41).

Secondary oxidation products like aldehydes, ketones, hydroxyketones, epoxides and dicarboxylic compounds can react with amino acids and proteins (42). These reactions produce acrylamide at a heating temperature of above 100˚C and increase with increasing temperature (43). Acrylamide is identified as a probable cause for the formation of carcinogens in human body (44).

It is postulated worldwide that PUFAS have potential health benefits and people are advised to substitute PUFAS for saturated fatty acids. There are a number of studies show that PUFAS, mainly ω6 and ω3 PUFAS, can reduce the risk of coronary heart disease

(CHD), and have beneficial effects in patients with diseases such as dyslipidemia, atherosclerosis, hypertension, diabetes mellitus, metabolic syndrome, obesity, and inflammatory diseases (45-47). However, some researchers have found that PUFAS may be a potential hazard to human health. Grootveld, Silwood and Claxson warned that when subjected to frying temperature, culinary oils rich in PUFAS could produce detectable toxic aldehydic components. Using NMR, they have shown that trans-2-nonenal and trans-2-pentenal could be absorbed from the gut in vivo, metabolized and excreted in the urine (48). Kim, Gallaher and Csallany also found that lipophilic aldehydes and other carbonyl products of lipid peroxidation were detected in rat and human urine, indicating similarity between the lipophilic product of lipid oxidation formed *in vivo* and *in vitro* (49). These findings go against the worldwide dietary advice to substitute polyunsaturated fats including the most abundant PUFA ω6 linoleic acid for saturated fats (50).

It has been found that aldehydes can diffuse and move in or between cells to react with biomolecules such as DNA and proteins, disrupting cell functions, gene expression, and even leading to cell death (51). Among the aldehydes, 4-hydroxyalkenals seem to be the most reactive and have caused great concern about their effect on health. Their three functional groups—a carbonyl group, a trans double bond and a hydroxy group—are the causes of their high reactivity and toxicity (52). The aldehydic carbonyl group is able to

react with primary amine groups in amino acids, phospholipids and proteins to produce Schiff bases. This reaction can modify the functional groups of these molecules in biological systems (51, 53). The aldehydic group can also react with NH_2 groups so 2,4-dinitrophenyl hydrazine (NH_2-NH-(C_6H_3)-(NO_2)$_2$ forms a hydrazine which can be used to obtain more stable derivatives for analytical purposes (25). Another functional group of 4-hydroxyalkenals, the conjugated double bond, can react with nucleophilic compounds containing thiol, amino, or imidazole groups by Michael addition. This reaction can occur in both food and biological system (54-56). The third functional group hydroxy group can be transformed to oxo group (57).

Because of their high reactivity, many studies of 4-hydroxyalkenals were made in biological systems and it has been shown that they are cytotoxic and mutagenic (58, 59). HNE, one kind of 4-hydroxyalkenals, is known to be the most abundant and major toxic compound produced during the oxidation of ω6 PUFAS, such as linoleic acid and arachidonic acid. HNE can covalently attach to proteins through the Michael addition and form stable adducts with cysteine, lysine and histidine amino acid residues (Figure 9). Therefore the structure of protein is altered, resulting in the loss of protein function and activity (2). In addition, HNE can modulate the expression of several genes and inhibit growth (6, 7). An increasing body of evidence shows that HNE is related to atherosclerosis, Alzheimer's disease, Parkinson's disease, Huntington disease and some

other cancers (1, 3, 4). In the concentration range of 1 to 20μM, HNE can inhibit DNA and protein synthesis. Even at a very low concentration (less than 0.1μM), HNE can affect cellular metabolism (25). Nowadays HNE is also used as a biomarker for pathophysiological processes (60).

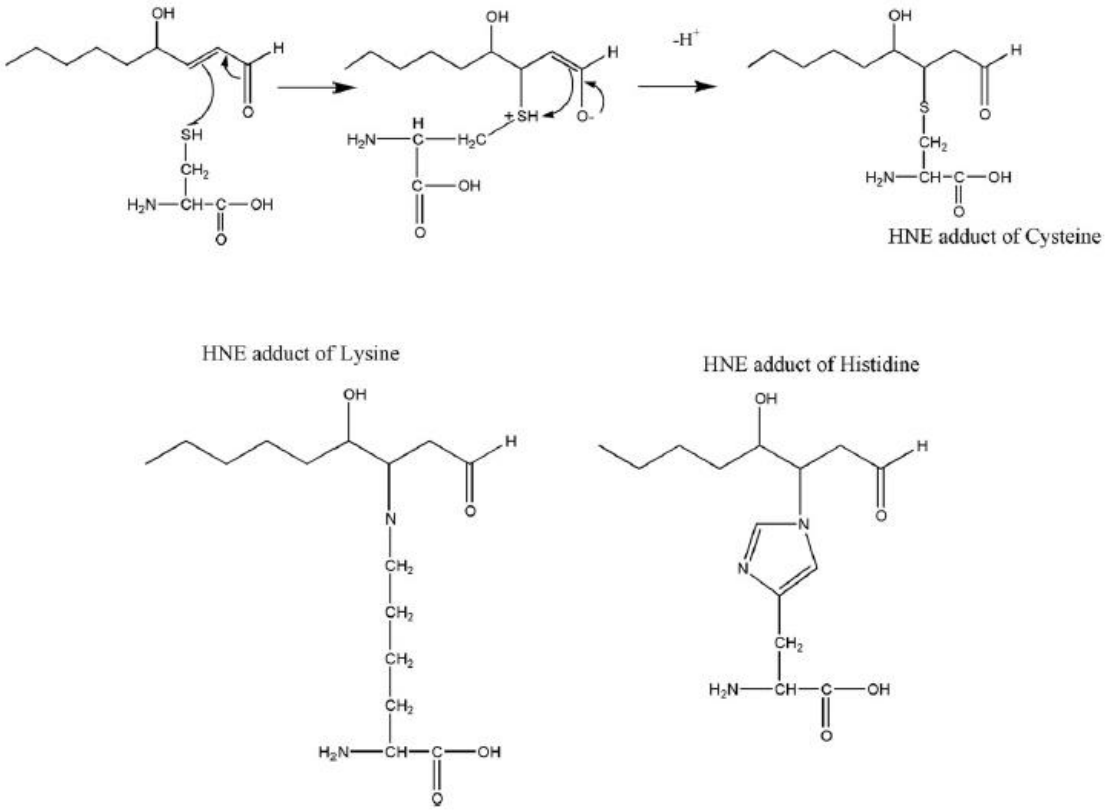


Figure 9. Michael addition reaction and HNE adduct products of cysteine, lysine, and histidine. (From Ref. (2))

Because of the increasing concern of the risk of HNE to human health, more and more studies were being conducted to investigate HNE formation in oils after heat treatment. The formation of HNE was monitored in soybean oil which was heated at

frying temperature185 ℃ for 2, 4, 6, 8 and 10 hours in a recent study by Seppanen and Csallany (61). Results showed that unheated soybean oil contained no HNE and a very low concentration of polar lipophilic secondary oxidation products. A great increase in the concentration of both HNE and the total lipophilic polar oxidation products was observed with increased heating time at frying temperature. A considerable concentration of HNE had already formed at 2 hours and the concentration continued to increase at 4 and 6 hours of heating time in soybean oil which contains a high level of linoleic acid (45-52%). After 6 hours, the concentration of HNE started to decrease due to possible thermal decomposition. In addition, it was found that the tocopherol concentration decreased as the lipid peroxidation and the secondary oxidation products increased.

The above mentioned authors also found that HNE could be incorporated into fried food from the frying oil which had been heated at 185 ℃ for 5 hours (8). Similar concentrations of HNE were found in the oil prior to and after frying and in the oil extracted from the fried potato. This showed that HNE was readily incorporated into food fried from the thermally oxidized oil.

By the same authors, the temperature dependence of the formation of HNE was demonstrated in high linoleic acid containing oils such as corn and soybean oils and low linoleic acid containing butter oil (62). The oils were heated at 190 ℃ and 218 ℃. The concentration of HNE at 218 ℃ increased greatly for all the three oils compared to the

lower temperature of heating 190 ℃ for the same heating period. In comparison, HNE concentration at higher temperature (218 ℃) was 4.9, 3.7, and 8.7 times higher than at the lower temperature (190 ℃) for corn, soybean and butter oils after only 30 min of heat treatment, respectively. This proved that HNE formation was temperature dependent in the tested oils.

The effect of intermittent heating on the formation of HNE in soybean oil was compared with continuous heating (11). Soybean oil samples were heated either for 1 h each day for five sequential days or for 5 h continuously at 185±5 ℃. The thermally oxidized samples were analyzed for the presence of HNE, HHE, HOE and HDE. It was found that the concentration of these four α, β-unsaturated-4-hydroxyaldehydes increased similarly under both intermittent and continuous heating conditions over a total of 5 h. These results demonstrated that the formation of HNE and other α, β-unsaturated-4-hydroxyaldehydes at frying temperature is a cumulative result of the peroxidation process of PUFA over time.

The formation of α,β-unsaturated-4-hydroxyaldyhydes was investigated in fatty acid methyl ester format (34). Fatty acid methyl esters (FAMEs) of stearic, oleic, linoleic and linolenic acids were heated separately at 185 ℃ for 0 to 6 hours. As a result, methyl stearate (MS) and methyl oleate (MO) did not produce any of the α,β-unsaturated-4-hydroxyaldehydes after thermally induced lipid peroxidation. The formation of HHE

was detected in both methyl linoleate (ML) and methyl linolenate (MLN), with concentration higher in MLN than in ML. HOE was detected in both ML and MLN, too. HNE was found only in ML and HDE was not detected in any of the four heat treated FAMEs.

Because of the incorporation of HNE into fried food and its toxicity, studies were carried out on the prevention of its formation. Gerde and Hammond treated soybean oils with polydimethylsiloxane (PDMS) heated at 180 ℃ for 48 h. They found that when the amount of PDMS was greater than that necessary to form a monolayer in the air-oil interface, PDMS had a positive effect in preventing the formation of HNE (63).

Though these above studies were carried out on HNE formation and prevention, there is almost no information on the effect of temperature, heating time and linoleic acid concentration on HNE formation in various commercial edible oils and fats.

Objectives

The objective of the present experiments was to measure the formation of α, β-unsaturated-4-hydroxyaldehydes (4-hydroxy-2-trans-nonenal (HNE), 4-hydroxy-2-trans-hexenal (HHE), 4-hydroxy-2-trans-octenal (HOE) and 4-hydroxy-2-trans-decenal (HDE)) resulting from various heating time, temperatures and the levels of unsaturation in oil/fat sample.

Part II: Experiments

Methods and Materials

Chemicals and Instruments

2, 4-Dinitrophenylhydazine was purchased from Eastman Kodak Co.(Rochester, NY). 2-thiobarbituric acid, thichloroacetic acid, HPLC-grade methanol, HPLC-grade water, HPLC-grade dichloromethane, and boron trifluoride-methanol solution were obtained from Sigma Chemical Company (St. Louis, MO); sodium thiosulfate and glacial acetic acid were from Fisher Scientific (Fair Lawn, NJ); hydrochloric acid and potassium iodide were from Mallinckrodt Baker Inc. (Paris, KY). HPLC-grade hexane was from EMD Chemicals, Inc. (Gibbstown, NJ). HNE was from Cayman Chemical Co. (Ann Arbor, MI). No. 1 filter paper and 0.45um syringe filters were purchased from Whatman Ltd. (Kent, England). Plates for thin layer chromatography (TLC) were purchased from EMD Millipore, Inc. (Billerica, MA).

Corn oil, soybean oil, canola oil (Crisco®, the J.M. Smucker Company, Orrville, OH) and peanut oil (Planters®, Kraft Foods Global, Inc. East Hanover, NJ) were purchased from retail stores (Roseville, MN). Lard and beef tallow were obtained from meat laboratory of the University of Minnesota.

The gas chromatograph used a 18835B capillary inlet system (5830A Gas Chromatograph, Hewlett-Packard, Saginaw, MI).

The HPLC system consisted of a sample injector (712 WISP, Waters, Milford, MA), a solvent delivery system (9050, Varian, Walnut Greek, CA) and a UV-Vis detector (9010, Varian). The HPLC column was Ultrasphere ODS (5×4.6 mm, 25 cm, Hichrom, Berkshire, UK). Detailed operating parameters are provided later in the methods section (G).

Thermal Treatments of the Oil Samples

Duplicate 5g ±0.05g oil or fat samples were placed in open test tubes (16×150mm) in a sand bath and continuously heated at 145, 165 and 185 ℃ for 1, 2, 3, 4, 5, and 6 hours. Target temperatures were reached in 30 minutes.

Peroxide Value

Peroxide value determination of the unheated oil samples was done following the method of the American Oil Chemists' Society (64). One g sample of unheated oils (in duplicate) were placed into Erlenmeyer flasks and dissolved with 15 mL of a solution containing 6 parts glacial acetic acid and 4 parts USP chloroform. One mL saturated aqueous potassium iodide solution was added and mixed by swirling for 1 minute. After 5 minutes, 15 mL distilled water was added and mixed as before. The mixture was titrated with 0.002N sodium thiosulfate until yellow color became faint. Several drops of starch solution were added and the titration was ended up with a clear endpoint.

The peroxide value was calculated by (volume of sodium thiosulfate × normality of sodium thiosulfate ×1000) / weight of fat (g) and expressed as milliequivalents of peroxide per 1000g of sample.

Fatty Acid Distribution by Gas Chromatography

Fatty acid distribution of unheated oils and fats was determined by gas chromatography (65). Three mL of BF_3-Methanol (14% BF_3 in methanol) was added to 2 drops of each unheated oils or fats in a 20mL test tube in duplicate. The test tubes were capped and shaken vigorously. Then they were placed in boiling water bath for 1 h. After cooling, 3 mL distilled water and 10mL hexane were added and the mixture was shaken for 10 minutes. After separation into 2 layers, the top hexane layer was removed and saved. The hexane samples were dried with the addition of 1-2 grams of sodium sulfate. Five μL of the dry hexane samples were injected into GC and the fatty acid distribution was measured by comparison with the retention times of fatty acid standards.

Thiobarbituric Acid (TBA) Test

In order to determine the rate and the extent of oxidation under different temperature and time conditions, the thiobarbituric acid test was used to measure the formation of TBARS in the samples. Duplicate samples of 5g (±0.05g) corn, soybean, canola, peanut oils, lard and beef tallow were placed in open test tubes (16×150mm) inserted into a sand bath and heated continuously at 145 ℃ or 165 ℃ or 185 ℃ for 1, 2, 3, 4, 5, and 6 hours.

The TBARS values of each sample were measured under the above mentioned time and temperature conditions.

TBA test is one of the oldest, most commonly used methods for determining lipid oxidation. It is based on the color reaction between TBA and secondary oxidation products such as aldehydes and ketones. TBA test measures all aldehydes and related carbonyl compounds including malonaldehyde (MDA), exhibiting colored complex absorption at 530-532nm. MDA is used to develop a standard curve and the test results were expressed as MDA equivalents (66, 67).

The thiobarbituric acid reactive substances (TBARS) were measured by the TBA test. The TBA test was done by the method of Buege and Aust (68). The standard curve was determined by combining 200 μL of malondialdehyde (MDA)/water with 4 mL of TCA/TBA/HCl solution (equal volumes of 15% w/v thichloroacetic acid (TCA), 0.375% w/v 2-thiobarbituric acid (TBA), and 0.25N HCl). MDA concentration was made by the dilution with distilled water as listed in Table **1** below. The mixture of MDA and TCA/TBA/HCl solution was heated for 15 minute in a boiling water bath. Absorbance of the sample was measured at 535 nm with a UV/Vis spectrophotometer (Spectronic 20, Bausch&Lomb). The standard curve is shown in Figure 10.

Table 1. The Concentration of MDA for TBARS standard curve

Tube Number	MDA(5×10^{-5}M) (μL)	Water (μL)	Concentration of MDA (μg/mL)
1	0	200	0
2	10	190	0.0086
3	25	175	0.0214
4	50	150	0.0429
5	100	100	0.086
6	150	50	0.129
7	200	0	0.171

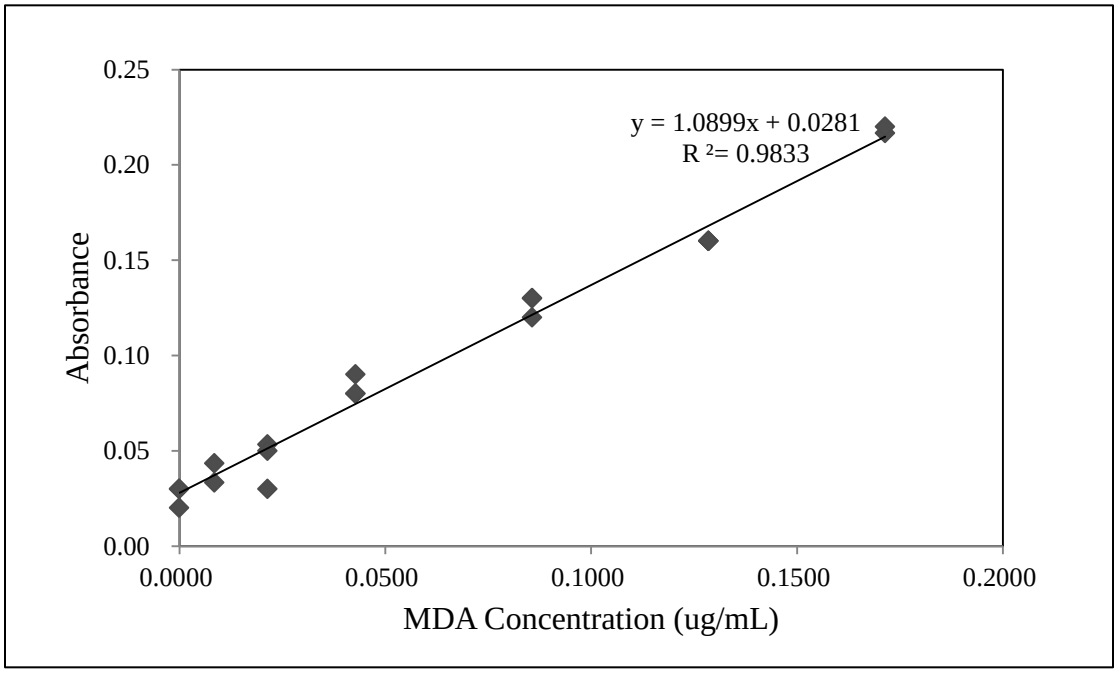


Figure 10. TBA assay standard curve

Measurement of the samples: 200 μL of oil was combined with 4 mL of TCA/TBA/HCl solution (equal volumes of 15% w/v thichloroacetic acid (TCA), 0.375% w/v 2-thiobarbituric acid (TBA), and 0.25N HCl). The mixture was heated for 15 minute

in a boiling water bath. Absorbance of the sample was measured at 535 nm with a UV/Vis spectrophotometer.

Method to Determine the Secondary Oxidation Products of Samples

The method was developed by Seppanen and Csallany (10). The principle of the method was that hydroxyalkenals can react easily with 2, 4-dinitrophenylhydrazine (DNPH) to form hydroxyalkenal-DNPH derivatives through Schiff base formation; the hydroxyalkenal-DNPH derivatives which actually are 2, 4-dinitrophenylhydrazones can be measured by ultraviolet (UV) light at 378nm. DNPH is a large compound and has high molar extinction coefficient at the given wavelength. Due to the formation of hydroxyalkenal-DNPH derivatives, hydroxyalkenals can be easily detected and measured.

A) Recrystallization of DNPH

One g DNPH was dissolved in 20 mL methanol and heated at 60 ℃ for 30 minutes. The dissolved DNPH was placed in an ice bath for at least 18h for crystallization. The crystallized DHPN was filtered by No. 1 filter paper and redissolved in about 20 mL methanol. The crystallization process was repeated at least two more times and the collected DNPH crystals were placed in a desiccator for 3 days for drying.

B) Preparation of DNPH Reagent

DNPH reagent was prepared freshly for each assay. Recrystallized DNPH (10 mg) was dissolved in 20mL 1 N hydrochloric acid and heated at 50 ℃ for 1 h. After cooling, the DNPH reagent was washed four times with 10 ml of HPLC-grade hexane (total 40mL) to remove impurities and the hexane layer was discarded.

C) Preparation of the DNPH reagent blank and the acetone-DNPH standard

The DNPH reagent blank and the acetone-DNPH standard were made by combining 3mL HPLC-grade water for the reagent blank or 3mL 1% acetone for the acetone-DNPH standard with 3mL freshly prepared DNPH reagent. The mixture was incubated at the speed of 120 oscillations/min shaking at room temperature overnight. After incubation, the DNPH derivatives were extracted three times with 5mL dichloromethane. The solvent was evaporated from the combined extracts under N_2 gas to a volume of 0.5mL.

D) Preparation of HNE-DNPH standard

One hundred μL (5mg/500μL of ethanol) HNE was added to 10mL freshly made DNPH reagent, the mixture was incubated at room temperature overnight in the dark with shaking at the speed of 120 oscillations/min. HNE-DNPH was extracted with 10mL dichloromethane three times. The combined dichloromethane extract was evaporated under N_2 gas to about 1.5mL. All of the concentrated HNE-DNPH in dichloromethane was applied to two Thin Layer Chromatography (TLC) plates. The polar region was

extracted from the TLC plates three times with 10mL methanol. The combined methanol extract was evaporated under N_2 gas to an exact volume of 10mL.

E) Preparation of DNPH-hydrazones of lipophilic aldehydes and related carbonyl compounds from oils and fats

One g of unheated or heat-treated oil sample (in duplicate) was reacted with 5mL of freshly prepared DNPH reagent same as above in a 25mL Erlenmeyer flask. The mixture was incubated at room temperature overnight in the dark with shaking at the speed of 120 oscillations/min.

The DNPH derivatives were extracted three times with 10mL methanol/water (75:25, vol/vol) and separated by centrifugation at 2000 rpm for 10 min. The DNPH derivatives in the combined methanol extracts were further extracted three times with 10mL dichloromethane and separated by centrifugation as before. The combined dichloromethane layer was evaporated under N_2 gas until the sample volume was about 1mL.

F) Preliminary separation of DNPH-hydrazones of lipophilic aldehydes and related carbonyl compound by Thin Layer Chromatography (TLC)

The concentrated dichloromethane extract (about 1mL) was applied to two silica gel thin-layer chromatographic plates. The sample was applied in a very thin line across the

plate with a 250μL micropipette attached to a Hamilton syringe with a piece of flexible rubber tubing. Half of the sample (about 0.5mL) was applied to each plate, then about 0.2mL dichloromethane was used to rinse the test tube and this was also applied to the plate. The DNPH reagent blank and acetone-DNPH standard were spotted next to the sample on the plate and they would help identify the position of the polar and nonpolar aldehyde-DNPH in samples. The plates were developed in HPLC-grade dichloromethane.

Figure 11 shows a diagram of a typical TLC plate. The nonpolar and polar aldehydes and other related carbonyl compounds were separated by the location of DNPH reagent and acetone-DNPH standards. Polar carbonyl compounds (PC), including the hydroxyaldehydes, were located between the origin and the DNPH reagent band. Nonpolar carbonyl compounds (NPC), such as alkanals, alkenals, alkadienals and ketones were located between the acetone-DNPH band and the solvent front. The polar and nonpolar regions were cut from the plate into small pieces and placed in 25 mL Erlenmeyer flasks. The compounds were extracted from the plate three times with 10mL methanol. The combined methanol extracts were evaporated under N_2 gas to the exact volume of 1mL in a volumetric flask and stored in amber vials tightly covered with Parafilm at -20 ℃ until HPLC analysis.

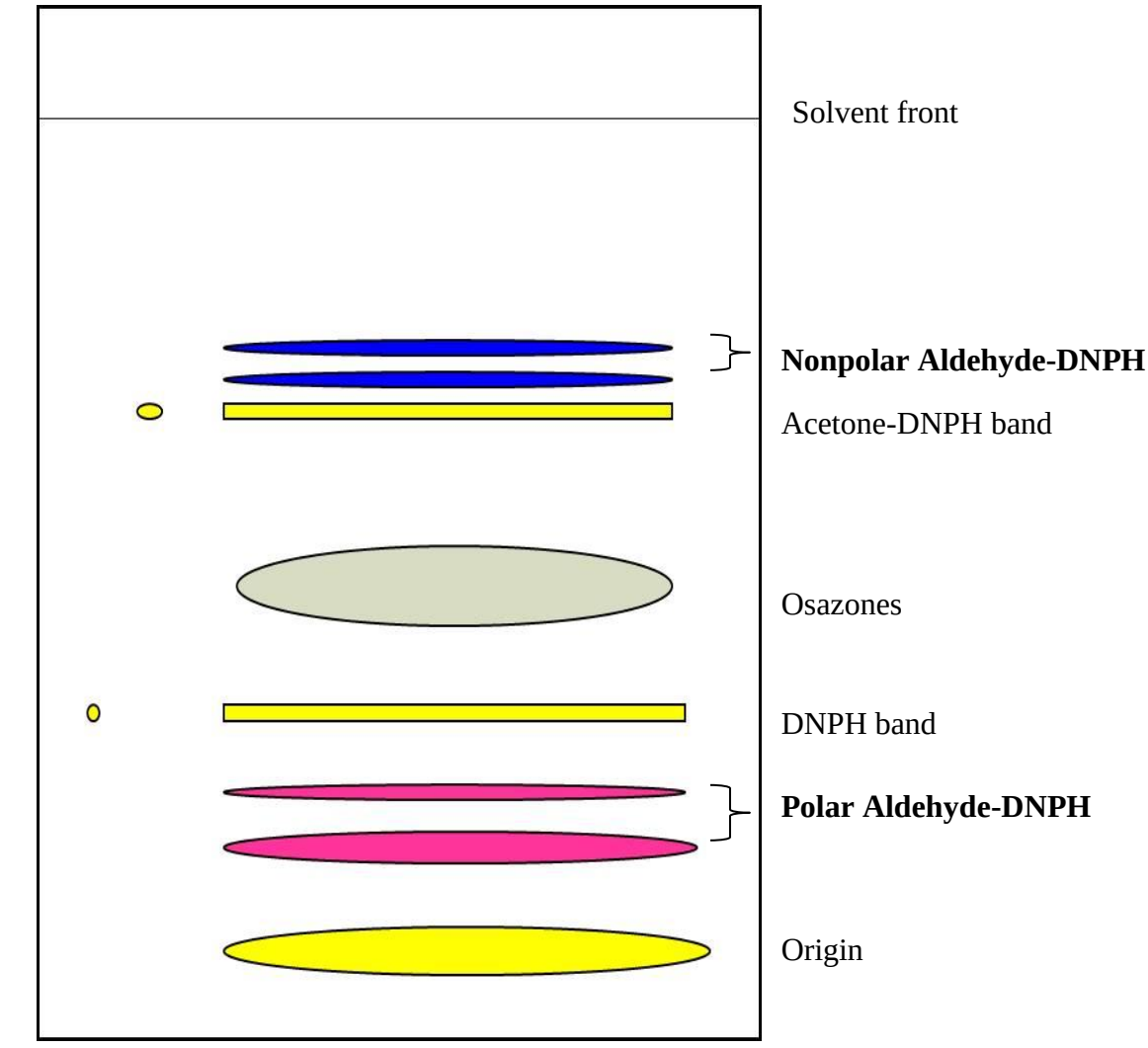


Figure 11. Position of polar and nonpolar aldehydes-DNPH derivatives after TLC Development. Plate was developed in HPLC-grade dichloromethane.

G) Separation and identification of DNPH derivatives of polar (PC) and nonpolar (NPC) lipophilic aldehydes and related carbonyl compounds from oils and fats by HPLC

One hundred μL aliquots of PC-DNPH derivatives and NPC-DNPH derivatives were injected into an HPLC reverse-phase C18 column, equiped with a guard column.

a) For polar compounds (PC), 10 min of isocratic elution with methanol/water (50:50, vol/vol) was followed by a linear gradient to 100% methanol for 20 min, then 100% methanol for an additional 10 min at a flow rate of 0.8mL/min. Absobance was measured at 378 nm.

b) For nonpolar compounds (NPC), an initial isocratic elution was methanol/water (75:25, vol/vol) for 10 min and was followed by a linear gradient to 100% methanol for 20 min, then 100% methaol for an additional 10 min at a flow rate of 0.8mL/min. Absorbance was also measured at 378 nm.

Each day, prior to injection of samples, a mixture of known standards or a single standard was injected into the HPLC column and eluted with the solvent system to be used that day in order to check the reproducibility of the HPLC system. Figure 12 shows a typical separation and the retention times of the pure standards in the PC solvent system.

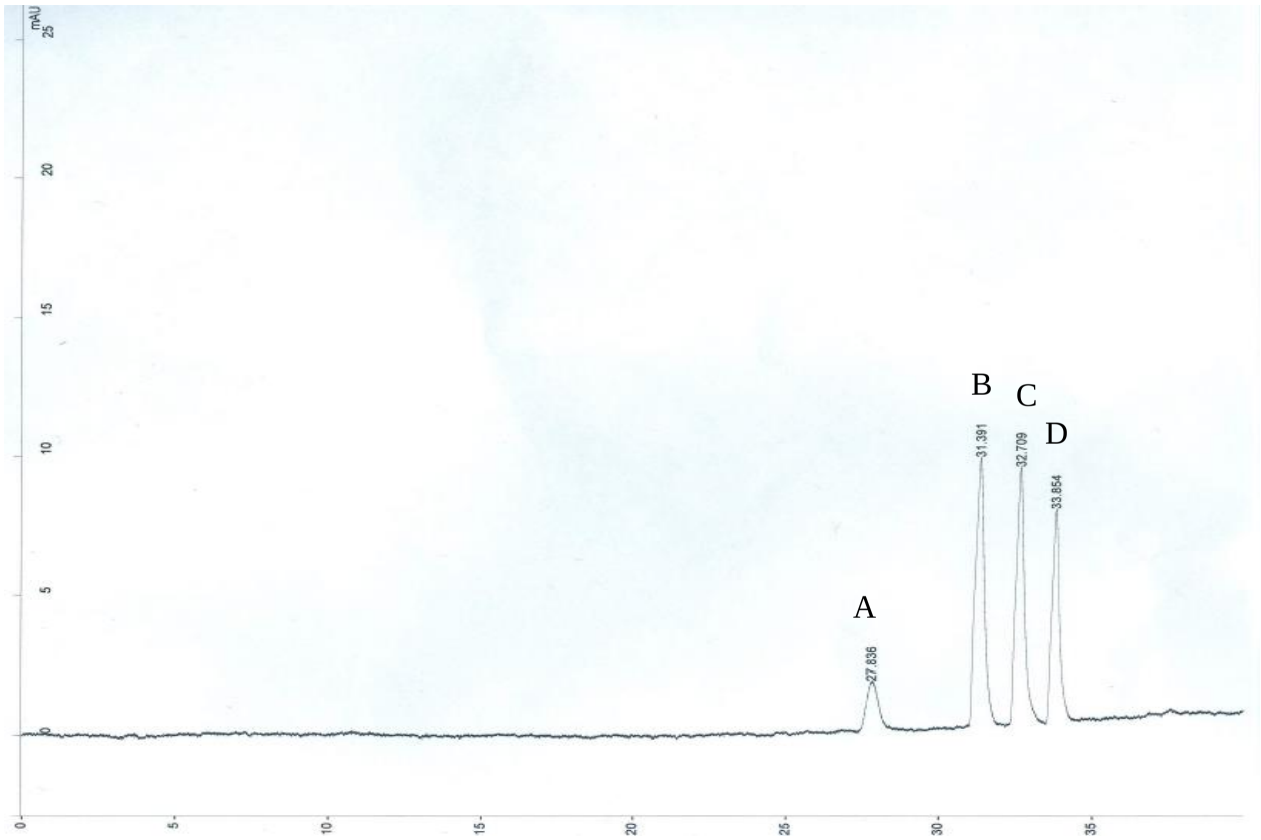


Figure 12. HPLC separation of a mixture of standards. DNPH derivatives of A: HHE, B: HOE, C: HNE and D: HDE.

Identification of the individual polar and nonpolar aldehydes and related carbonyl compounds from oils are accomplished by:

a) Comparing the retention times of known standards to the retention times of peaks derived from the oil or fat samples.

b) Co-chromatography of sample mixed with a small amount of pure standards. The chromatogram was then examined for 1) single retention time of sample and pure standard 2) the ratio of increased peak area to the added pure standard compared to the original area of the sample.

Aldehydes were quantified by comparing the peak area of aldehyde- DNPH with the peak area of hexanal-DNPH standard and expressed as g hexanal equivalent/g oil. For HHE, HOE, HNE and HDE, hexanal equivalent was converted to ng hydroxyaldehyde (HHE/ HOE/ HNE/ HDE)/g oil by calculation based on the molecular weights of hexanal and hydroxyaldehyde (HHE/ HOE/ HNE/ HDE). For total polar and nonpolar lipophilic aldehydes, since the individual aldehyde molecular weights are unknown, the results were represented as μg hexanal equivalent/g oil.

Statistical Analysis

ANOVA was used to determine if there were significant differences between the groups. A Tukey test was conducted to calculate the p values. Statistically significant differences were determined at $p \leq 0.05$.

Results

A. Preliminary Experiments

a) Peroxide Value

Before heat treatment, the initial oxidation concentrations of all samples were measured by peroxide value. (69). **Table 2** shows the peroxide values of commercial corn, soybean, canola, peanut oils, lard and beef tallow measured in duplicate per sample. The peroxide values were in the range between 1.26-3.56 milliequivalents of peroxide per 1000g. These values are relatively low which indicate that all samples were minimally oxidized at the start of the thermal oxidation process.

Table 2. Peroxide value of unheated oils and fats

Oil or fat	Peroxide value (milliequivalents of peroxide per 1000g)
Corn	3.56 ±0.08
Soybean	2.94 ±0.03
Canola	1.26 ±0.15
Peanut	2.48 ±0.09
Lard	3.66 ±0.09
Beef tallow	2.98 ±0.39

b) Fatty Acid Distribution

The fatty acid distributions of commercial corn, soybean, peanut, canola oils, lard and beef tallow were measured to determine the concentrations of linoleic and linolenic

acids in the samples since these fatty acids are precursors for the formation of α, β-unsaturated-4-hydroxyaldehydes (34).

Table 3 shows the retention times of pure palmitic, stearic, oleic, linoleic, linolenic and arachidic acids using a GC method as described in the method section.

Table 3. Retention times of pure fatty acids using GC

Fatty acid	Retention time (min)
Palmitic acid	1.11
Stearic acid	1.69
Oleic acid	1.82
Linoleic acid	2.05
Linolenic acid	2.49
Arachidic acid	2.77

By comparing the retention times of fatty acids between samples and the pure standards, the fatty acid distributions of corn, soybean, peanut, canola oils, lard and beef tallow in this experiment are shown in **Table 4**.

Table 4. Percent fatty acid distribution for unheated commercial corn, soybean, peanut, canola oils, lard and beef tallow (Unit: %)

	Corn	Soybean	Peanut	Canola	Lard	Beef tallow
Palmitic acid	18.73	16.62	10.43	4.55	32.37	27.96
Stearic acid	2.69	7.15	2.47	1.61	24.44	27.07
Oleic acid	33.10	28.07	57.48	63.71	34.71	39.85

Linoleic acid	44.19	42.97	26.97	20.04	8.27	1.91
Linolenic acid	0.71	4.56	0.17	9.40	-	-
Arachidic acid	0.58	0.63	1.50	0.55	-	-

It can be seen in **Table 4** that the linoleic acid in corn oil had the highest concentration in unheated oil followed by soybean oil, peanut oil, canola oil, lard and beef tallow. It should be noticed that canola oil had a highest level of linolenic acid concentration followed by soybean oil. However, both corn and peanut oils had less than 1% linolenic acid. And in lard and beef tallow linolenic acid was not determined.

c) Thiobarbituric Acid Reactive Substances (TBARS)

Figure 13 and Figure 14 show the results of TBARS formation of the 6 oils: corn, soybean, peanut, canola oils, lard and beef tallow at 3 different temperatures and various times including 0, 1, 2, 3, 4, 5 and 6 hours of thermal treatments.

From the results, it can be seen that the TBARS measured as the MDA equivalents increased with higher temperatures and longer heating time for all the oils. The maximum TBARS values were reached at 185 ℃ for 5 or 6 hours for all the oils and fats. Among all the oils, the oxidation of canola and soybean oils were extremely high compared to corn oil, peanut oil, lard and beef tallow. Corn oil, peanut oil, lard and beef tallow had relatively predictable increases with the increased heating time and temperatures. Beef tallow, as predicted, did not change too much during the heat treatments over 6 hours in all 3 temperatures. Canola oil showed the highest TBAR values, which was unexpected

since canola oil is high in oleic acid and relatively low in linoleic acid (20%). The reason may be that canola oil has a higher concentration of linolenic acid (9.4%) which has three double carbon bonds and is more susceptible to oxidation than the other oils. This explanation was also applicable to soybean oil. Following canola oil, soybean oil was the second one in terms of MDA equivalents, and then followed by corn oil, peanut oil, lard and beef tallow. This ranking was consistent with the concentration of linoleic acid.

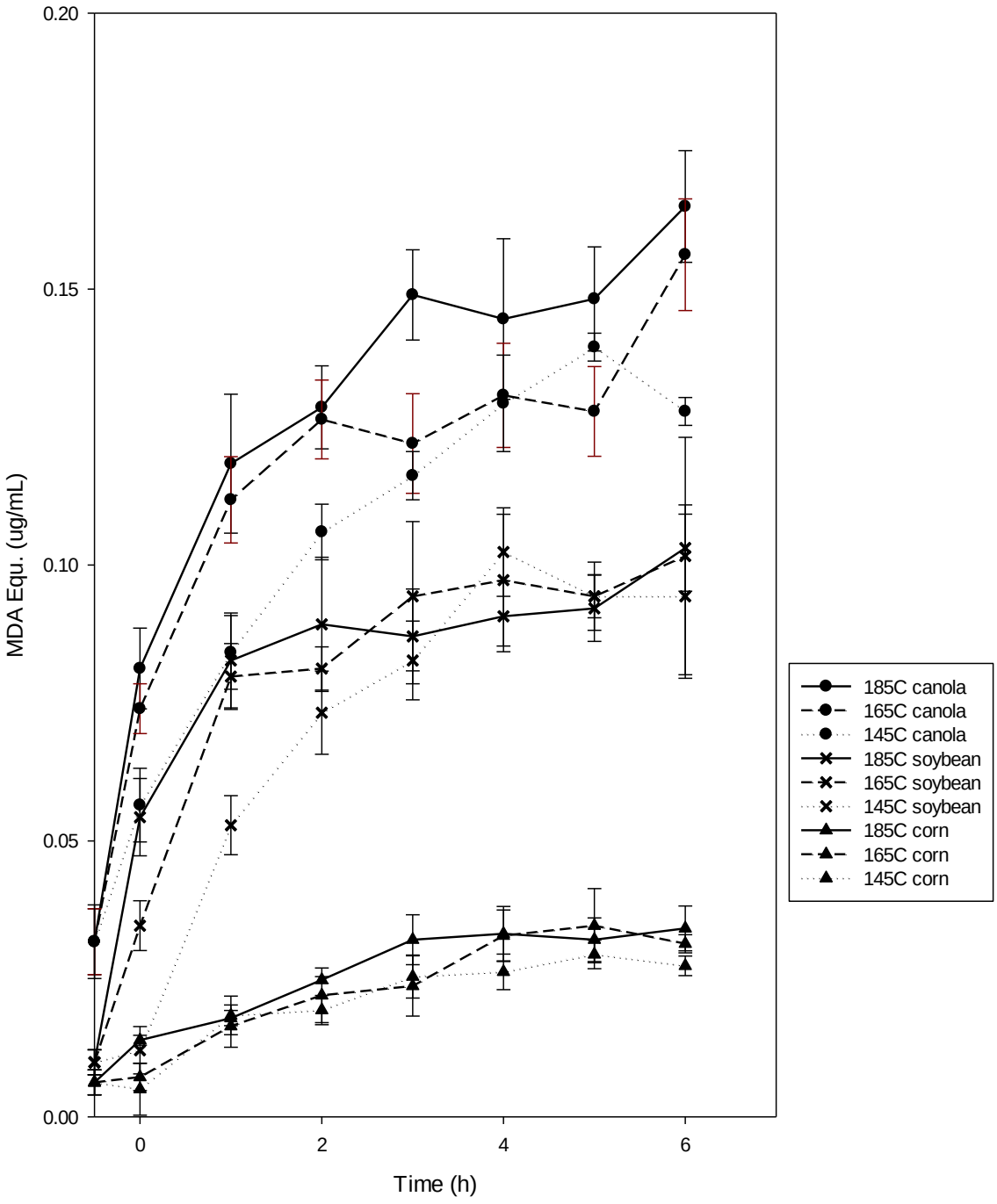


Figure 13. TBARS formation at various temperatures and heating time for commercial corn, soybean, and canola oils

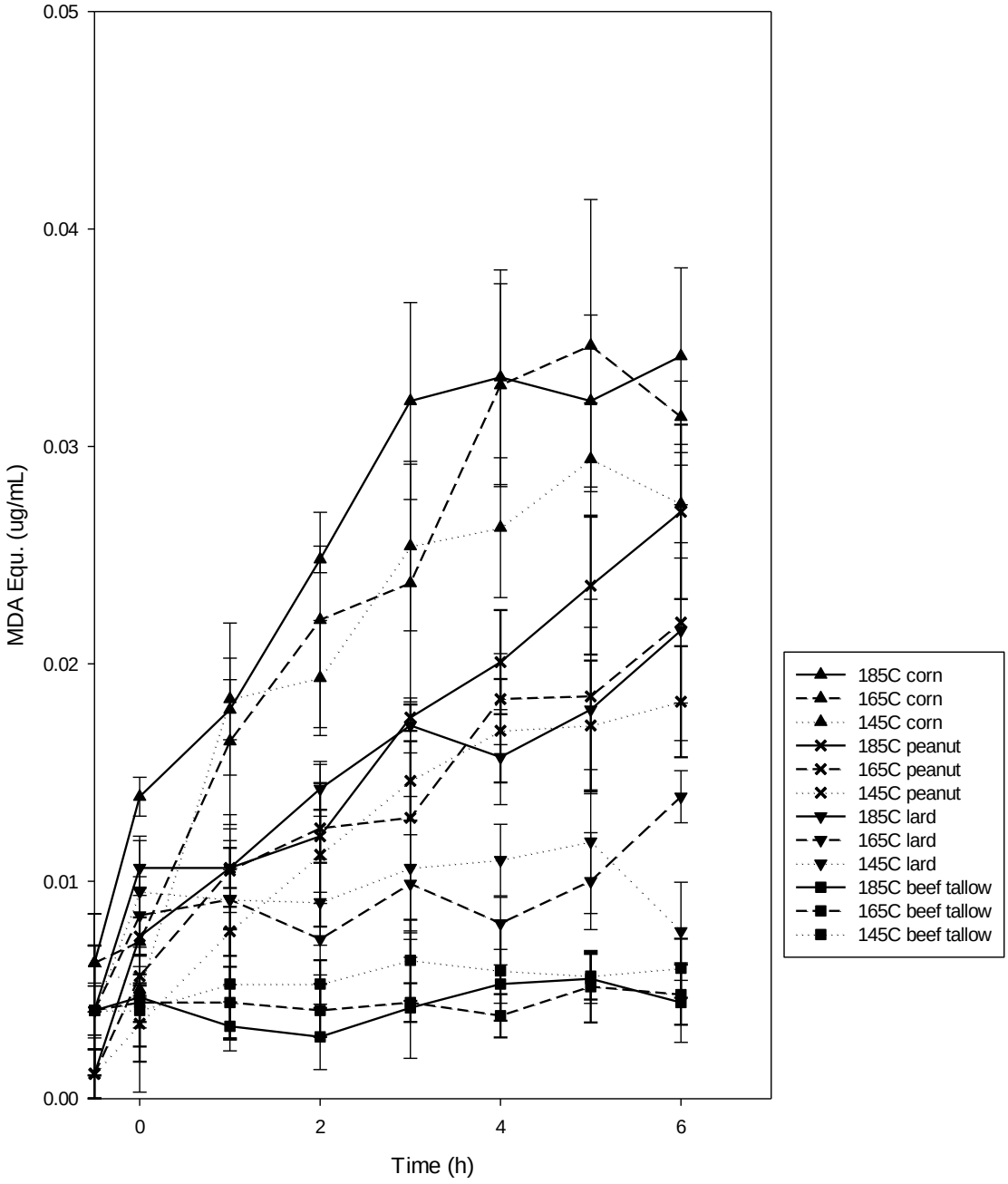


Figure 14. TBARS formation at various temperatures and heating time for corn, peanut oils, lard and beef tallow

(Corn oil is shown again as reference because units are different on this figure)

Based on the results of oxidation rates measured by TBARS of the 6 oils and fats, the following conditions were selected to measure the formation of nonpolar and polar lipophilic aldehydes including the α, β-unsaturated-4-hydroxyaldehydes such as HHE, HOE, HNE and HDE: temperatures **145, 165 and 185 ℃** and heating time **0, 1, 3 and 5 hours**.

B. Formation of HNE, the Major Toxic α, β-Unsaturated 4-Hydroxyaldehyde, in Commercial Corn, Soybean, Peanut, Canola oils, Lard and Beef Tallow Due to 0, 1, 3 and 5 Hours of Thermal Oxidation at 145 ℃,165 ℃ and 185 ℃

HNE is the major α, β-unsaturated 4-hydroxyaldehyde which was measured in each of the above mentioned oils and fats by HPLC. Figure 15 to Figure 21 show the HNE formation in corn, soybean, peanut, canola oils, lard and beef tallow at the selected heating time and temperatures.

a) Corn oil

Figure 15 shows the HNE formation in heated corn oil at 145, 165 and 185 ℃ for 1, 3 and 5 hours and the concentration of HNE in the unheated oil at 0 time. HNE was not detected in the unheated corn oil. At 145 ℃, the formation of HNE increased slightly along with heating time, however there were no significant differences between 1, 3 and 5 hours. At 165 ℃ and 185 ℃, the formation of HNE increased remarkably and there was significant difference between the heating periods. The HNE formation at 165 ℃ for 5-hour heating was 5 times higher than the HNE formation at 165 ℃ for 1-hour heating. HNE formation at 185 ℃ for 5-hour heating was 7 times higher than HNE formation at 185 ℃ for 1-hour heating. In terms of 1-hour heating, however, HNE formation did not reach significant differences between the three temperatures. Both at 3 and 5 hours of heating time, the HNE formation at 185 ℃ was the highest and again with a lower level at

145 ℃. HNE formation was greatly influenced both by temperature and the time of heating period.

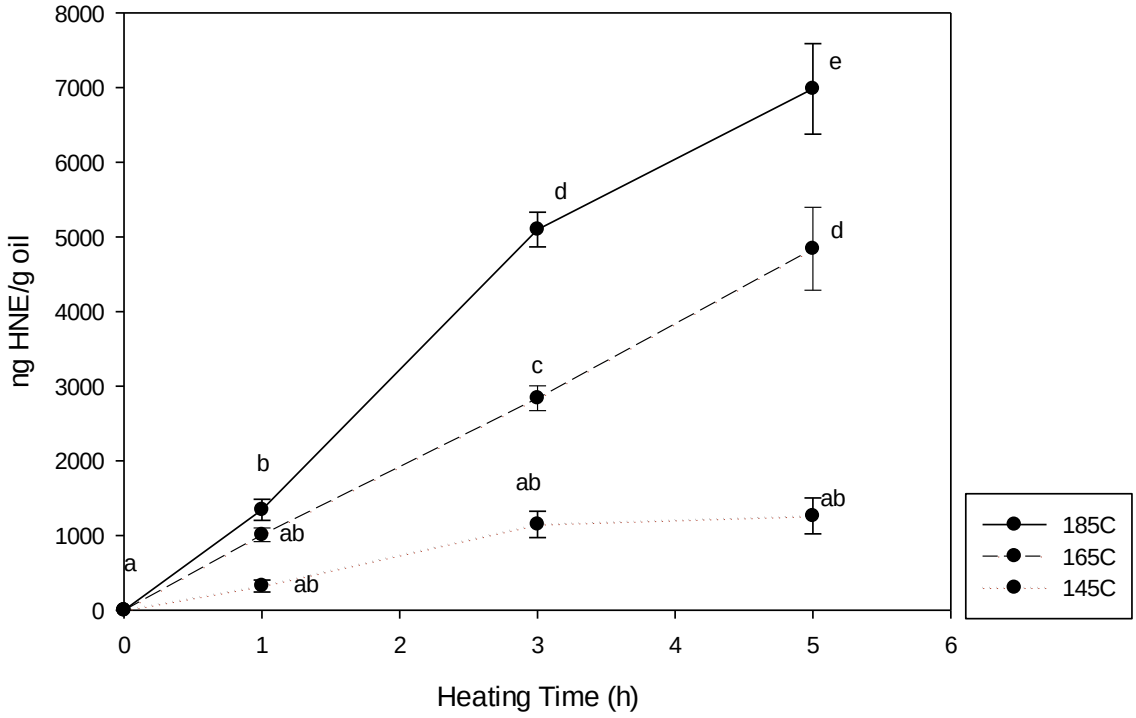


Figure 15. The HNE formation in commercial corn oil at various heating time and temperatures

b) Soybean oil

Figure 16 shows the HNE formation in heated soybean oil at 145, 165 and 185 ℃ for 1, 3 and 5 hours. No HNE was detected in the unheated soybean oil at 0 time of heating. For all three above temperatures, the formation of HNE increased along with heating time. At 145 ℃, HNE formation increased with no significant difference between 1, 3 and 5 hours. However at 165 ℃ and 185 ℃, the HNE formation increased dramatically along with heating time. HNE formation at 185 ℃ for 5 hours was 6 times higher than HNE formation at 185 ℃ for 1 hour. At 165 ℃, HNE formation with 5-hour heating was also roughly 6 times higher than HNE formation with 1-hour of heating. In the same heating period, higher temperatures led to higher HNE formation. It is interesting to note that HNE formation at 165 ℃ for 5 hours heating was almost as high as that at 185 ℃ for the same heating time. This may be due to the fact that soybean oil is relatively high in linolenic acid (4.56%) which would probably induce the oxidation even at somewhat lower temperature to a faster rate.

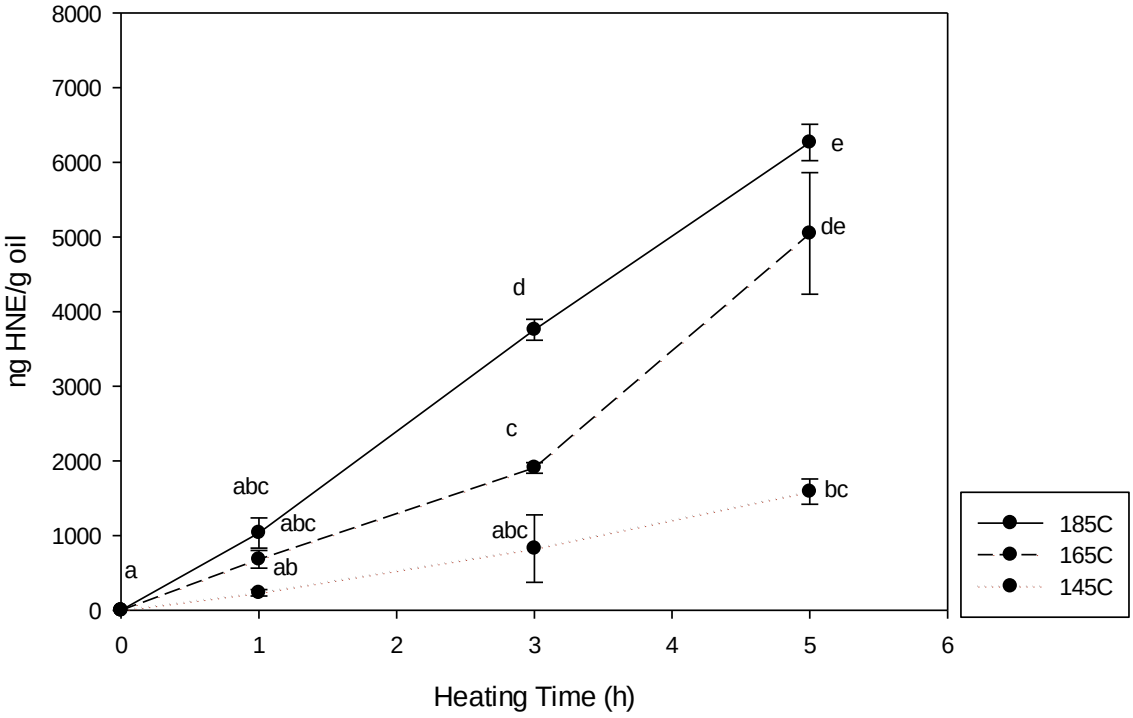


Figure 16. The HNE formation in commercial soybean oil at various heating time and temperatures

c) Peanut oil

Figure 17 shows the HNE formation in heated peanut oil at 145, 165 and 185 ℃ for 1, 3 and 5 hours. No HNE formation was detected in unheated peanut oil at 0 time of heating. For all three above temperatures, the increase of HNE formation along with the increased heating time was significant, which was different from corn and soybean oils at the lowest heating temperature 145 ℃. After heating at 185 ℃ for 5 hours, HNE formation was 5 times higher than heating for 1 hour. HNE formation at 165 ℃ after 5 hours of heating was 3 times the concentration of HNE formation after 1 hour heating. However at 145 ℃ the HNE formation after 5 hours heating was 4 times higher compared with HNE formation after 1 hour heating. The reaction rate of oxidation as expected was lower than that in corn and soybean oils due to the difference in fatty acid distribution of peanut oil. There were no significant differences between the three temperatures for 1 and 3 hours, and the HNE formation was close at 145 ℃ and 165 ℃ under these two heating time. However, the HNE concentration was significantly higher at 185 ℃ than 145 and 165 ℃ after 5 hours of heating.

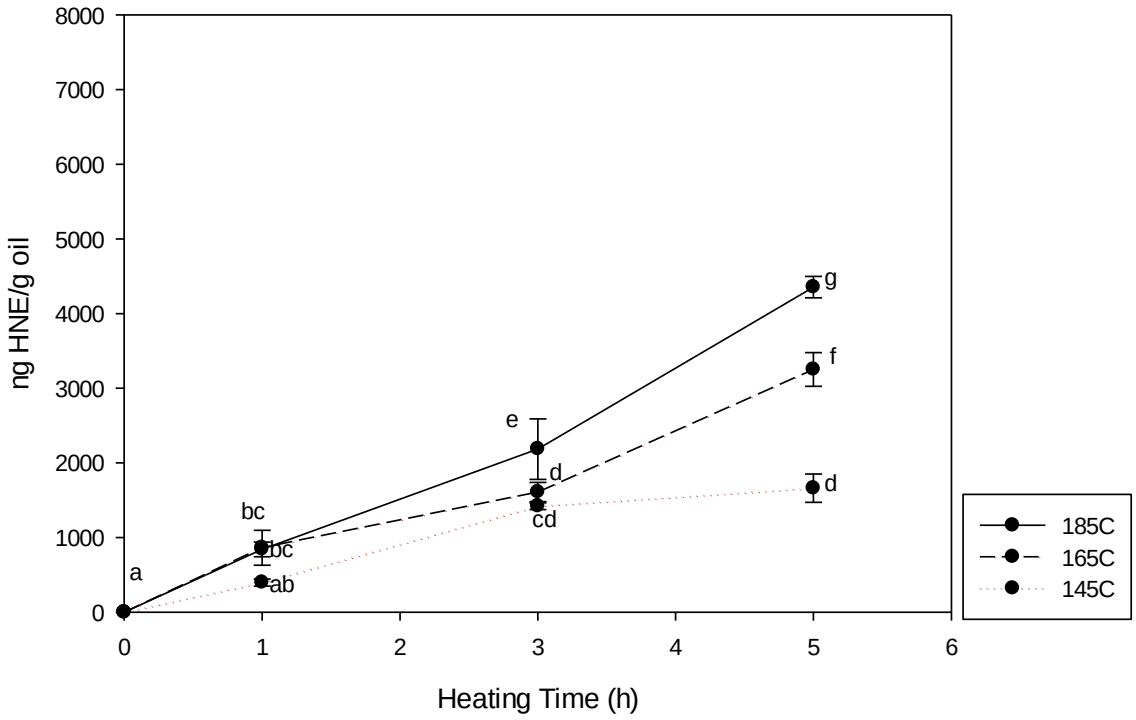


Figure 17. The HNE formation in commercial peanut oil at various heating time and temperatures

d) Canola oil

Figure 18 shows the HNE formation in heated canola oil at 145, 165 and 185 ℃ for 1, 3 and 5 hours. No HNE formation was detected in the unheated canola oil at 0 time of heating. At each of the temperatures, the HNE formation was very low. The HNE formation hardly increased along with heating time from 1 hour to 5 hours at 145 ℃ and 165 ℃. At 185 ℃, the HNE formation after 5 hours of heating was only 2 times as much as that after 1 hour of heating. HNE formation at 185 ℃ was significantly different from HNE formation at 145 ℃ after 3 hours of heating but the rate was also very low. HNE formation at 185 ℃ was significantly higher than HNE formation at 145 ℃ and 165 ℃ after 5 hours heating. Canola oil had low HNE formation even at 185 ℃ after 5 hours of heating. This is expected because linoleic acid concentration in this oil is low (only 20%) compared to the high linoleic acid containing oils such as corn and soybean oils.

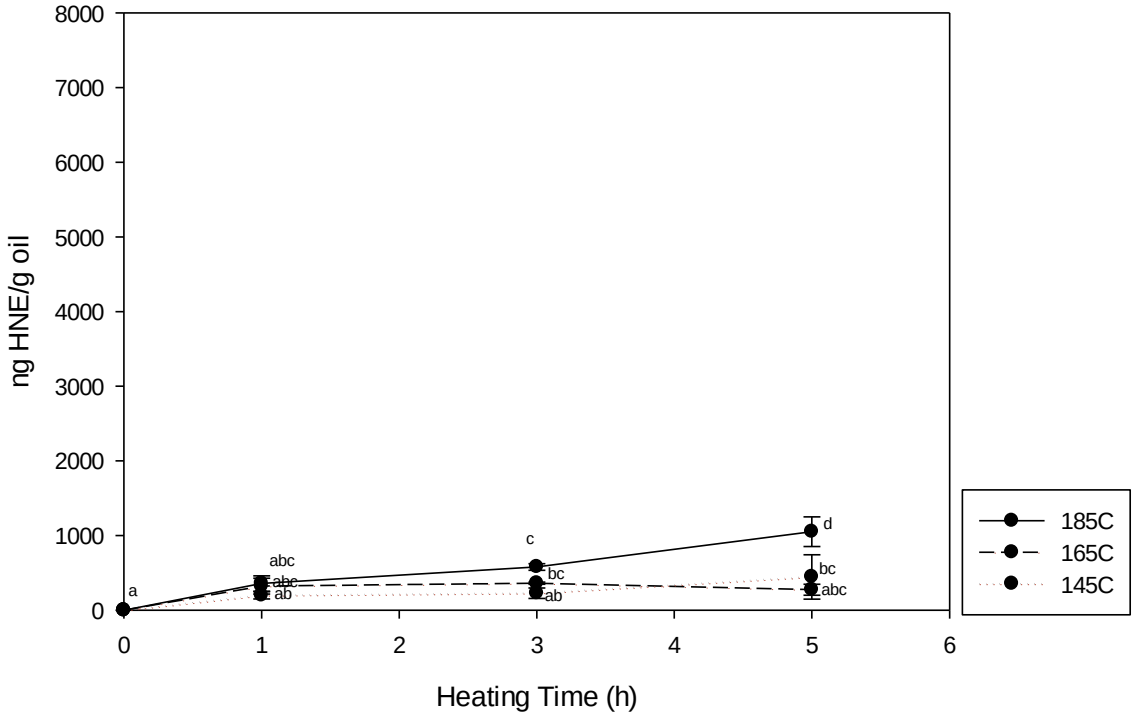


Figure 18. The HNE formation in commercial canola oil at various heating time and temperatures

e) Lard

Figure 19 shows the HNE formation in heated lard at 145, 165 and 185 ℃ for 1, 3 and 5 hours. No HNE formation was detected in the unheated lard at 0 time of heating. The HNE formation increased heavily at all the three temperatures, especially at 185 ℃. After 5 hours of heating at 185 ℃, the HNE formation was 4 times as high as the HNE formation after 1 hour heating. HNE formation at 165 ℃ and 145 ℃ after 5 hours of heating was approximately 4 times the concentration of HNE formation after heating for 1 hour. The HNE formation at 185 ℃ was much higher than the heating for 1 or 3 hours at 165 ℃ and 145 ℃.

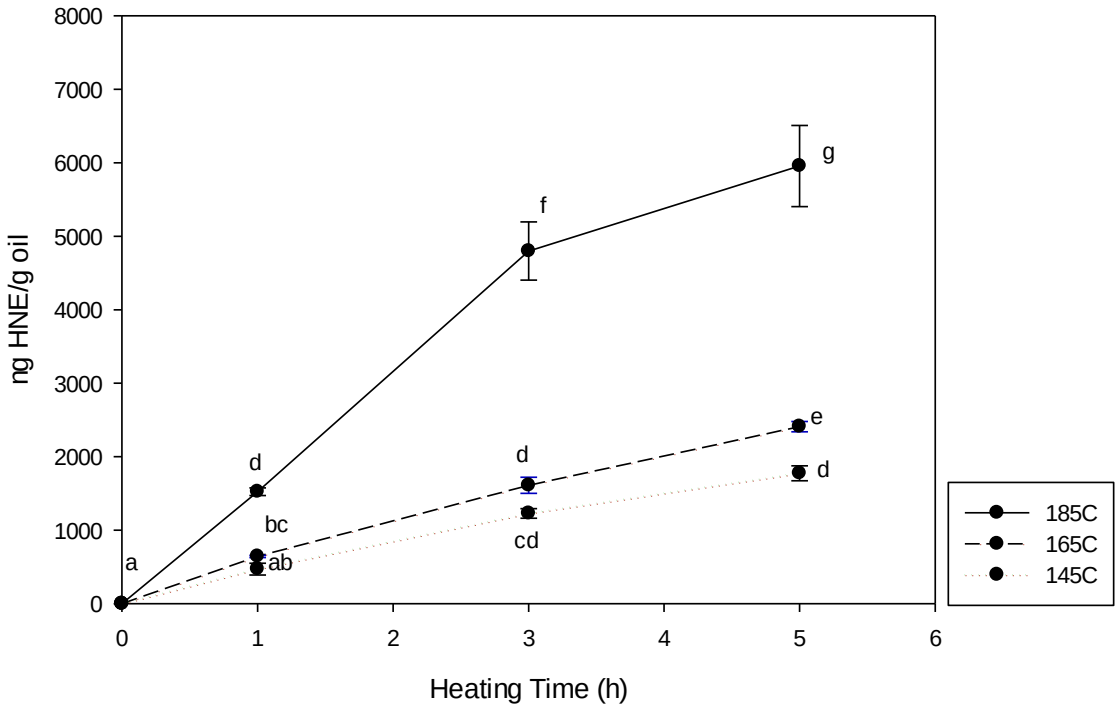


Figure 19. The HNE formation in lard at various heating time and temperatures

f) Beef tallow

Figure 20 shows the HNE formation in heated beef tallow at 185 ℃ for 5-hour of heating. Beef tallow has very low, only 1.9%, linoleic acid concentration which is a precursor of HNE, therefore HNE formation is minimal. Only the condition that may produce relatively measurable HNE formation was investigated at present. HNE formation at 185 ℃ after 5-hour heating time of beef tallow was measurable but its formation was very low.

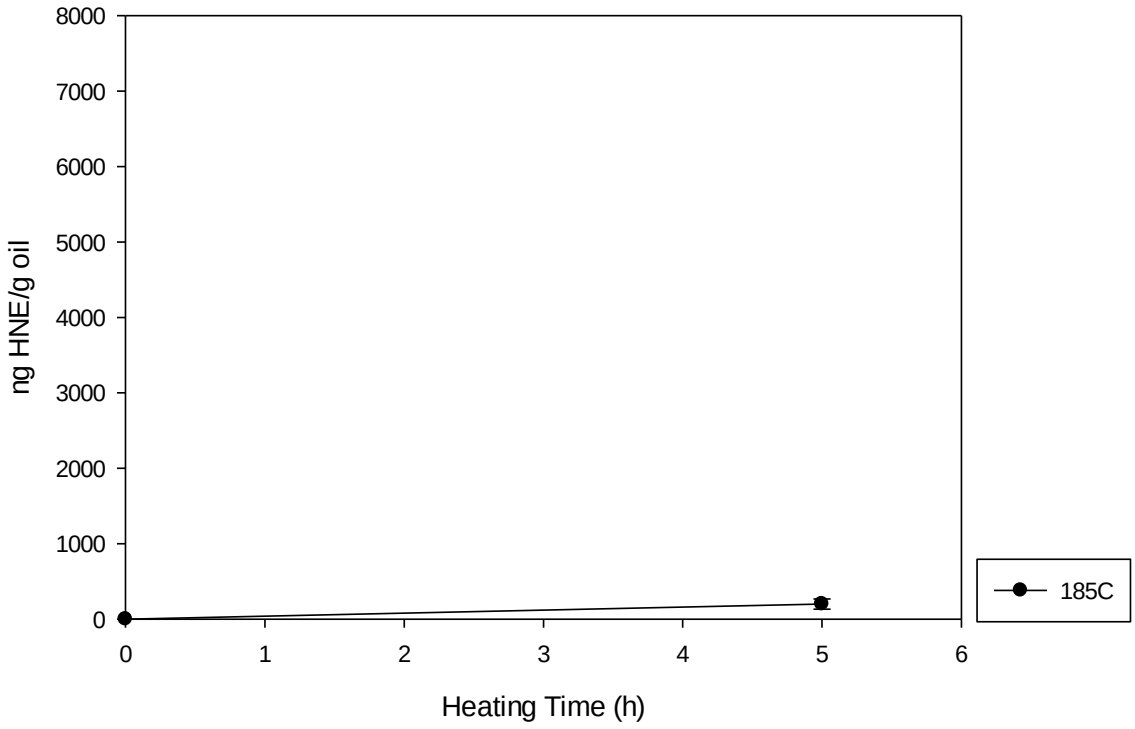


Figure 20. The HNE formation in beef tallow after 185 ℃ and 5 hours of heating period

g) Comparison of HNE concentration in commercial corn, soybean, peanut, canola oils, lard and beef tallow heat-treated for 1, 3 and 5 hours at 185 ℃ or 165 ℃ or 145 ℃

Figure 21 (a), (b) and (c) shows the comparison of HNE formation in corn, soybean, peanut, canola oils, lard and beef tallow under three different temperature treatments at various times. The linoleic acid concentrations of corn oil, soybean oil, peanut oil, canola oil, lard and beef tallow were 44%, 43%, 27%, 20%, 8% and 2%, respectively. For beef tallow, due to its very low linoleic acid concentration, the HNE formation was measured only at 185 ℃ for 5 hours of heat treatment. As it was expected that even at the higher temperature and longest heating time, HNE formation in beef tallow was very low compared to the other oils and fats. It was surprising to see that canola oil which contained 20% linoleic acid had much lower HNE formation at each temperature than peanut oil which had 27% linoleic acid. The differences of HNE formation at 145 ℃ and 165 ℃ between corn, soybean and peanut oils were not significant except for the lower level of HNE in peanut oil after 5 hours of heat treatment at 165 ℃. In contrast, at the highest temperature 185 ℃, the HNE formation showed differences between corn, soybean and peanut oils, reflecting the differences of the linoleic acid concentration in these oils. It was surprising to see in lard that the elevated levels of HNE formation under all levels of temperature and time of heat treatment, even that its linoleic acid

concentration was only 8% which was much lower than canola oil that contained 20% linoleic acid. Since lard is an animal fat, maybe some indigenous compound induced some catalytic effects on increasing the HNE formation. More studies are needed to be done to resolve this question in the future.

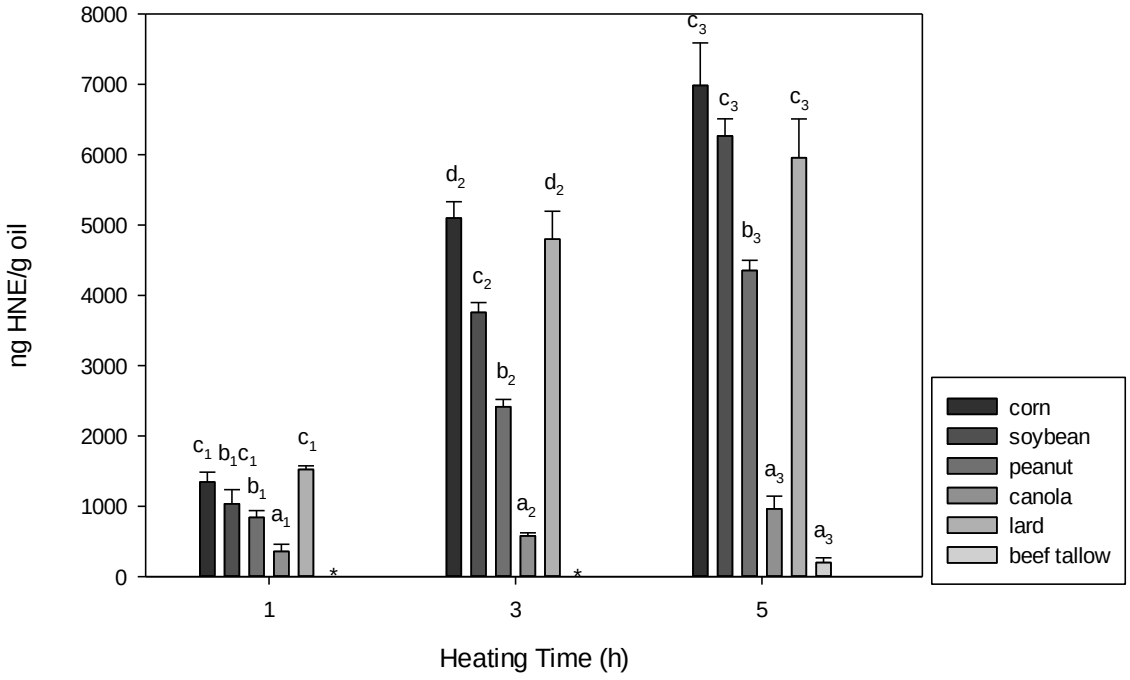


Figure 21 (a). Comparison of HNE concentration in 6 different oils and fats heat treated for 1, 3 and 5 hours at 185 ℃.

* means no detection

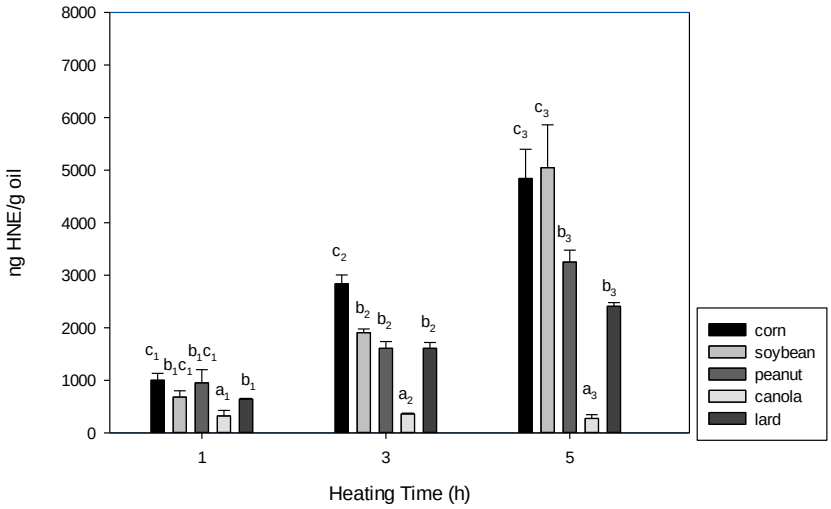


Figure 21 (b). Comparison of HNE concentration in 5 different oils and fats heat-treated for 1, 3 and 5 hours at 165 ℃.

* Beef tallow was not measured at 165 ℃.

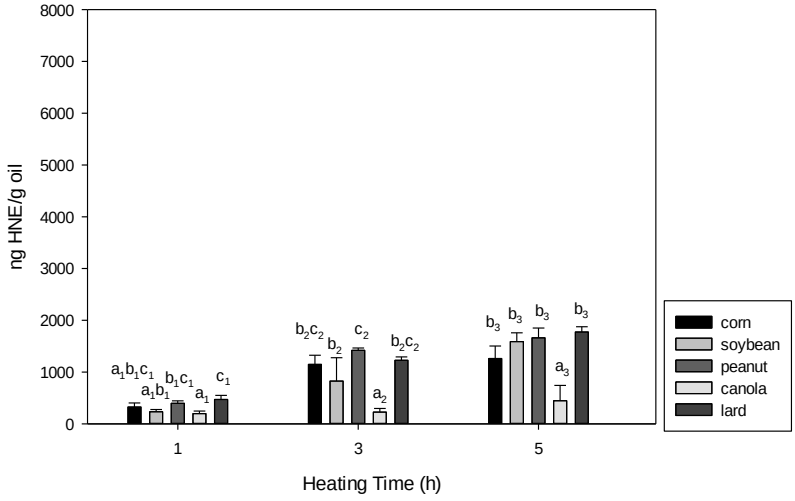


Figure 21 (c). Comparison of HNE concentration in 5 different oils and fats heat-treated for 1, 3 and 5 hours at 145 ℃.

* Beef tallow was not measured at 145 ℃.

C. Formation of HHE, HOE and HDE, the Minor Toxic α, β- unsaturated-4-hydroxyaldehydes, in Commercial Corn, Soybean, Peanut and Canola Oils Due to 0, 1, 3 and 5 Hours of Thermal Oxidation at 145 ℃,165 ℃ and 185 ℃

a) Corn oil

Figure 22 to Figure 24 show the HHE, HOE and HDE formation respectively in corn oil heated for 1, 3 and 5 hours at 145, 165 and 185 ℃. No HHE was detected in the unheated corn oil.

The HHE formation was already detected after 1 hour of heat treatment at 165 ℃ and 185 ℃ in corn oil. The concentration of HHE formation was very low during all the heat treatments. It did not reach significant differences between the various heating conditions. This result was expected because corn oil is very low in linolenic acid (only 0.7%) which is the precursor for HHE.

The HOE formation is shown in Figure 23. In general, HOE formation increased along with higher temperatures and longer heating periods. At 145 ℃, there was no significant difference between the 3 heating periods. At 165 and 185 ℃, HOE formation increased significantly by comparison to 1 hour and 5 hours of heating periods. The HOE formation was not significantly elevated after the heating time 3 and 5 hours at both 165 ℃ and 185 ℃.

Figure 24 shows the formation of HDE in corn oil. Increasing trend of HDE formation could be the same at 165 and 185 ℃ for all 3 heating periods but significant

differences were hardly achieved due to the very low concentration of HDE. HDE formation at 165 and 185 ℃ was much higher than that at 145 ℃ for the same heating time. It should be noted that in contrast to HHE, HOE and HDE can also be formed from linoleic acid which is high in corn oil (34).

Comparing HHE, HOE and HDE formation, the HOE and HDE formation were much higher than the HHE formation.

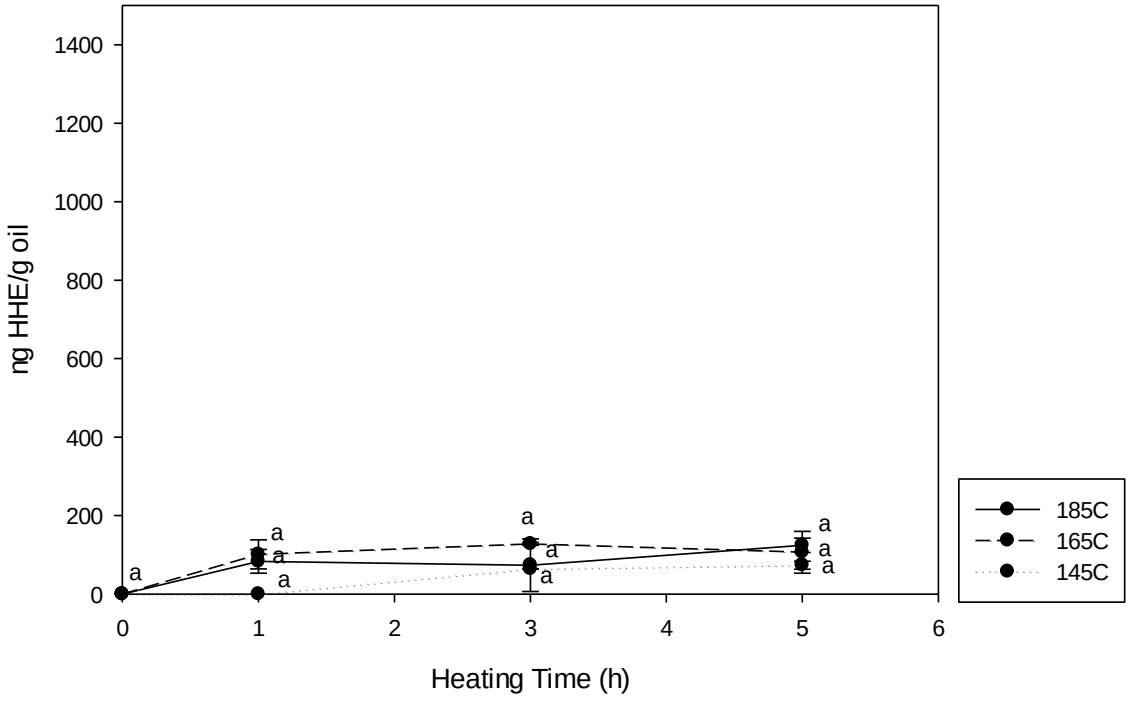


Figure 22. The HHE formation in commercial corn oil at various heating time and temperatures

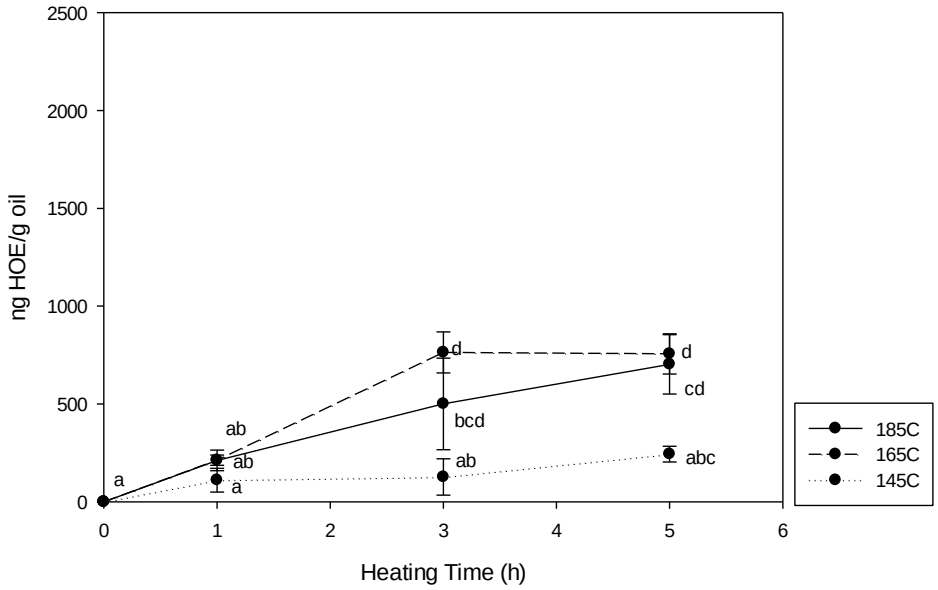


Figure 23. The HOE formation in commercial corn oil at various heating time and temperatures

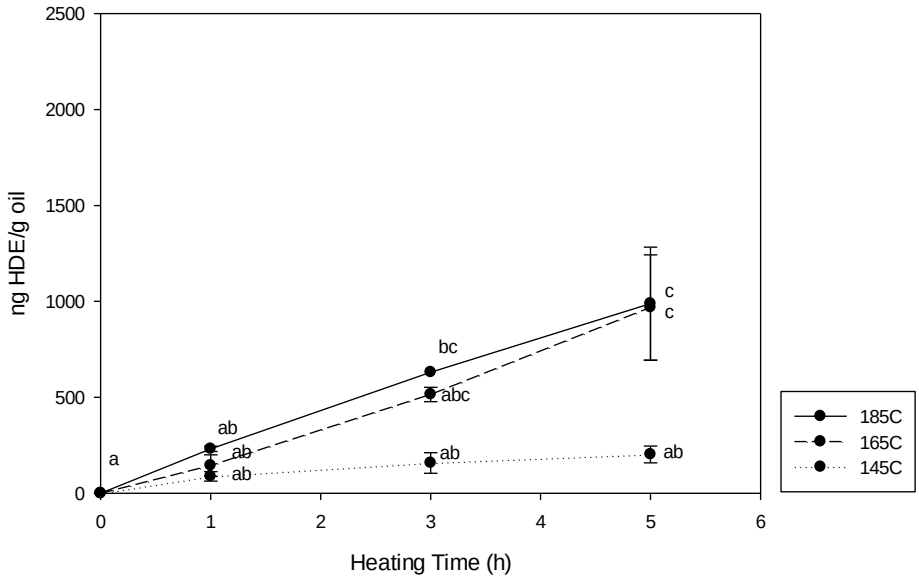


Figure 24. The HDE formation in commercial corn oil at various heating time and temperatures

b) Soybean oil

Figure 25 to Figure 27 show the HHE, HOE and HDE formation respectively in soybean oil heated for 1, 3 and 5 hours at 145, 165 and 185 ℃.

The HHE formation in soybean oil is shown in Figure 25. No HHE was detected in unheated soybean oil. Because soybean oil contained relatively high concentration of linolenic acid (4.5%) which is a precursor to HHE, the HHE formation in heated soybean oil was as expected. It increased along with higher temperatures and longer heating periods. At 185 ℃, the formation of HHE at 3 hours reached a maximum and remained the same level at 5 hours of heat treatment. At the lowest temperature 145 ℃, the formation of HHE after 5 hours of heating was 5 times higher than that at 1 hour heating. Differences in heating time showed great effect on the formation of HHE.

Figure 26 shows the HOE formation in soybean oil. It should be noted again that the precursors for HOE formation are both linoleic and linolenic acids. In general, the HOE formation increased along with higher temperatures and longer heating periods. At 5 hours of heating, the concentration of HOE formation at 145 ℃ and 165 ℃ increased, however it reached maximum concentration at 185 ℃. But the increase of heating time to 5 hours had no significant effect on HOE formation.

Figure 27 shows the formation of HDE in soybean oil. Its precursors could be both linoleic and linolenic acids. Its increasing trend due to temperature and the time of heat treatment was similar to HOE, but the concentration of HDE was lower than that of HOE.

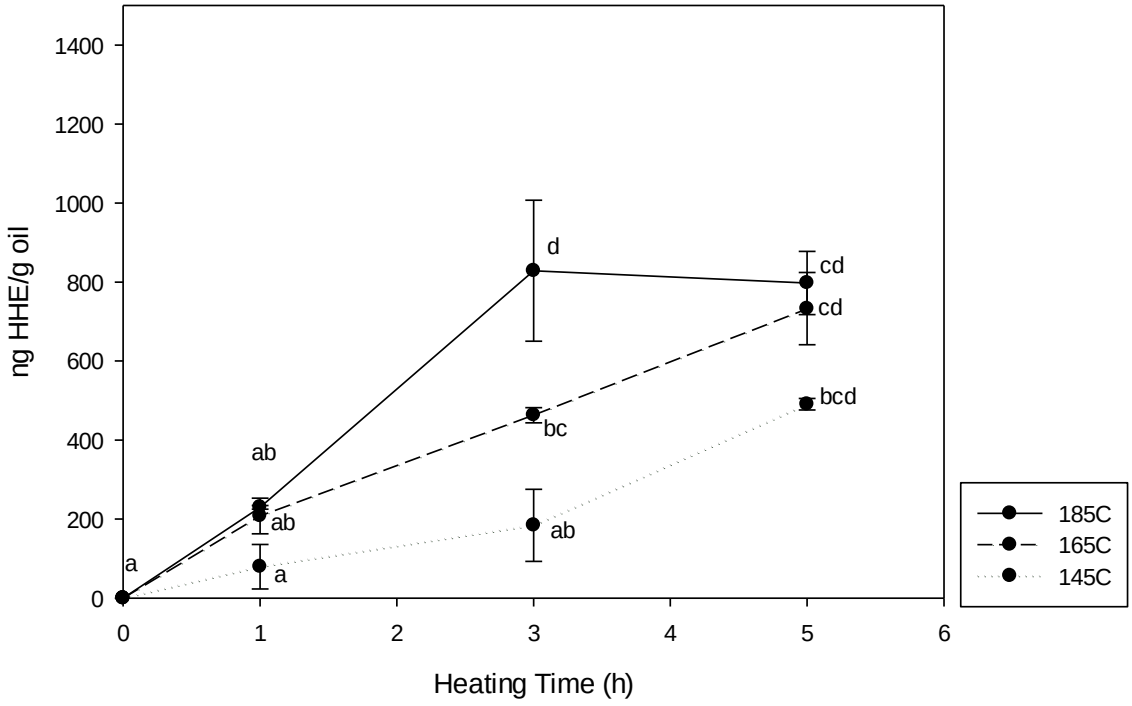


Figure 25. The HHE formation in commercial soybean oil at various heating time and temperatures

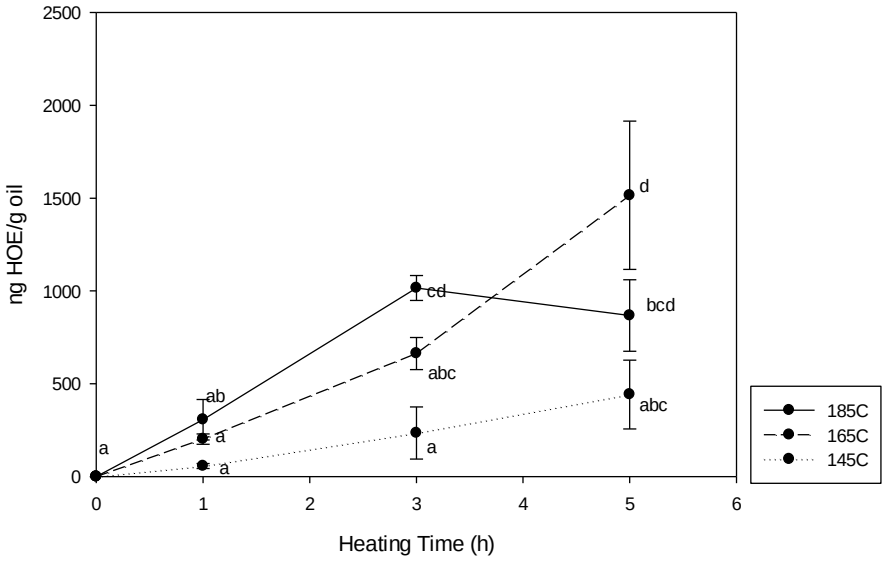


Figure 26. The HOE formation in commercial soybean oil at various heating time and temperatures

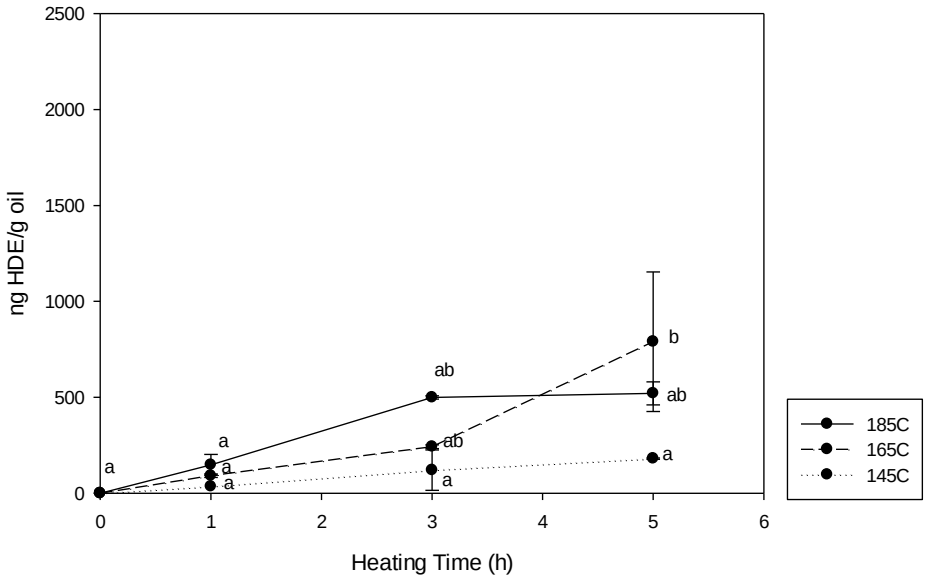


Figure 27. The HDE formation in commercial soybean oil at various heating time and temperatures

c) Peanut oil

Figure 28 to Figure 30 show the HHE, HOE and HDE formation respectively in heated peanut oil at 185 ℃. In the unheated peanut oil, HHE, HOE and HDE were not detected. Furthermore, at lower temperatures less than 185 ℃ with 1, 3 and 5 hours heating periods, no HHE, HOE and HDE were detected. Even at 185 ℃ after 5-hour heating, the formations of HHE and HOE in peanut oil were relatively very little (Figure 28 and Figure 29).

The HDE formation in peanut oil heated at 185 ℃ for 1, 3 and 5 hours is shown on Figure 30. The HDE formation increased steadily with heating time and it was significantly different between 3 and 5 hours of heating time compared to 1 hour of heating. The very low formation of the three minor α, β- unsaturated-4-hydroxyaldehydes is not surprising since linoleic acid concentration of peanut oil was 27.0%, however its linoleic acid level was only 0.17% (**Table 4**).

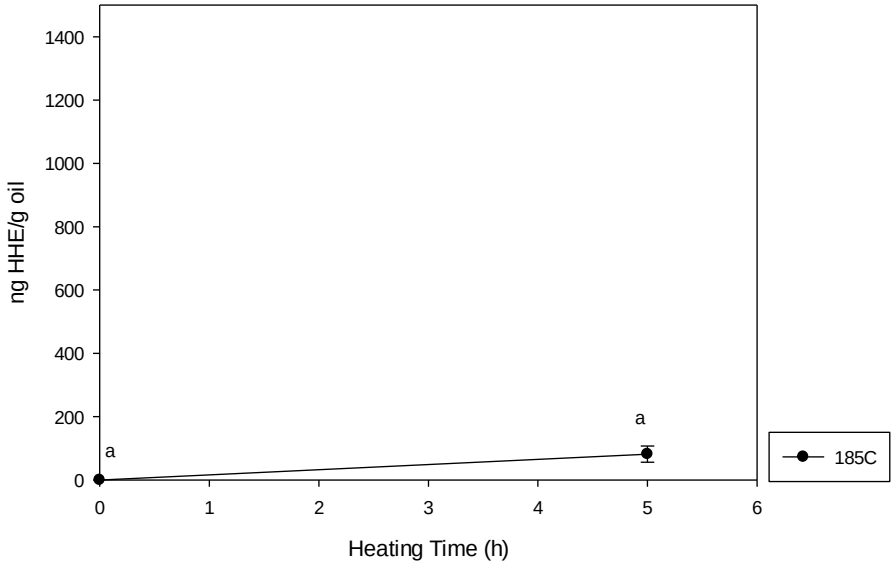


Figure 28. The HHE formation in commercial peanut oil after 185 ℃ and 5 hours of heating period

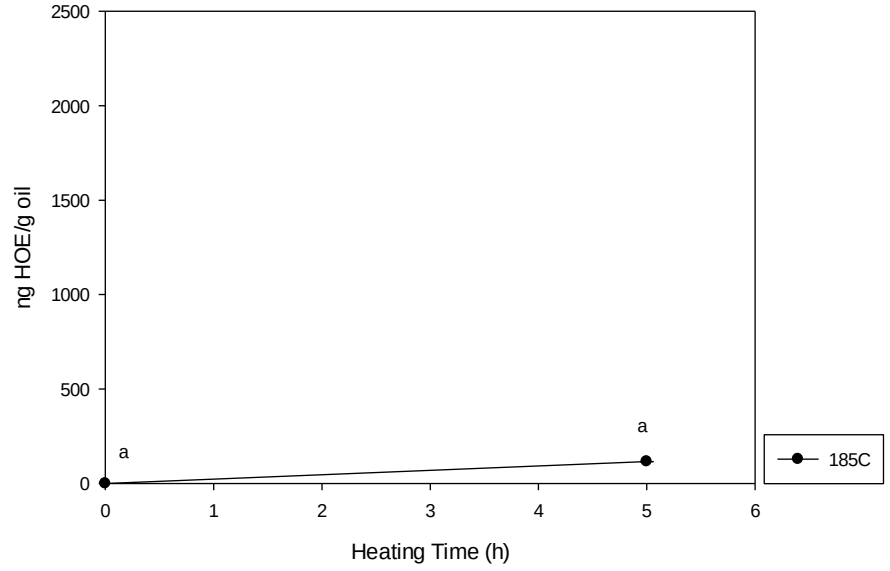


Figure 29. The HOE formation in commercial peanut oil after 185 ℃ and 5 hours of heating period

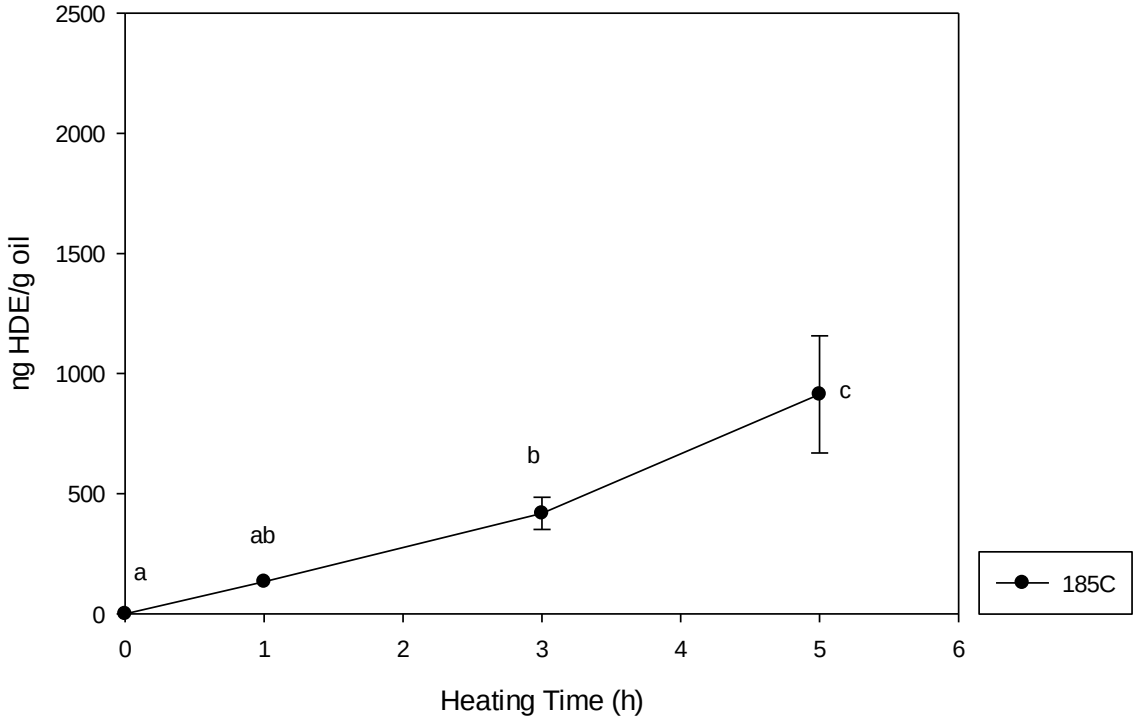


Figure 30. The HDE formation in commercial peanut oil after 185 ℃ and 1, 3 and 5 hours of heating periods

d) Canola oil

Figure 31 to Figure 33 show the HHE, HOE and HDE formation respectively in heated canola oil for 1, 3 and 5 hours at 145, 165 and 185 ℃. In the unheated canola oil, HHE, HOE and HDE were not detected.

The HHE formation is shown in Figure 31. In general, the formation of HHE increased mildly with higher temperatures and longer heating periods. At 165 ℃, the HHE formation seemed to decrease slightly along with the increased heating time to 5 hours, but it did not reach significance compared with 3 hours of heating. It seems that significant differences were not achieved between the groups because of extremely low concentrations. Since linolenic acid is a precursor for HHE and canola oil contained 9.3% of it, it is not surprising that some HHE formation can be detected under the time and temperature treatments.

The HOE and HDE formation (Figure 32 and Figure 33) were similar to HHE formation, both of their formation increased slightly along with higher temperatures and longer heating periods. The high levels were found at 185 ℃ after 5 hours of heating period.

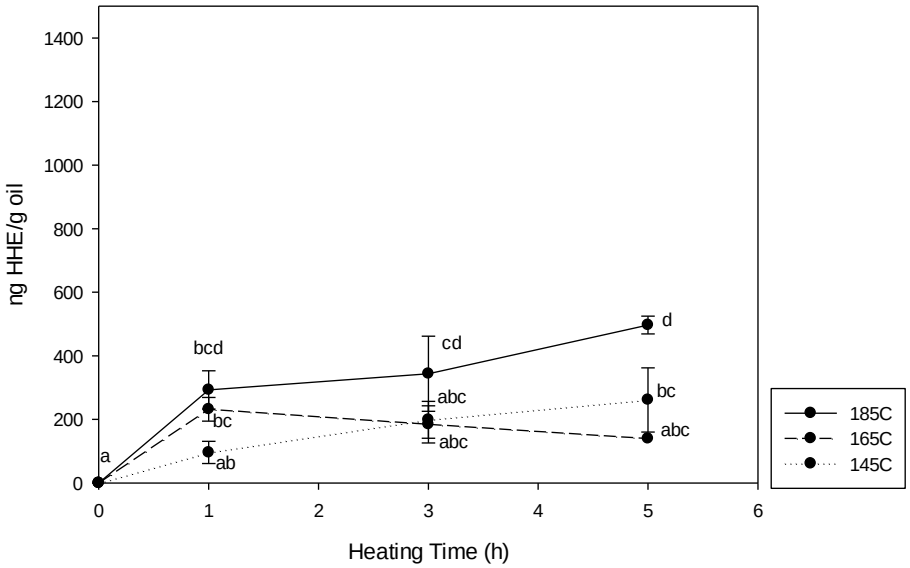


Figure 31. The HHE formation in commercial canola oil at various heating time and temperatures

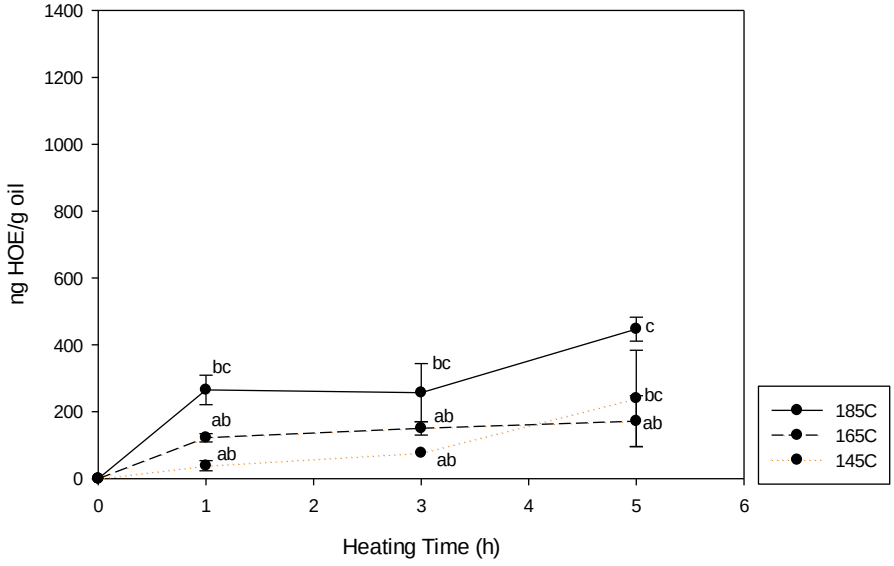


Figure 32. The HOE formation in commercial canola oil at various heating time and temperatures

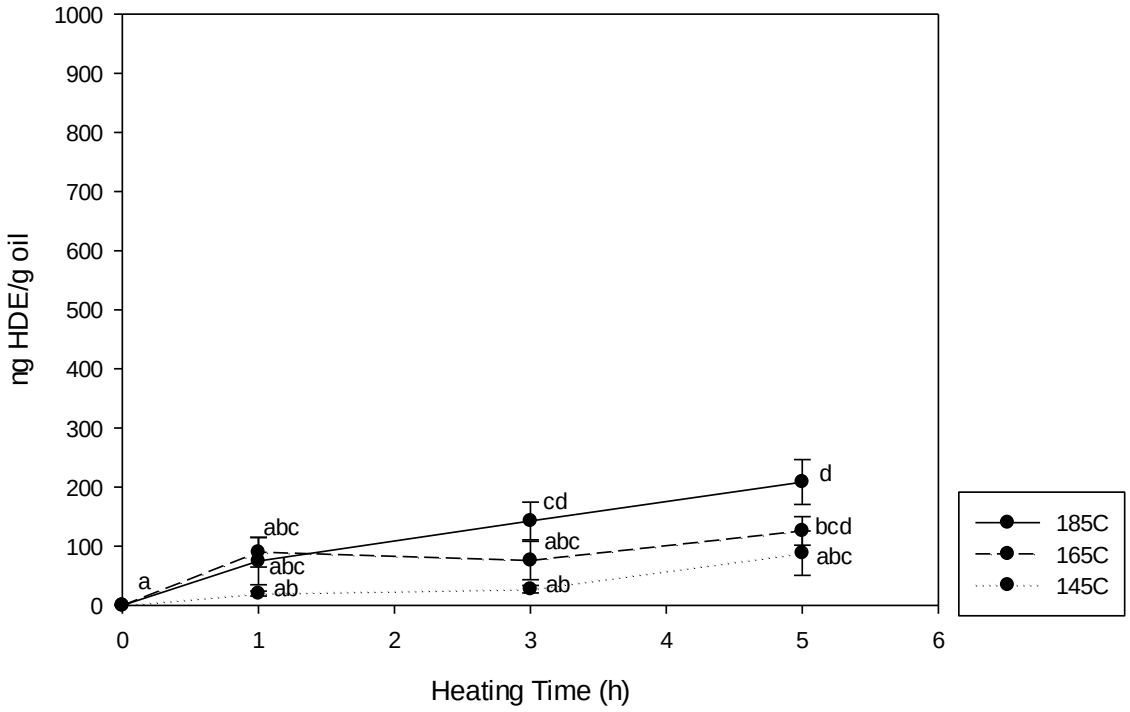


Figure 33. The HDE formation in commercial canola oil at various heating time and temperatures

e) Comparison between HHE, HOE and HDE concentration in commercial corn, soybean, peanut and canola oils after heat treated for 1, 3 and 5 hours at 185 ℃ or 165 ℃ or 145 ℃

Figure 34 (a), (b) and (c) show the comparison of HHE concentration in corn, soybean, peanut and canola oils treated at 185 ℃, 165 ℃ and 145 ℃ for 1, 3 and 5 hours respectively.

Soybean and canola oils had relatively high levels of HHE concentration compared to corn and peanut oils at every temperature and heating time. HHE formation in soybean oil was generally somewhat higher than that in canola oil. Corn oil had very low HHE concentrations at all temperature and heating time, except at 145 ℃ with 1 hour of heating it was not detected. HHE concentration was only detected in peanut oil when it was heated for 5 hours at 185 ℃. These results were consistent with the linolenic acid concentration in these oils, since linolenic acid is a precursor for HHE. Soybean and canola oils contain high levels of this fatty acid, while corn and peanut oils contained very low levels, 0.70 and 0.17% respectively.

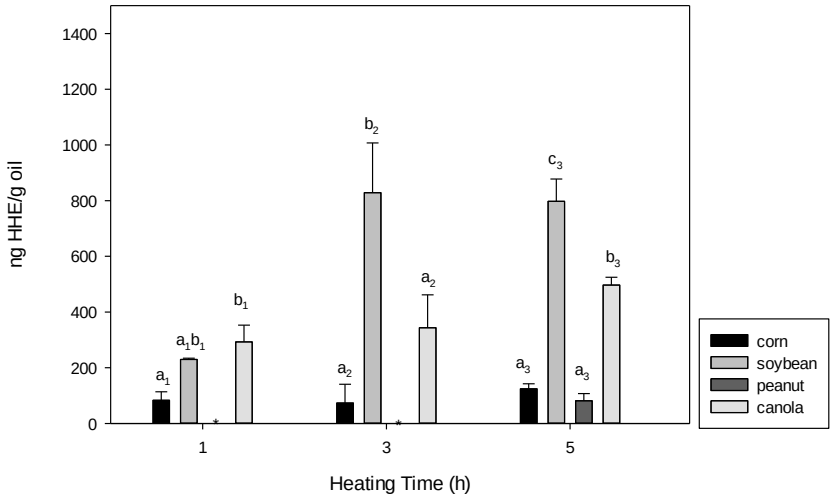


Figure 34 (a). Comparison of HHE concentrations between four different oils heat treated for 1, 3 and 5 hours at 185 ℃.

* means no detection.

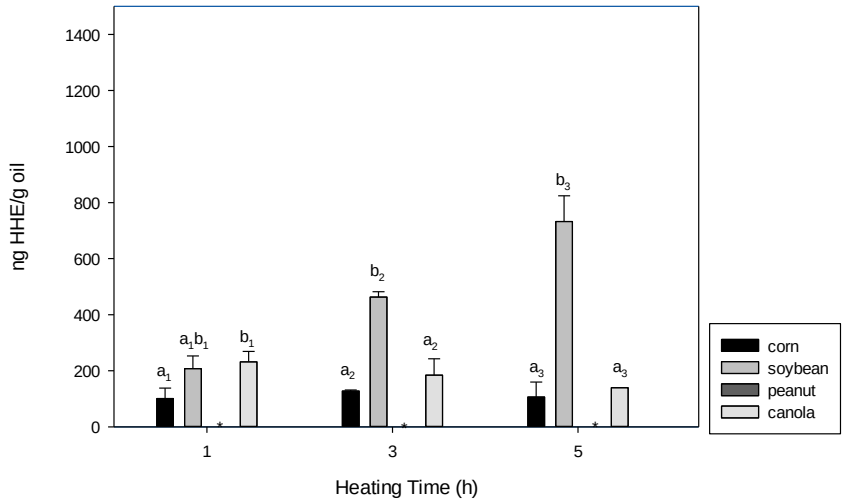


Figure 34 (b). Comparison of HHE concentrations between four different oils heat treated for 1, 3 and 5 hours at 165 ℃.

* means no detection.

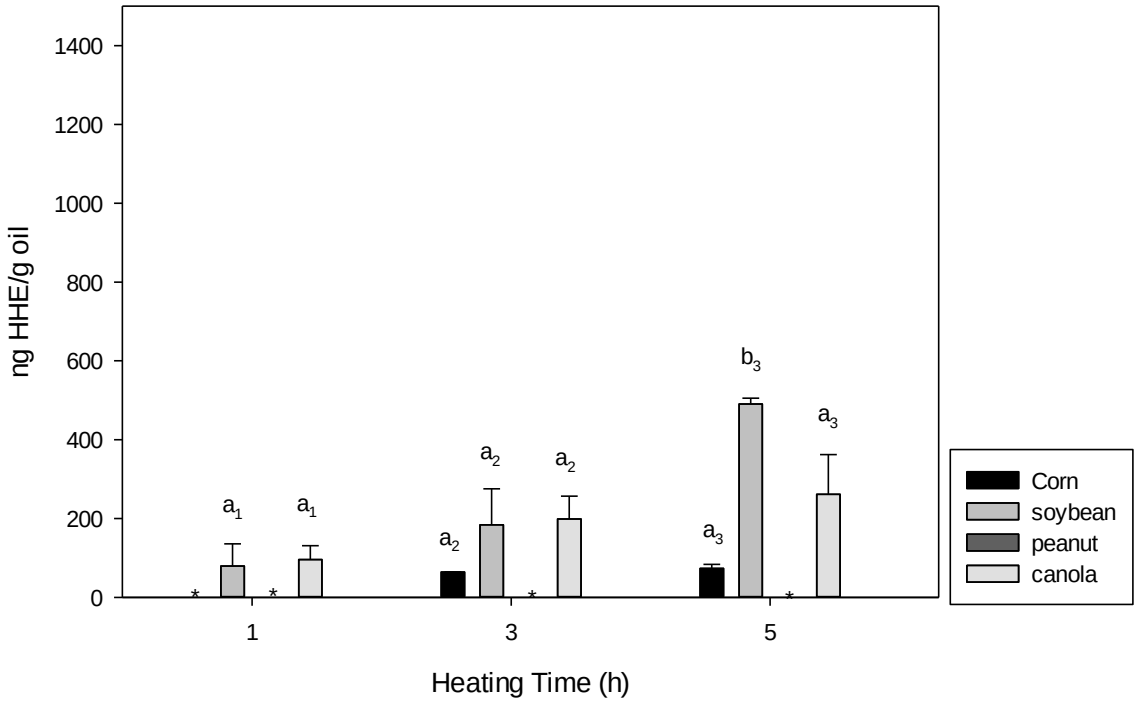


Figure 34 (c). Comparison of HHE concentrations between four different oils heat treated for 1, 3 and 5 hours at 145 ℃.

* means no detection.

Figure 35 (a), (b) and (c) show the comparison of HOE concentration between corn, soybean, peanut and canola oils treated at 185 ℃, 165 ℃ and 145 ℃ for 1, 3 and 5 hours respectively. HOE concentration in soybean oil was the highest in all 3 temperatures after 5 hours of heat treatments. After 3 hours of heat treatment, soybean and corn oils seem to have similar concentrations. After 1 hour of heat treatment, all oils except peanut oil had similar low concentrations. For peanut oil, HOE concentration was measurable only at 185 ℃ after 5 hours of heat treatment. Previous study in this laboratory showed that HOE comes from both linoleic acid and linolenic acid (34). Soybean oil has the highest combined level of linoleic and linolenic acid concentration (42.9% and 4.5% respectively), therefore it is not surprising that its HOE concentration was the highest among all the four tested oils. Corn oil has a high level of linoleic acid concentration, therefore it was the second in the ranking of HOE formation.

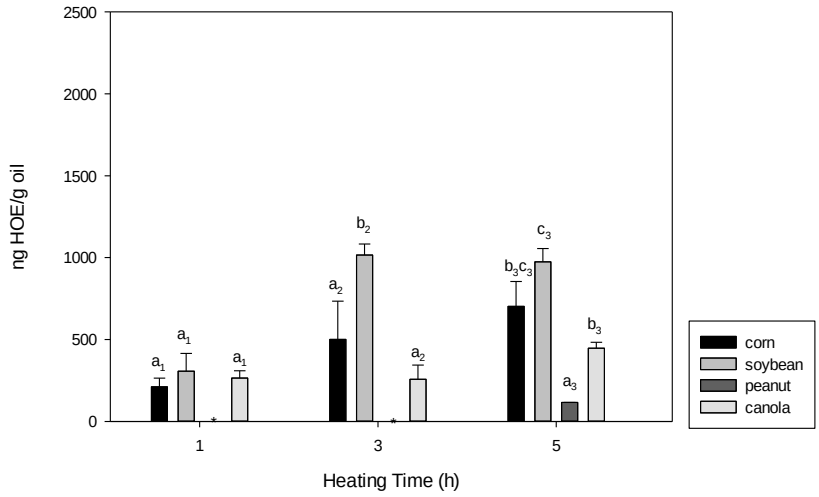


Figure 35 (a). Comparison of HOE concentrations between four different oils heat treated for 1, 3 and 5 hours at 185 ℃.

* means no detection.

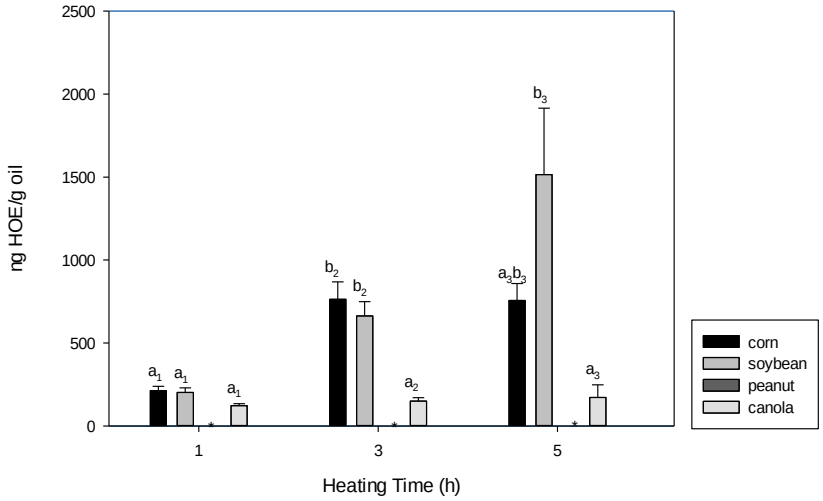


Figure 35 (b). Comparison of HOE concentrations between four different oils heat treated for 1, 3 and 5 hours at 165 ℃.

* means no detection.

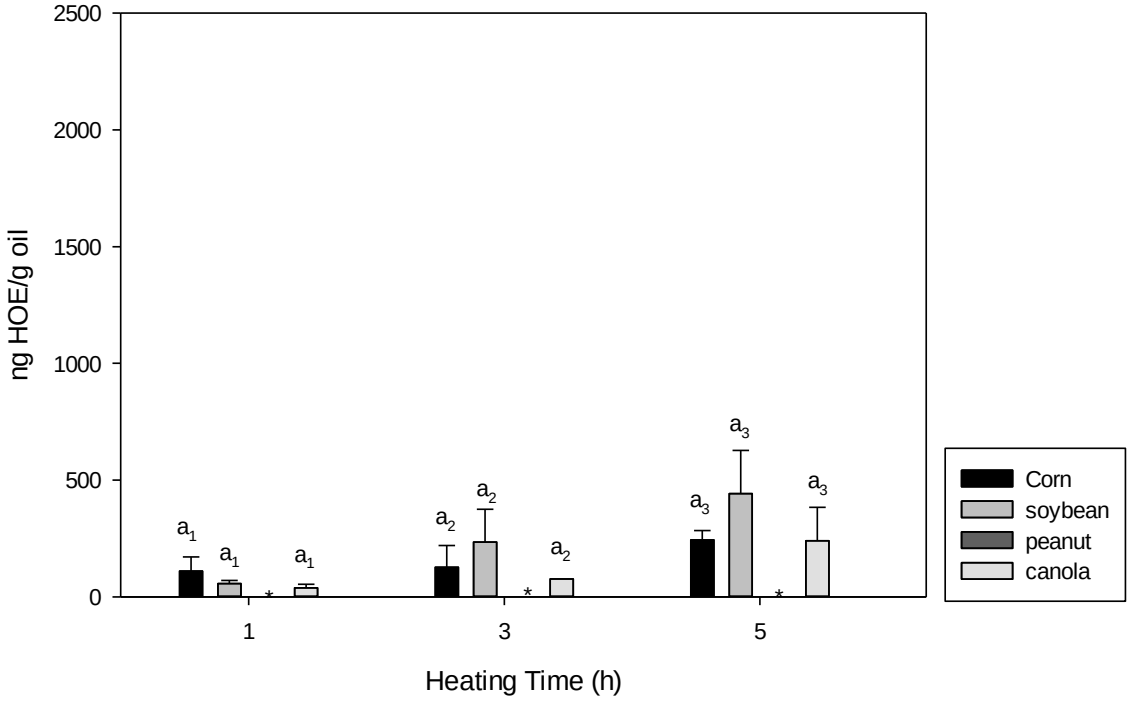


Figure 35 (c). Comparison of HOE concentrations between four different oils heat treated for 1, 3 and 5 hours at 145 ℃.

* means no detection.

Figure 36 (a), (b) and (c) show the comparison of HDE concentration between corn, soybean, peanut and canola oils treated at 185 ℃, 165 ℃ and 145 ℃ for 1, 3 and 5 hours respectively. Interestingly, in peanut oil HDE was detected after all these heating periods 1, 3 and 5 hours at 185 ℃, but was not detected at 165 ℃ and 145 ℃ after heating even as long as 5 hours. HDE seemed to form at all the temperatures and all the heating time treatments in corn oil, followed by soybean oil and then canola oil with minimal formation. Due to the small amount of HDE formation, significant differences could be detected only at 3 or 5 hours of heat treatments at 185 ℃. During these conditions, canola oil produced significantly less HDE than the other three oils.

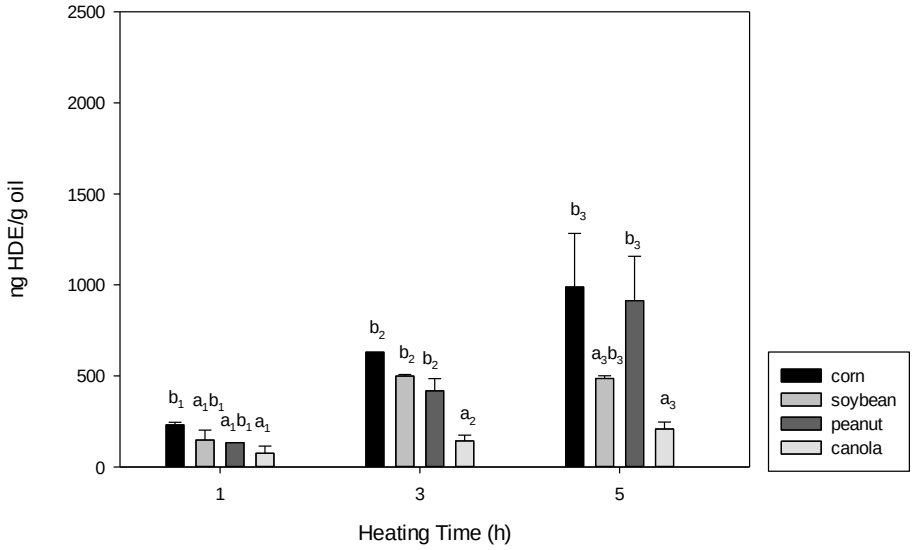


Figure 36 (a). Comparison of HDE concentrations between four different oils heat treated for 1, 3 and 5 hours at 185 ℃.

* means no detection.

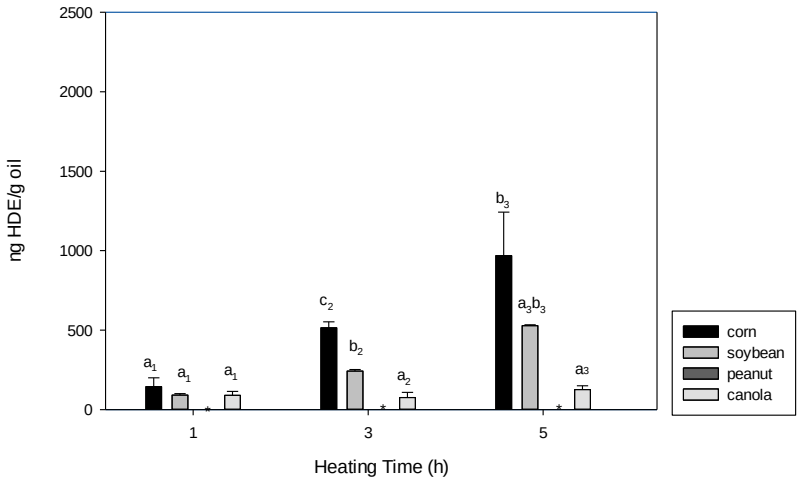


Figure 36 (b). Comparison of HDE concentrations between four different oils heat treated for 1, 3 and 5 hours at 165 ℃.

* means no detection.

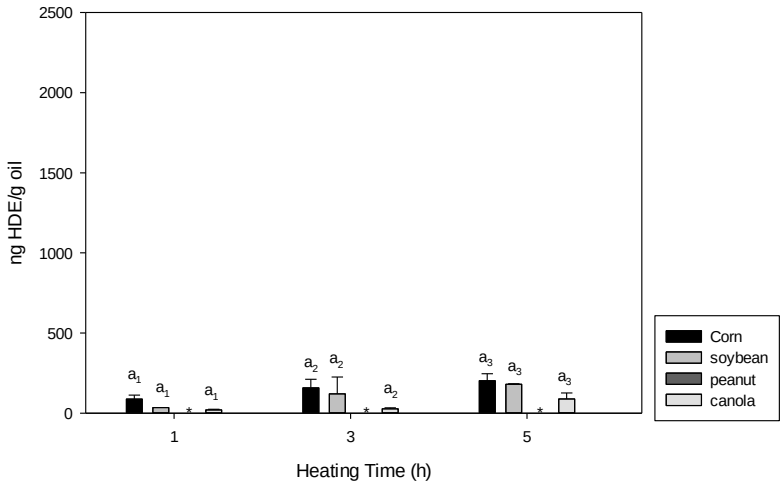


Figure 36 (c). Comparison of HDE concentrations between four different oils heat treated for 1, 3 and 5 hours at 145 ℃.

* means no detection.

D. Comparison between the Formation of HHE, HOE, HNE and HDE in Commercial Corn, Soybean, Peanut and Canola Oils Heat Treated for 5 Hours at 185 ℃

a) Corn oil

Figure 37 shows the comparison between the formation of HHE, HOE, HNE and HDE in corn oil heat treated at 185 ℃ for 5 hours. The formation of HNE was much higher than the other three hydroxyaldehydes. The concentration of HNE was about 56 times higher than the concentration of HHE, 10 times higher than the concentration of HOE and 7 times higher than the concentration of HDE. HNE was the major α, β-unsaturated-4-hydroxyaldehyde in corn oil followed by the other three hydroxyaldehydes with much smaller formations, but significant differences between the minor compounds were not achieved.

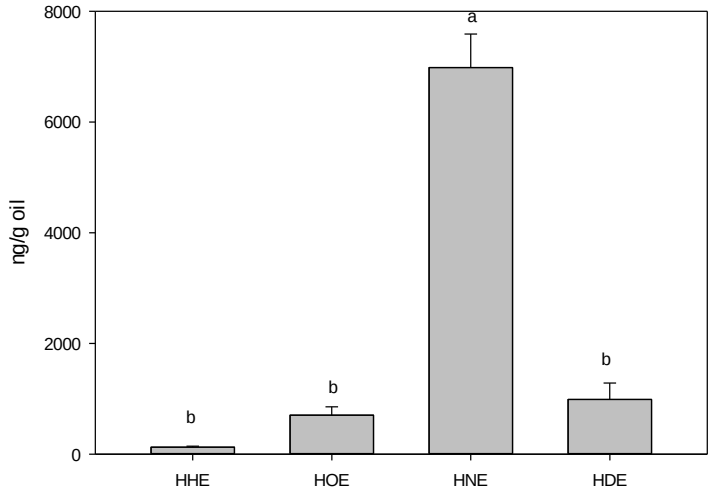


Figure 37. Comparison between the formation of HHE, HOE, HNE and HDE in commercial corn oil heat treated for 5 hours at 185 ℃

b) Soybean oil

Figure 38 shows the comparison between the formation of HHE, HOE, HNE and HDE in soybean oil heat treated at 185 ℃ for 5 hours. The concentration of HNE was about 7 times higher than the concentration of HHE, 6.6 times higher than the concentration of HOE and 11 times higher than the concentration of HDE. There were no significant differences between the formation of HHE, HOE and HDE.

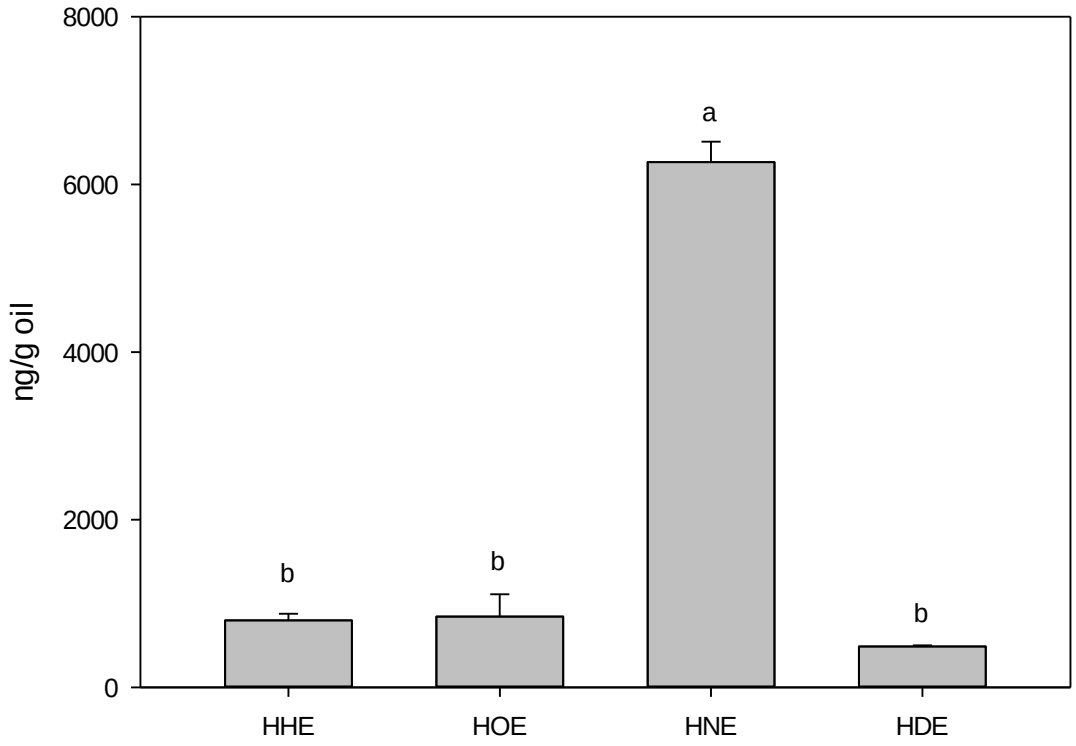


Figure 38. Comparison between the formation of HHE, HOE, HNE and HDE in commercial soybean oil heat treated for 5 hours at 185 ℃

c) Peanut oil

Figure 39 shows the comparison between the formation of HHE, HOE, HNE and HDE in peanut oil heat treated at 185 ℃ for 5 hours. The concentration of HNE was about 53 times higher than the concentration of HHE, 38 times higher than the concentration of HOE and 5 times higher than the concentration of HDE. There was no significant difference between the formation of HHE and HOE. But HDE formation was significantly higher than HHE and HOE. HNE in peanut oil was much higher than the other three hydroxyaldehydes.

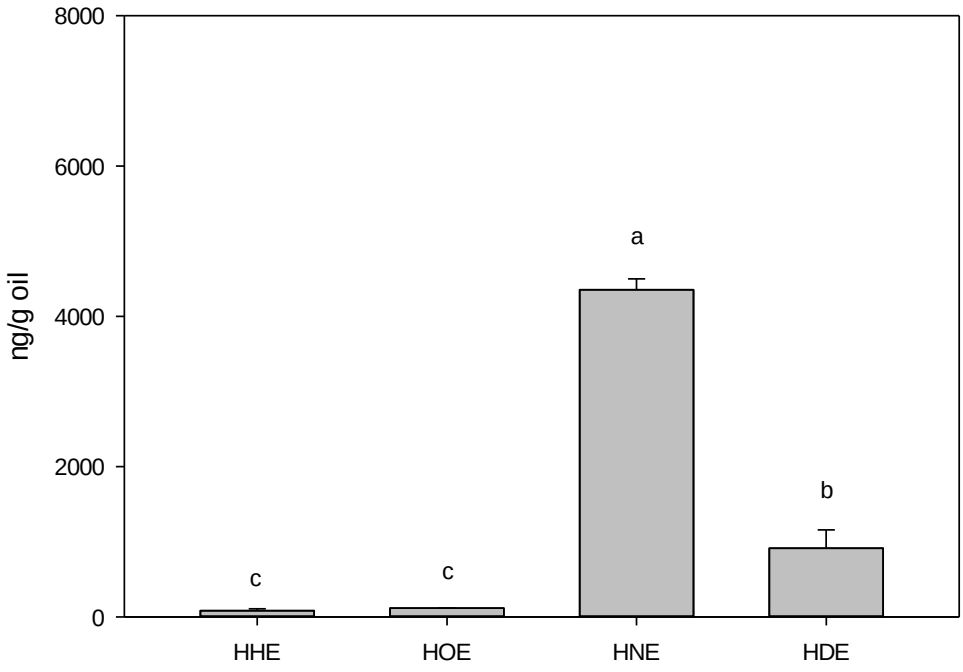


Figure 39. Comparison between the formation of HHE, HOE, HNE and HDE in commercial peanut oil heat treated for 5 hours at 185 ℃

d) Canola oil

Figure 40 shows the comparison between the formation of HHE, HOE, HNE and HDE in canola oil heat treated at 185 ℃ for 5 hours. The concentration of HNE was the highest among the four compounds in canola oil. The concentration of HNE was relatively low compared to corn and soybean oils. Interestingly, HDE formation was significantly lower than both the formation of HHE and HOE. HNE was 2.1 times higher than the concentration of HHE, 2.3 times higher than the concentration of HOE and 5 times higher than the concentration of HDE.

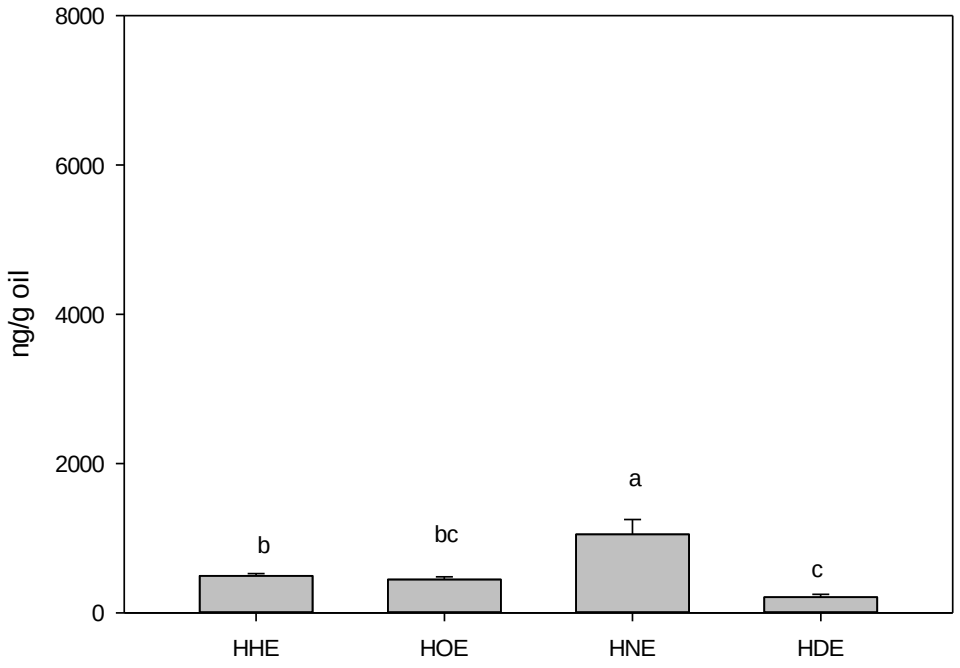


Figure 40. Comparison between the formation of HHE, HOE, HNE and HDE in commercial canola oil heat treated for 5 hours at 185 ℃

e) Comparison of HHE, HOE, HNE and HDE formation in commercial corn, soybean, peanut and canola oils heated at 185 ℃ for 5 hours

Figure 41 shows the comparison between the four hydroxyaldehydes HHE, HOE, HNE and HDE formation in corn, soybean, peanut and canola oils, respectively.

HHE concentration was significantly higher in soybean oil than in canola oil. And HHE concentrations in soybean and canola oils were significantly higher than in corn and peanut oils. It was not surprising because soybean and canola oils contain relatively high level of linolenic acid which is a precursor for HHE.

Peanut oil had significantly lower level of HOE than corn, soybean and canola oils. HOE formation is related to the concentration of both linoleic and linolenic acids in the oil (34).

The highest level of HNE was found in corn oil followed by soybean and peanut oil. HNE was significantly lower in canola oil which contained the lowest level of linoleic acid which is the precursor for HNE.

Interestingly, HDE formation in the four oils was almost opposite to HHE formation. Both corn and peanut oils had the highest concentration but were not significantly different from soybean oil. However HDE formation in canola oil was significantly lower than that in both corn and peanut oils. HDE formation is also related to both linoleic and linolenic acid concentrations in the oil.

In total, HNE concentration was much higher than the concentration of the other three hydroxyaldehydes in corn, soybean and peanut oils. HHE concentration was found to be very low except in soybean and canola oils which contain relatively high level of linolenic acid..

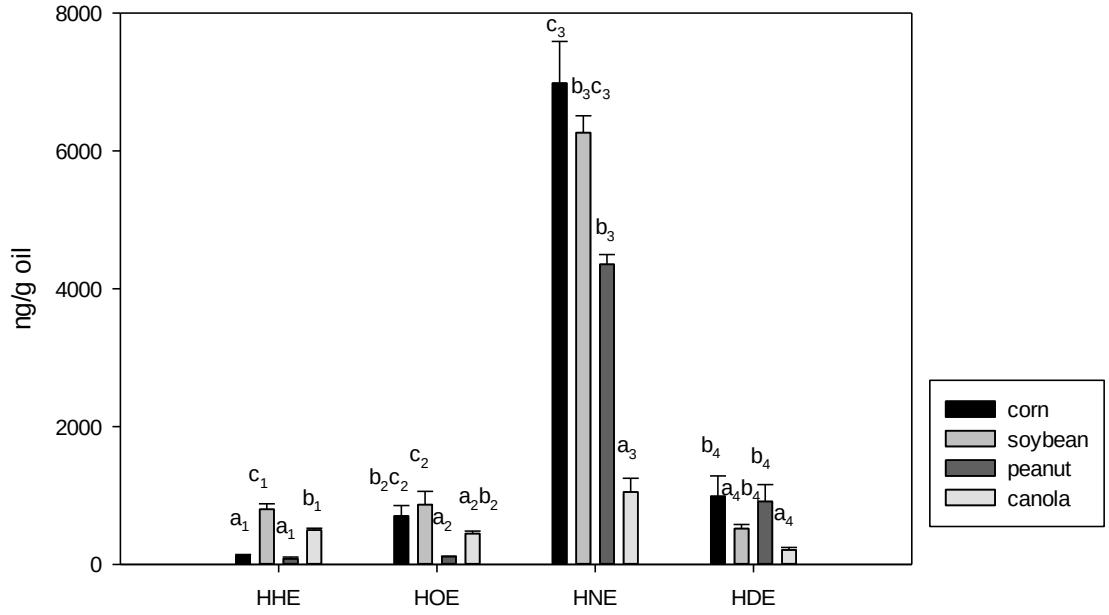


Figure 41. Comparison between the concentration of HHE, HOE, HNE and HDE in commercial corn, soybean, peanut and canola oils heat treated for 5 hours at 185 ℃

f) Comparison of the rates of HHE, HOE, HNE and HDE formation in commercial corn, soybean, peanut, canola oils, lard and beef tallow heated at 185, 165 and 145 ℃

By doing linear regression analysis, the rates for HHE, HOE, HNE and HDE formation in commercial corn, soybean, peanut, canola oils, lard and beef tallow heated at 185, 165 and 145 ℃ for 5 hours were obtained (**Table 5**). Higher temperature led to a faster rate of HHE, HOE, HNE and HDE formation. It can be seen that in corn, soybean, peanut and canola oils, the rates of HNE formation were much higher than that of HHE, HOE and HDE formation at all the three temperatures. The rates of HHE formation in corn and peanut oils were much lower than that of HOE and HDE formation. With regard to the rate of HNE formation in different oils and fats, except lard, corn oil had the fastest rate of HNE formation compared with the other oils and fats at the same temperature. Then soybean oil had a faster rate of HNE formation than peanut, canola oils and beef tallow. This result was consistent with the linoleic acid concentration in the oils and fats. The rates of HHE formation in soybean and canola oils were relatively higher than that in corn and peanut oils, since soybean and canola oils had higher levels of linolenic acid which indicates that HHE was mainly from linolenic acid.

Table 5. Rates of HHE, HOE, HNE and HDE formation in commercial corn, soybean, peanut, canola oils, lard and beef tallow heated at 185, 165 and 145 ℃ for 5 hours

	Temperature (℃)	HNE (ng/g oil/h)	HHE (ng/g oil/h)	HOE (ng/g oil/h)	HDE (ng/g oil/h)
corn	185	1473	26	149	201
	165	964	29	180	187
	145	288	16	49	45
soybean	185	1247	192	208	117
	165	904	150	279	99
	145	304	88	85	37
peanut	185	821	16	23	167
	165	630	-	-	-
	145	370	-	-	-
canola	185	209	109	91	44
	165	93	59	45	28
	145	88	59	44	15
lard	185	1306	-	-	-
	165	500	-	-	-
	145	372	-	-	-
beef tallow	185	40	-	-	-

E. The Formation of the Sum Total of the Four α, β-Unsaturated 4-Hydroxyaldehydes including HHE, HOE, HNE and HDE in Commercial Corn, Soybean, Peanut and Canola Oils at 145, 165 and 185 ℃ over 5 hours Heating Period

Figure 42 to Figure 45 show the formation of the sum of the 4 α, β-unsaturated-4-hydroxyaldehydes including HHE, HOE, HNE and HDE in corn, soybean, peanut and canola oils. Since HNE formation is the major α, β-unsaturated-4-hydroxyaldehyde among all these four related compounds, the sum of the four compounds mostly followed the HNE formation under the same temperatures and heating periods.

At 165 ℃ and 3 hours of heat treatment, the rate was very high for corn and soybean oils, while the rate was low for peanut oil especially for canola oil. At 185 ℃ and 5 hours of heat treatment, the sum of all the four hydroxyaldehydes reached a maximum concentration. However the rate between 3 and 5 hours seemed to slow down for soybean oil. This could be due to the relatively high level of linolenic acid which is a precursor for HHE and linolenic acid seems to decompose faster than HHE at that temperature. Looking at canola oil which was 2 times higher in linolenic acid concentration than soybean oil, the rate still increased under the same condition. It is possible because that higher concentration of precursor for HHE led to a faster HHE formation than it was being decomposed under similar condition compared with that of soybean oil.

a) Corn oil

Figure 42 shows the formation of the sum of HHE, HOE, HNE and HDE in corn oil at 145, 165 and 185 ℃ over 5 hours of heating period. There was a significant difference in the sum of hydroxyaldehydes formation for 3 hours of heat treatment at 165 ℃ compared to that at 145 ℃. It increased even more at 185 ℃ for 5 hours of heat treatment.

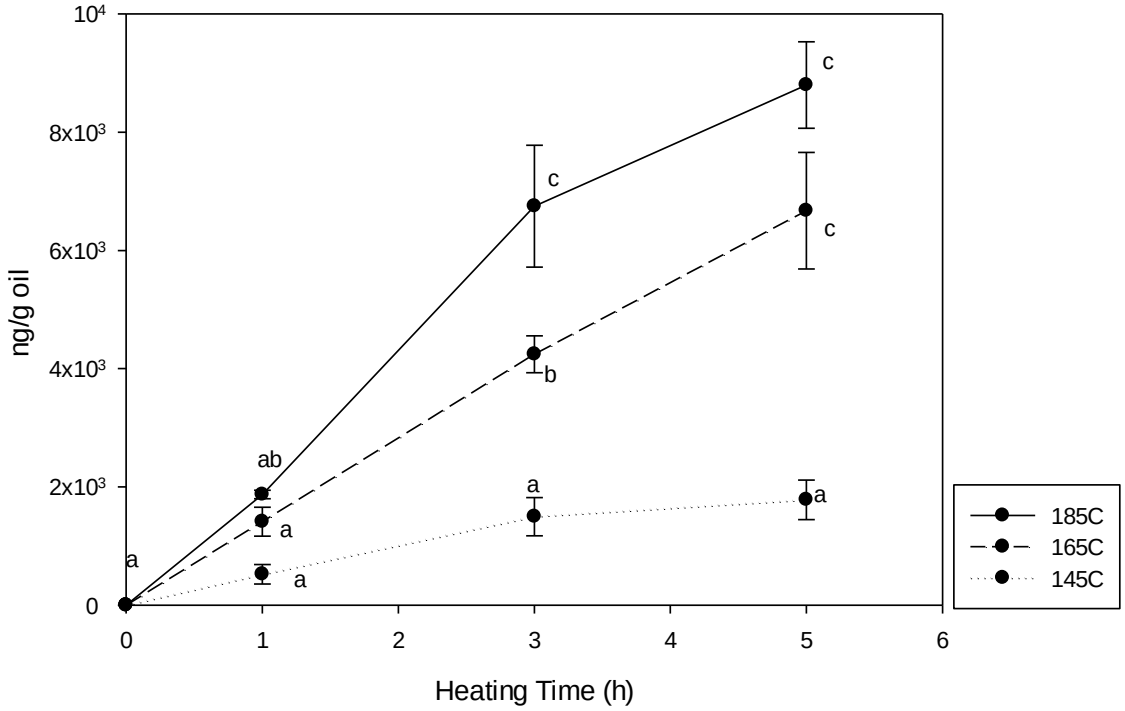


Figure 42. The formation of the sum of four α, β-unsaturated- 4-hydroxyaldehydes including HHE, HOE, HNE and HDE in corn oil at 145, 165 and 185 ℃ over 5 hours heating period

b) Soybean oil

Figure 43 shows the formation of total α, β-unsaturated- 4-hydroxyaldehydes (HHE, HOE, HNE and HDE) in soybean oil. They increased with higher temperatures and longer heating time. The increasing trends at 185 and 165 ℃ were more obvious than the increasing trend at 145 ℃.

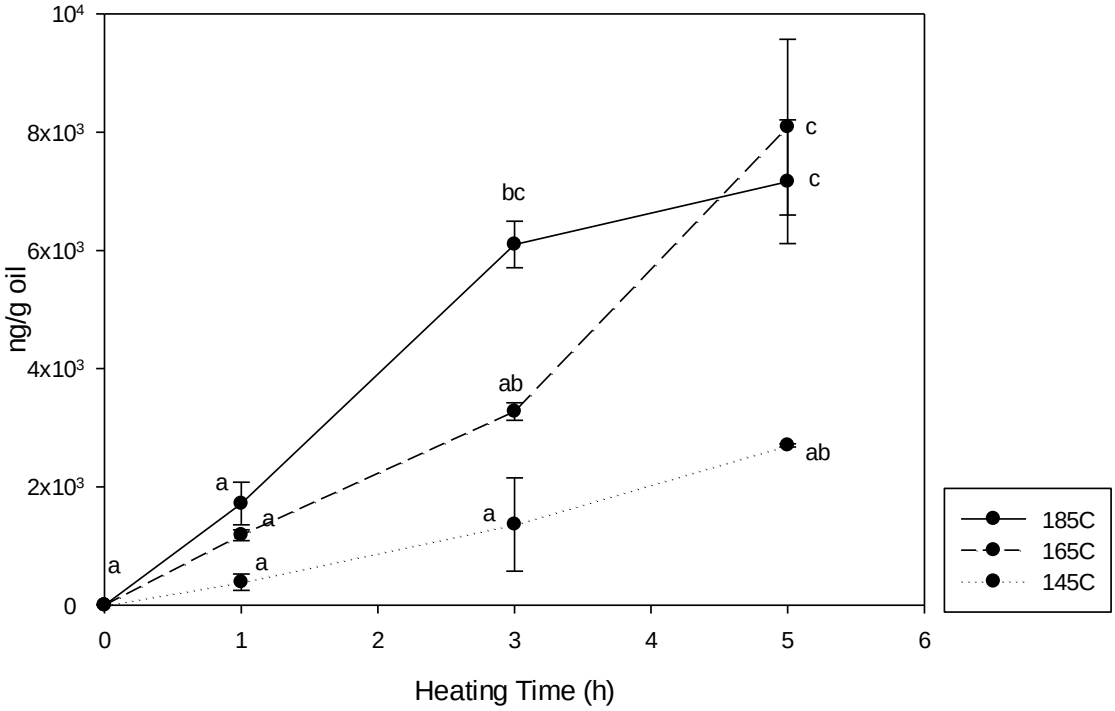


Figure 43. The formation of the sum of four α, β-unsaturated- 4-hydroxyaldehydes including HHE, HOE, HNE and HDE in soybean oil at 145, 165 and 185 ℃ over 5 hours heating period

c) Peanut oil

Figure 44 shows the formation of total α, β-unsaturated- 4-hydroxyaldehydes (HHE, HOE, HNE and HDE) in peanut oil. They increased with higher temperatures and longer heating time. The increasing trend at 145 ℃ was mild compared with that at 165 and185 ℃.

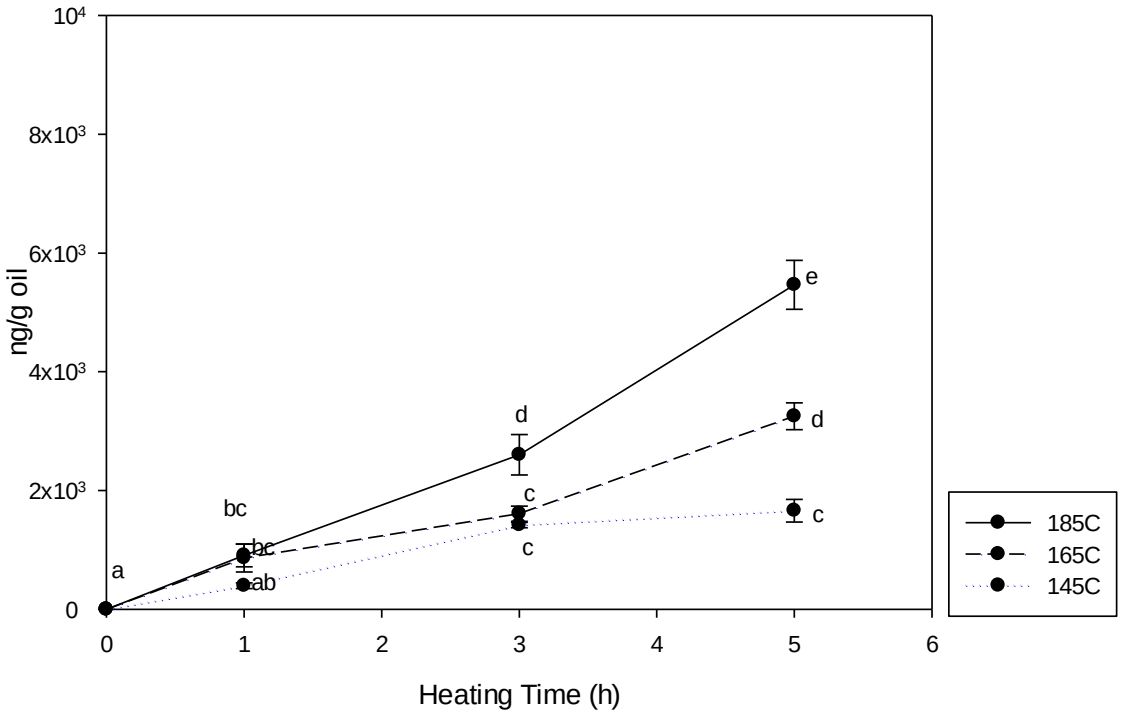


Figure 44. The formation of the sum of four α, β-unsaturated-4- hydroxyaldehydes including HHE, HOE, HNE and HDE in peanut oil at 145, 165 and 185 ℃ over 5 hours heating period

d) Canola oil

Figure 45 shows the formation of total α, β-unsaturated- 4-hydroxyaldehydes (HHE, HOE, HNE and HDE) in canola oil. They increased with higher temperatures and longer heating time. The increasing trend at 185 ℃ was noticeable compared with that at 145 and165 ℃.

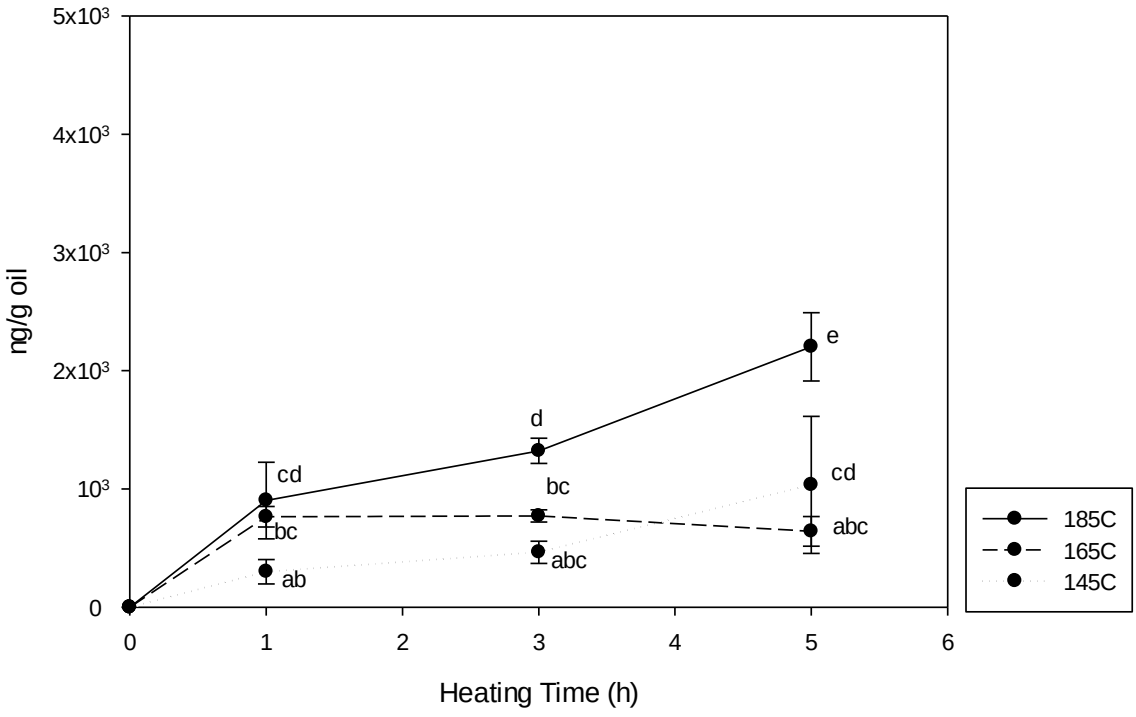


Figure 45. The formation of the sum of four α, β-unsaturated- 4-hydroxyaldehydes including HHE, HOE, HNE and HDE in canola oil at 145, 165 and 185 ℃ over 5 hours heating period

F. The Formation of the Sum Total of Individual Polar Lipophilic Aldehydes in Commercial Corn, Soybean, Peanut, Canola oils, Lard and Beef Tallow Heat Treated for up to 5 Hours at 145, 165 and 185 ℃

Figure 46 to Figure 51 show the formation of the sum total of polar lipophilic aldehydes in corn, soybean, peanut, canola oils, lard and beef tallow, respectively. In unheated lard and beef tallow, small amounts of total polar aldehydes were detected which is possible due to the heat treatment used in rendering and melting process. In the unheated vegetable oils, polar aldehydes were not detected. For the heat-treated oils and fats, the formation of the sum total of polar aldehydes increased with higher temperatures and longer heating time. The increasing trends of total polar aldehydes in corn, soybean, peanut oils and lard were prominent, while the increasing trends of total polar aldehydes in canola oil and beef tallow were low. The sum total of polar aldehydes in beef tallow was so low and it was only detected after 5 hours of heat treatment at 185 ℃.

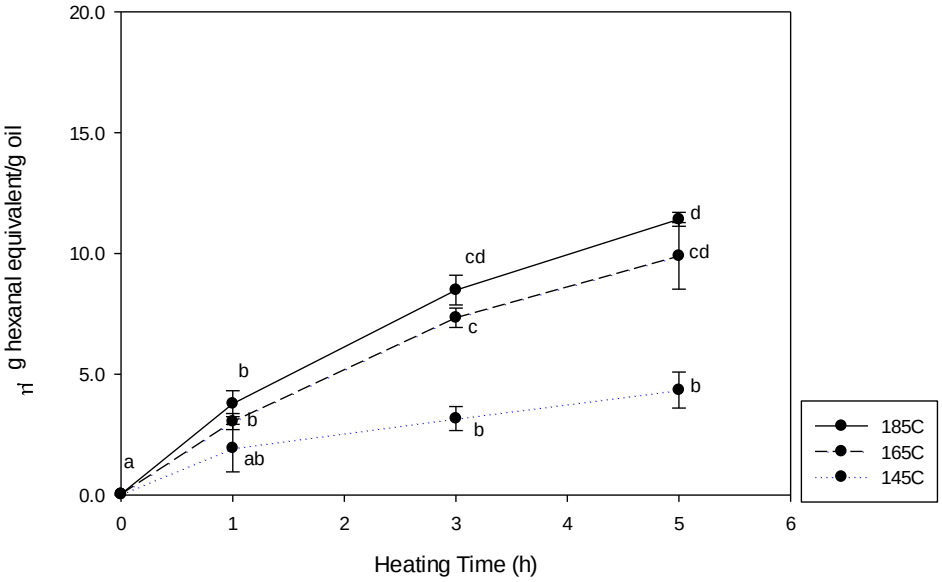


Figure 46. The formation of the sum total of polar lipophilic aldehydes in commercial corn oil heat treated for up to 5 hours at 145, 165 and 185 ℃

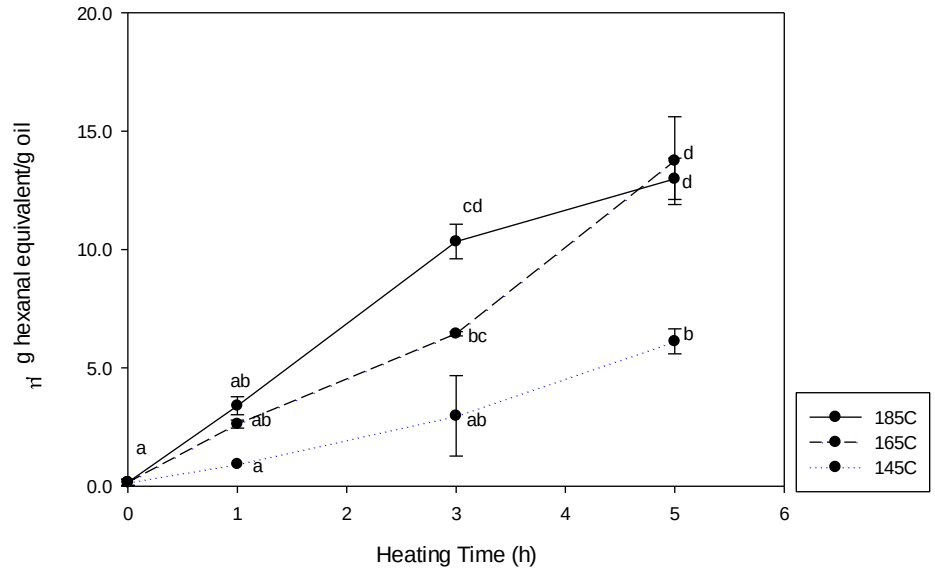


Figure 47. The formation of the sum total of polar lipophilic aldehydes in commercial soybean oil heat treated for up to 5 hours at 145, 165 and 185 ℃

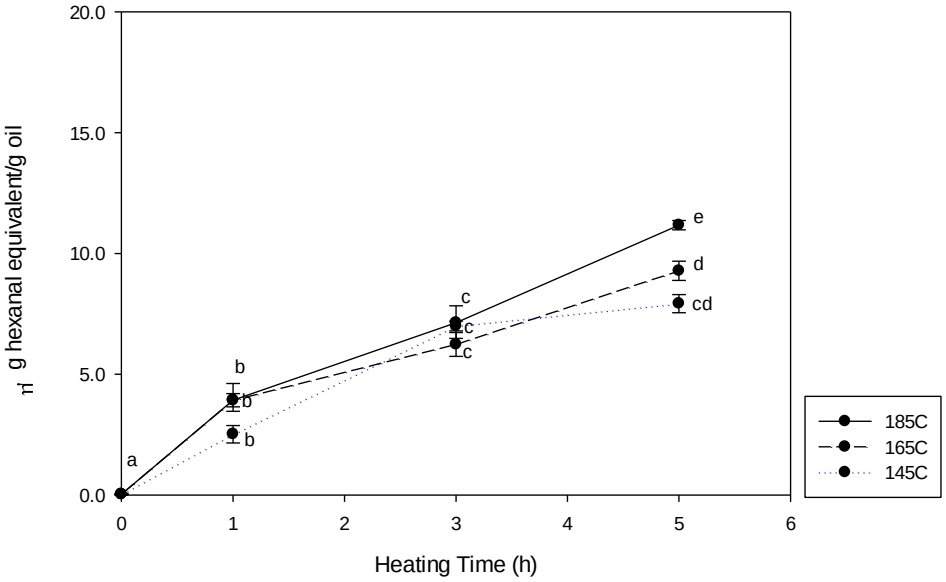


Figure 48. The formation of the sum total of polar lipophilic aldehydes in commercial peanut oil heat treated for up to 5 hours at 145, 165 and 185 ℃

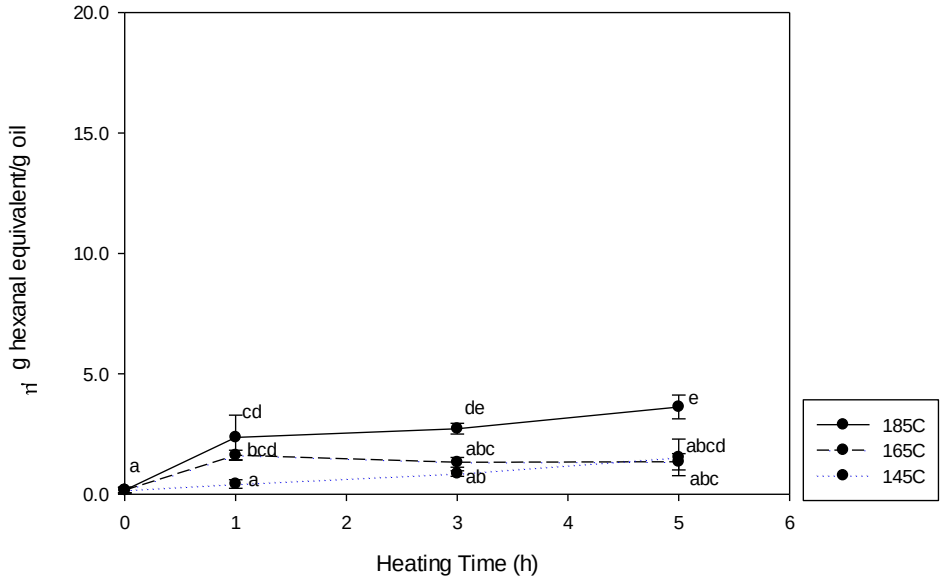


Figure 49. The formation of the sum total of polar lipophilic aldehydes in commercial canola oil heat treated for up to 5 hours at 145, 165 and 185 ℃

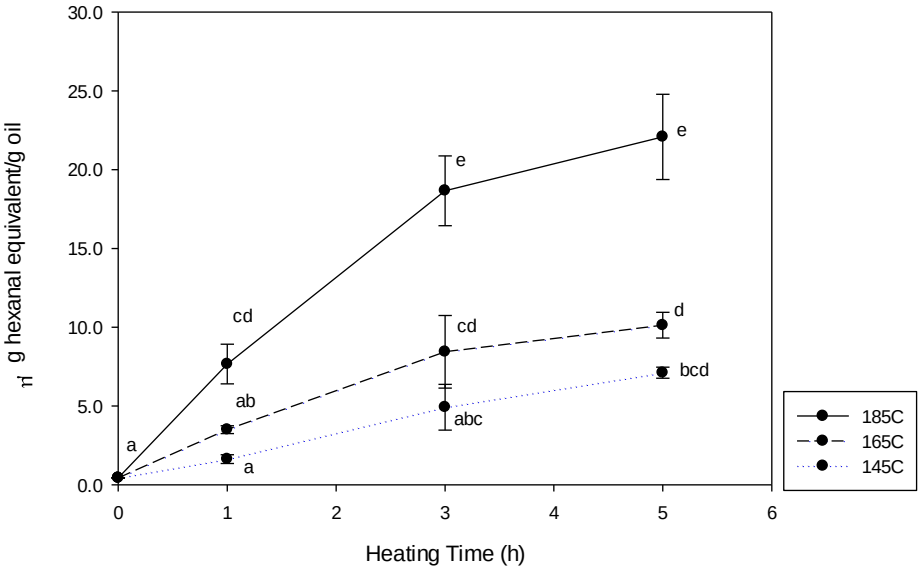


Figure 50. The formation of the sum total of polar lipophilic aldehydes in lard heat treated for up to 5 hours at 145, 165 and 185 ℃

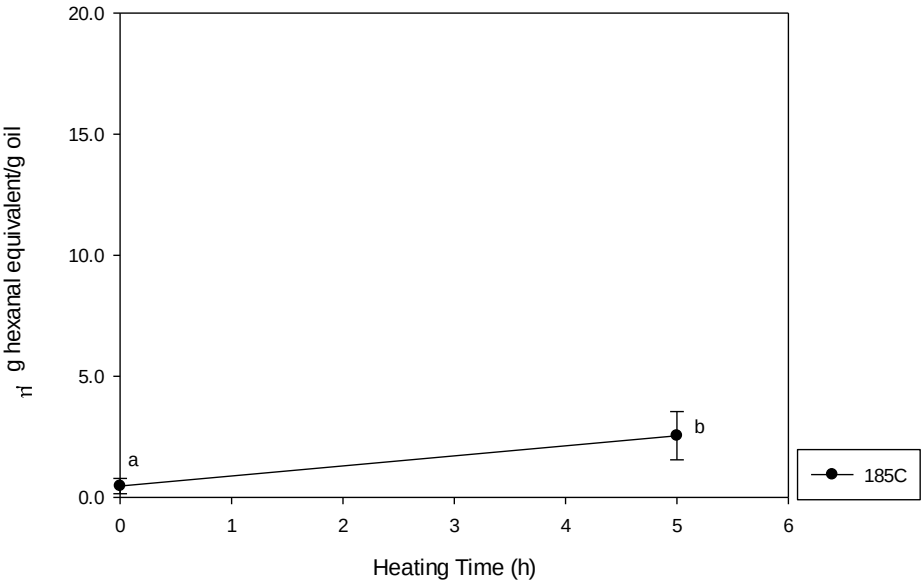


Figure 51. The formation of the sum total of polar lipophilic aldehydes in beef tallow heat treated for up to 5 hours at 185 ℃

Figure 52 (a), (b) and (c) show the comparison of the sum total of polar lipophilic aldehydes formation between commercial corn, soybean, peanut, canola oils, lard and beef tallow which were heat treated for 1, 3 and 5 hours at 185, 165 and 145 ℃. Total polar aldehydes in beef tallow were only measured at 185 ℃ for 5 hours. At 185 ℃ for 5 hours of heat treatment, a very slow formation of polar lipophilic aldehydes was found in beef tallow. At all of the three temperatures and heating time, canola oil was found to contain the lowest level of total polar aldehydes compared with the other oils and lard.

After 1 hour of heating at 145 ℃ (Figure 52 (c)), corn, peanut oils and lard seemed to contain the highest concentration of total polar aldehydes. After 3 and 5 hours of heating at 145 ℃, the concentrations of total polar aldehydes in peanut oil and lard were the highest.

Figure 52 (b) shows that at 165 ℃ for 3 hours of heat treatment, no significant differences were found between corn, soybean, peanut oils and lard. After 5 hours of heating at the same temperature, soybean oil was significantly higher than corn, peanut oils and lard.

Figure 52 (a) shows that at 185 ℃ for 1 hour of heat treatment, lard was significantly higher than the four vegetable oils. At 3 hours of heat treatment, canola oil remained the same as at 1 hour of heating. Corn, soybean and peanut oils were significantly higher than canola oil and significantly lower than lard. After 5 hours of heating, the significant

differences remained the same as at 3 hours of heating but beef tallow had similarly significant concentration as canola oil.

The relatively high concentration of the sum total of individual polar aldehydes under all heat treatment conditions in lard was not expected because its unsaturated fatty acid concentration was very low – linoleic acid concentration was only 8.2% and linolenic acid concentration was not measurable. Both of these fatty acids are the precursors for the polar aldehydes. It was postulated that since this is an animal fat it might contain some catalysts which helped increase polar aldehydes formation especially at the highest temperature of 185 ℃ and the increasing heating time.

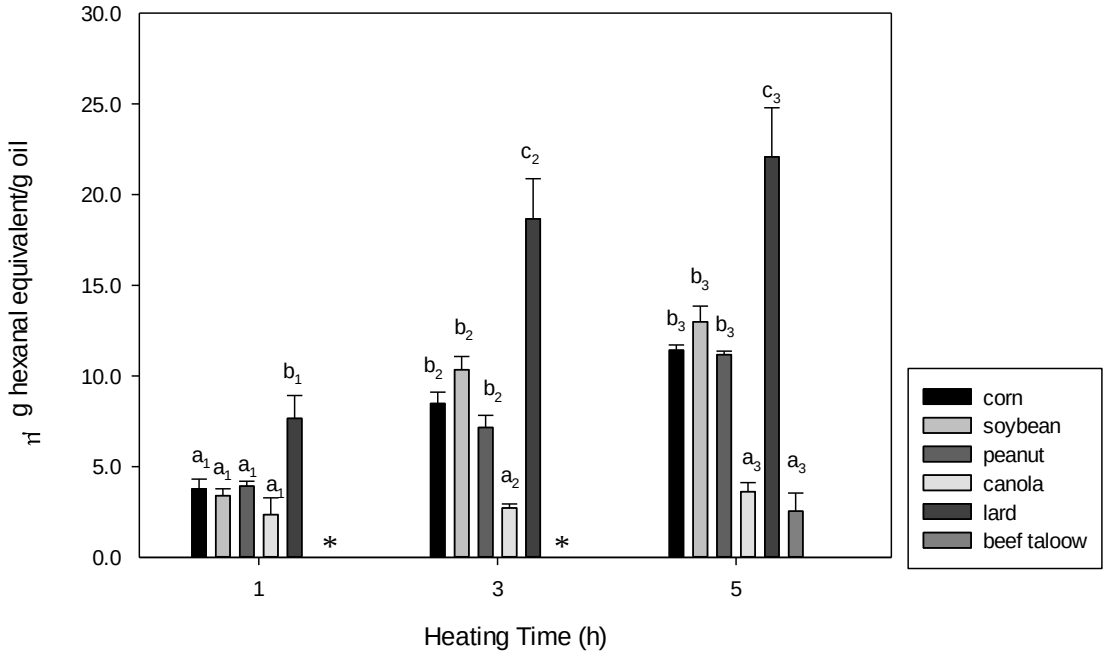


Figure 52 (a). The formation of the sum total of individual polar lipophilic aldehydes in commercial corn, soybean, peanut, canola oils, lard and beef tallow heat treated for 1, 3 and 5 hours at 185 ℃

* means no measurement.

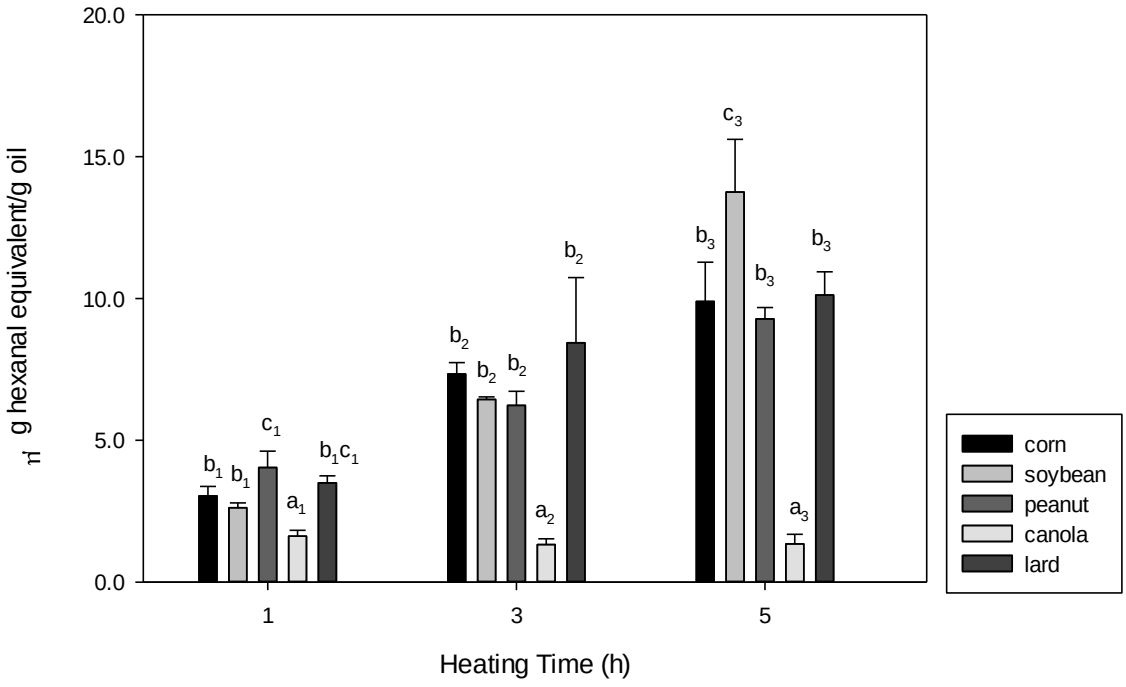


Figure 52 (b). The formation of the sum total of individual polar lipophilic aldehydes in commercial corn, soybean, peanut, canola oils, lard and beef tallow heat treated for 1, 3 and 5 hours at 165 ℃

* Beef tallow was not measured.

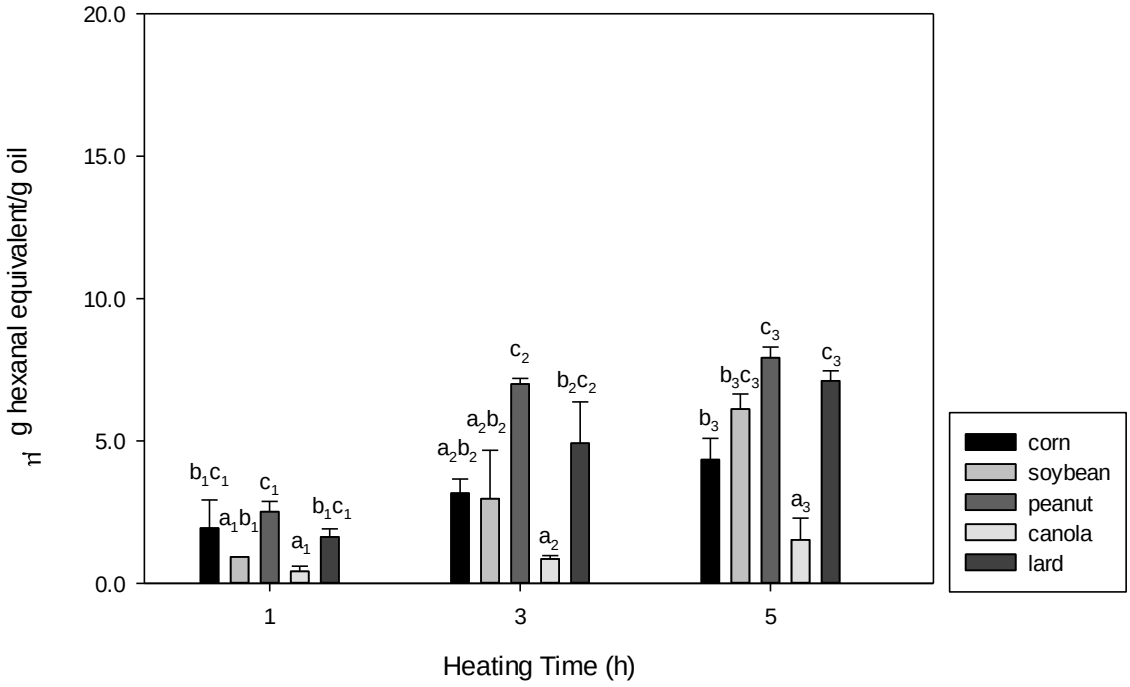


Figure 52 (c). The formation of the sum total of individual polar lipophilic aldehydes in commercial corn, soybean, peanut, canola oils, lard and beef tallow heat treated for 1, 3 and 5 hours at 145 ℃

* Beef tallow was not measured.

Table 6 shows the rates of total polar aldehydes formation in commercial corn, soybean, peanut, canola oils, lard and beef tallow heated at 185, 165 and 145 ℃ for 5 hour. Higher temperature led to a faster rate of total polar aldehydes formation in these oils and fats. Corn, soybean, peanut oils and lard had relatively faster rates of total polar aldehydes formation than canola oil and beef tallow.

Table 6. Rates of total polar aldehydes formation in commercial corn, soybean, peanut, canola oils, lard and beef tallow heated at 185, 165 and 145 ℃ for 5 hours

	Temperature (℃)	Total Polar Aldehydes (μg hexanal equivalent/g oil/h)
corn	185	2.14
	165	1.88
	145	0.74
soybean	185	2.63
	165	2.65
	145	1.19
peanut	185	2.11
	165	1.66
	145	1.61
canola	185	0.54
	165	0.11
	145	0.26
lard	185	4.13
	165	1.87
	145	1.37
beef tallow	185	0.42

G. The Formation of the Sum Total of Individual Nonpolar Lipophilic Aldehydes in Commercial Corn, Soybean, Peanut, Canola oils, Lard and Beef Tallow Heat Treated for up to 5 Hours at 145, 165 and 185 ℃

Figure 53 to Figure 58 show the formation of the sum total of nonpolar lipophilic aldehydes in corn, soybean, peanut, canola oils, lard and beef tallow, respectively. In unheated oils and fats, the sum total of nonpolar lipophilic aldehydes was 100 μg hexanal equivalent/g oil. For vegetable oils such as corn, soybean, peanut and canola oils, the sum total of nonpolar aldehydes increased slightly with increasing heating time. The nonpolar aldehydes were mostly volatile and likely evaporated as they were forming in the oils and fats during the heating process. There was almost no significant difference between the heating time and temperatures. For the animal fats like lard and beef tallow, the sum total of nonpolar aldehydes decreased with longer heating time. The decreasing trend was more obvious at higher temperatures. This suggests that the secondary oxidation products of nonpolar aldehydes were volatile short-chain aldehydes. Results were represented as μg hexanal equivalent/g oil since the individual aldehyde molecular weights are unknown.

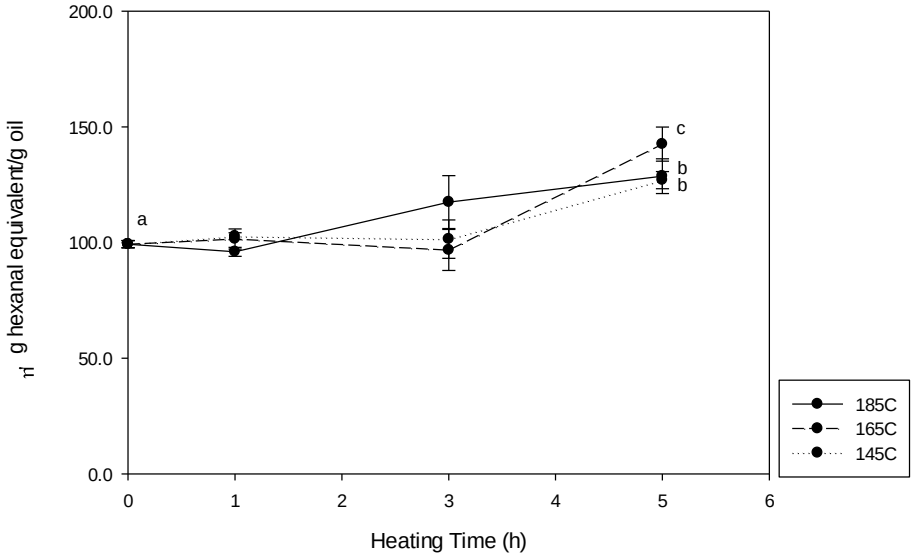


Figure 53. The formation of the sum total of nonpolar lipophilic aldehydes in commercial corn oil heat treated for up to 5 hours at 145, 165 and 185 ℃

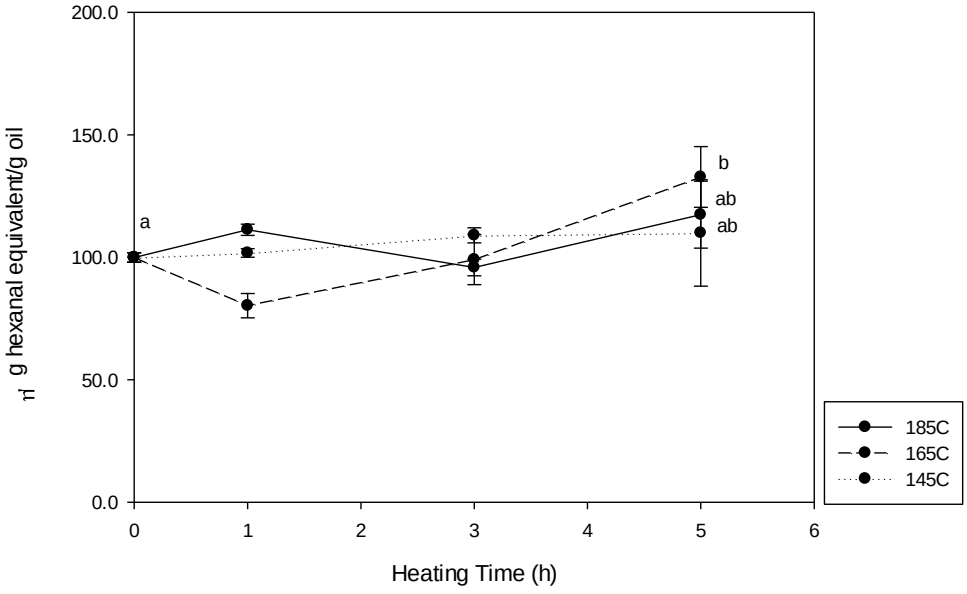


Figure 54. The formation of the sum total of nonpolar lipophilic aldehydes in commercial soybean oil heat treated for up to 5 hours at 145, 165 and 185 ℃

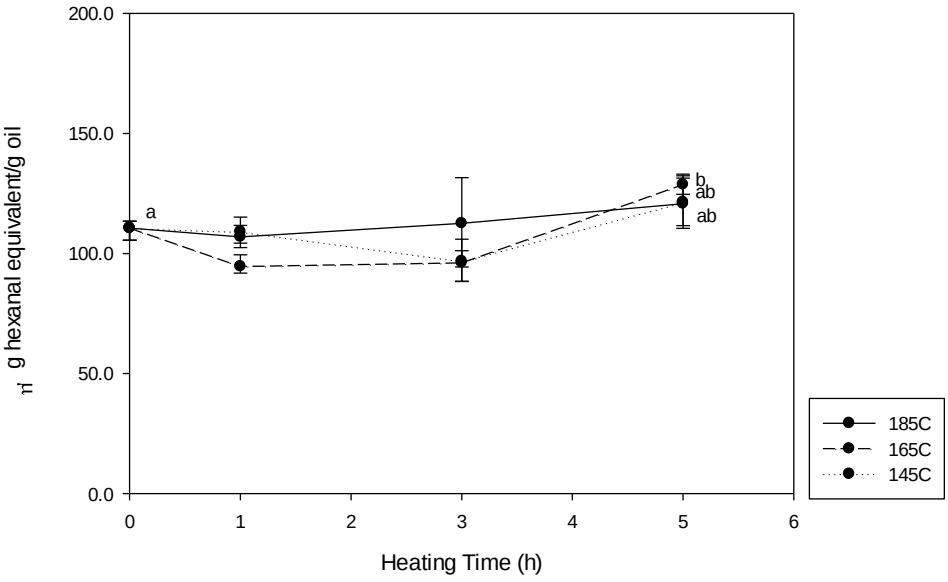


Figure 55. The formation of the sum total of nonpolar lipophilic aldehydes in commercial peanut oil heat treated for up to 5 hours at 145, 165 and 185 ℃

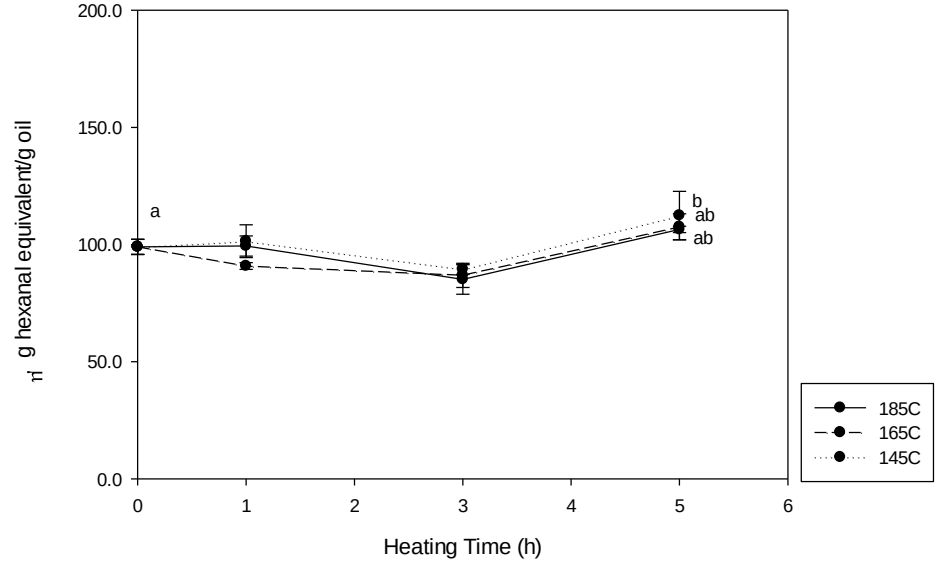


Figure 56. The formation of the sum total of nonpolar lipophilic aldehydes in commercial canola oil heat treated for up to 5 hours at 145, 165 and 185 ℃

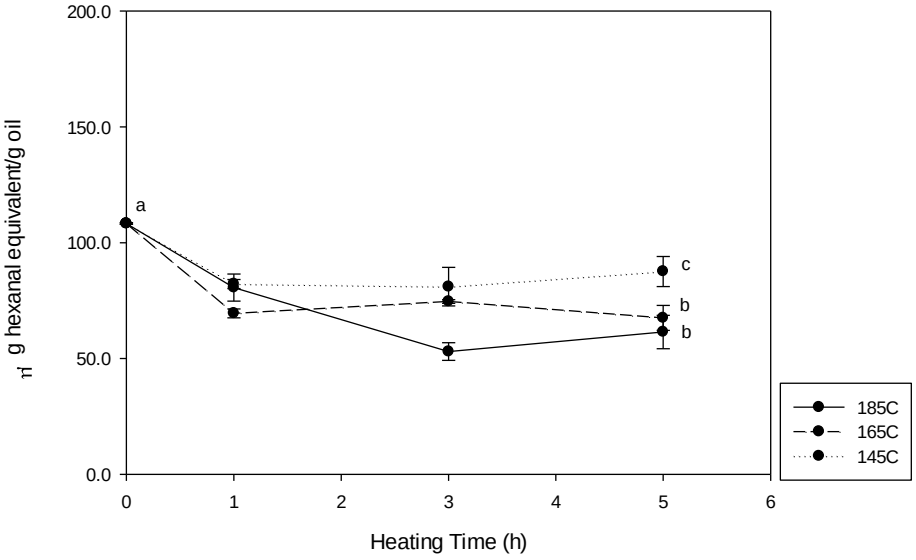


Figure 57. The formation of the sum total of nonpolar lipophilic aldehydes in lard heat treated for up to 5 hours at 145, 165 and 185 ℃

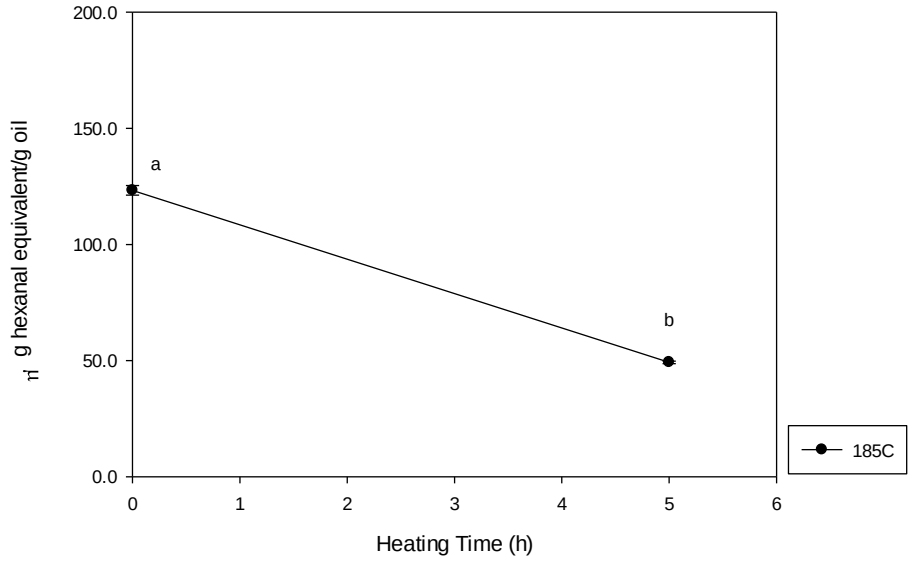


Figure 58. The formation of the sum total of nonpolar lipophilic aldehydes in beef tallow oil heat treated for up to 5 hours at 185 ℃

Figure 59 (a), (b) and (c) show the comparison of the formation of the sum total of individual nonpolar lipophilic aldehydes between commercial corn, soybean, peanut, canola oils, lard and beef tallow which were heat treated for 1,3 and 5 hours at 185 ℃, 165 ℃ and 145 ℃.

At the shortest heating time of 1 hour and lowest temperature 145 ℃ (Figure 59 (c)), the concentrations of the total nonpolar aldehydes of the four oils were significantly higher than that of lard. Lard remained the lowest concentration compared to the four oils as the heating time increased to 3 and 5 hours.

At 165 ℃ (Figure 59 (b)), with the increasing heating time, lard remained containing the significantly lower concentration of nonpolar aldehydes than that of the four oils. At this temperature and heat treatment of 5 hours, the concentrations of the total nonpolar aldehydes of corn, soybean and canola oils were significantly higher than that of canola oil. And canola oil was significantly higher than lard.

At frying temperature of 185 ℃ for 1, 3 and 5 hours of heat treatment (Figure 59 (a)), lard remained significantly lower than all of the four vegetable oils. In the case of 3 and 5 hours of heat treatment, corn, soybean and peanut oils seemed to be very similarly higher than canola oil, while lard was significantly lower than all the four oils. Beef tallow was only measured for total nonpolar aldehydes at 185 ℃ after 5 hours of heat treatment. It seems that especially at the higher temperature and longer heating period, the nonpolar

aldehydes evaporation rate was faster than the rate at lower temperature and shorter heating period.

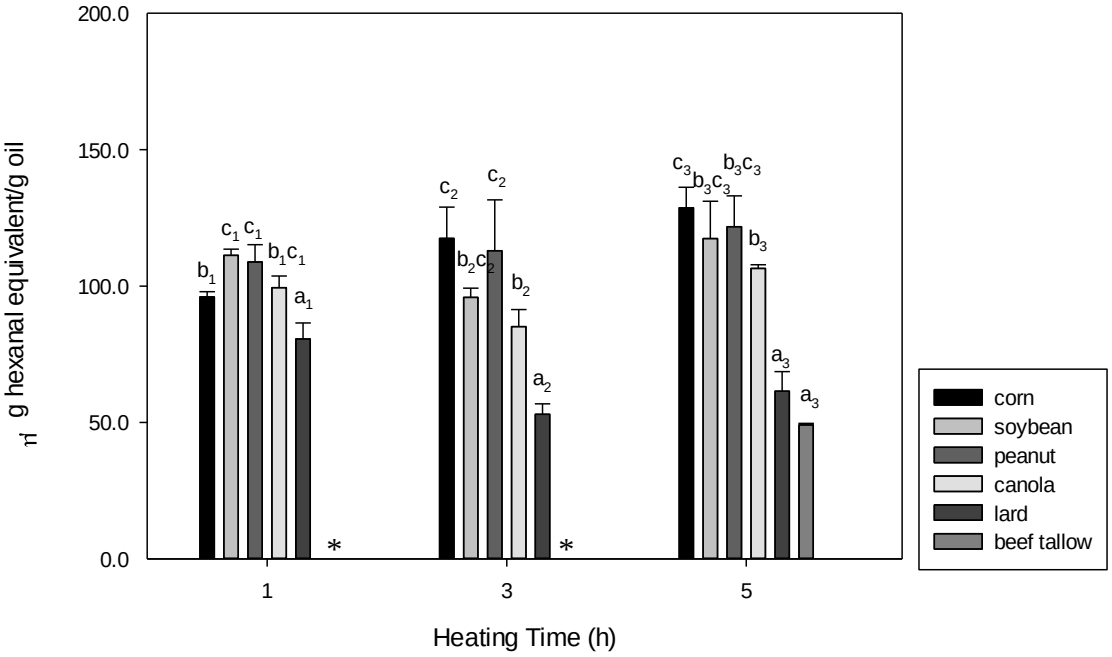


Figure 59 (a). The formation of the sum total of individual nonpolar lipophilic aldehydes in commercial corn, soybean, peanut, canola oils, lard and beef tallow heat treated for 1, 3 and 5 hours at 185 ℃

* means no measurement.

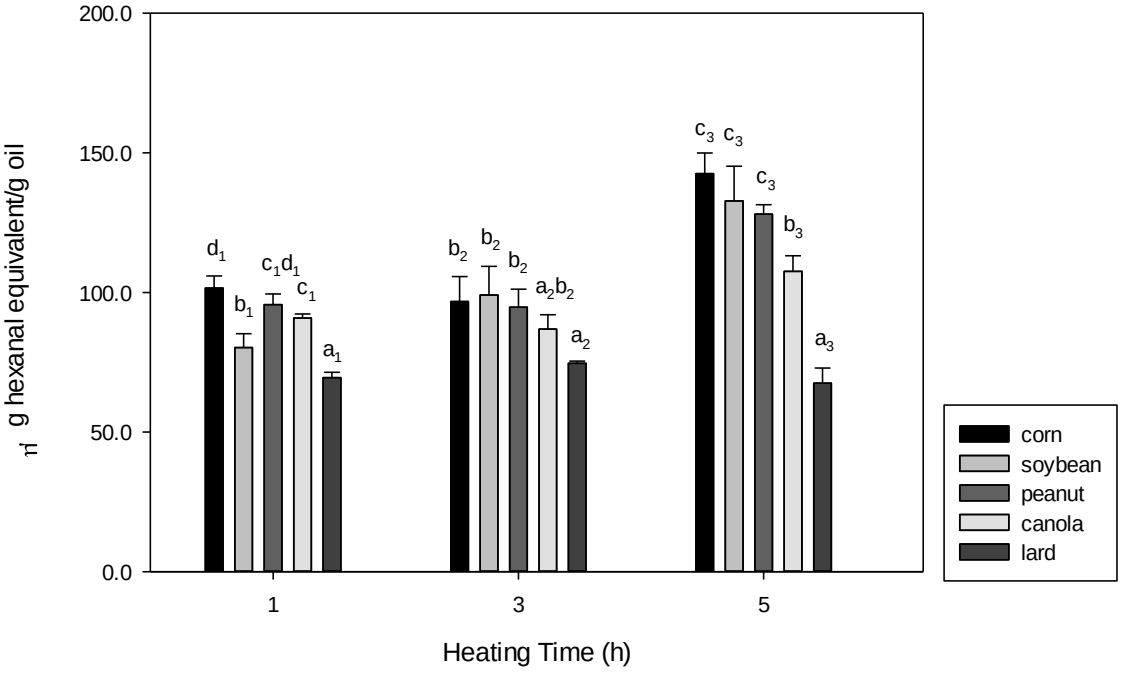


Figure 59 (b). The formation of the sum total of individual nonpolar lipophilic aldehydes in commercial corn, soybean, peanut, canola oils, lard and beef tallow heat treated for 1, 3 and 5 hours at 165 ℃

* Beef tallow was not measured.

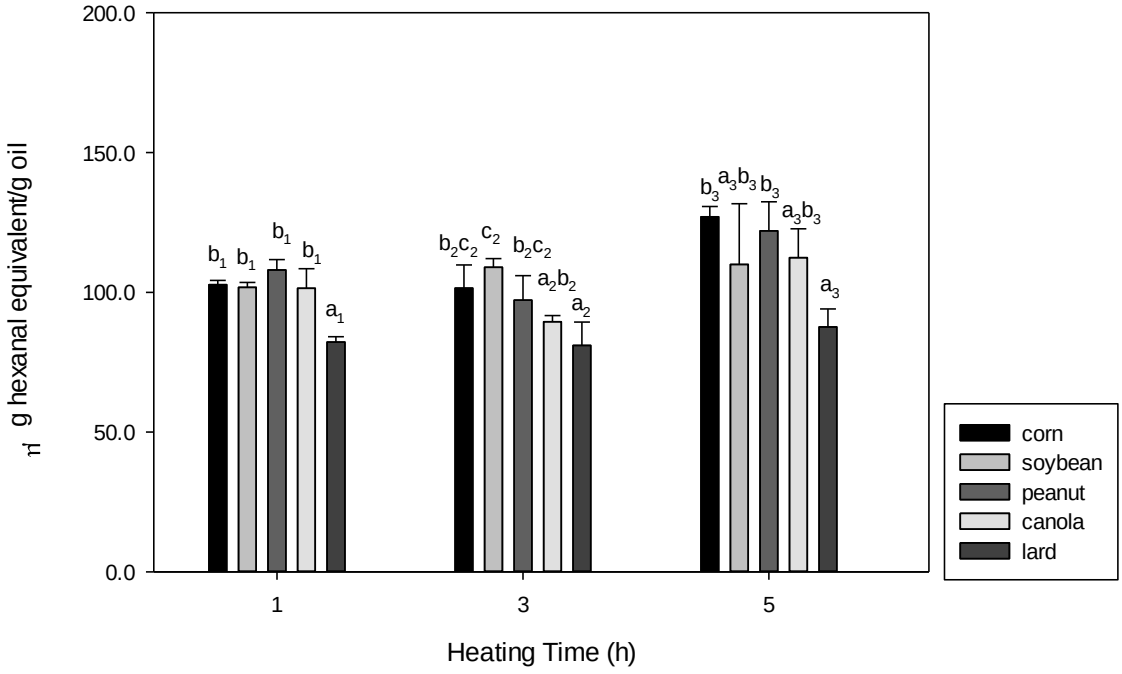


Figure 59 (c). The formation of the sum total of individual nonpolar lipophilic aldehydes in commercial corn, soybean, peanut, canola oils, lard and beef tallow heat treated for 1, 3 and 5 hours at 145 ℃

* Beef tallow was not measured.

Table 7 shows the rates of total nonpolar aldehydes formation in commercial corn, soybean, peanut, canola oils, lard and beef tallow heated at 185, 165 and 145 ℃ for 5 hour. In the vegetable oils, corn, soybean, peanut and canola oils, the rates of total nonpolar aldehydes

formation were positive which means the total nonpolar aldehydes formation increased with longer heating time. While in the fats, lard and beef tallow, the rates of total nonpolar aldehydes were negative. Because nonpolar aldehydes are volatile and evaporate easily, the HPLC method used in this experiment only measured the nonpolar aldehydes retained in the oils and fats after heat treatment.

Table 7. Rates of total nonpolar aldehydes formation in commercial corn, soybean, peanut, canola oils, lard and beef tallow heated at 185, 165 and 145 ℃ for 5 hours

	Temperature (℃)	Total Nonpolar Aldehydes (μg hexanal equivalent/g oil/h)
corn	185	6.68
	165	7.74
	145	4.98
soybean	185	2.10
	165	7.76
	145	2.17
peanut	185	2.53
	165	3.88
	145	1.84
canola	185	0.65
	165	1.67
	145	1.87
lard	185	-8.68
	165	-5.37
	145	-2.52
beef tallow	185	-14.82

Discussion

From the present experiments, we can conclude that the formation of the α, β-unsaturated 4-hydroxyaldehydes, HHE, HOE, HNE and HDE, increased with elevated temperatures from 145 ℃ to 165 ℃ and to 185 ℃ in commercial corn, soybean, peanut and canola oils. At all of these temperatures, with the increased time of heat treatment from 0 to 1, 3 up to 5 hours, the oxidation rate increased which led to the higher concentration of hydroxyaldehydes. The combination of the three factors, unsaturation, temperature, and time of heat treatment, resulted in highly increased HHE, HOE, HNE and HDE formation. The concentration of HNE, the most toxic compound of the four α, β- unsaturated 4-hydroxyaldehydes, was much higher than other three 4-hydroxyaldehydes in all samples. It was found that the oils which contained the higher concentration of linoleic acid had higher HNE formations compared to the oils with lower level of linoleic acid. This was expected because linoleic acid is a precursor of HNE. Soybean and canola oils which contained relatively high levels of linolenic acid had higher HHE formation than the low linolenic acid containing oils and fats. In beef tallow, HNE formation was found to be very low even after 5 hours of heating at 185 ℃.At lower temperatures, it could not even be detected. Lard seemed to be an exception. Its HNE formation was very high but its linoleic acid concentration was relatively low. The reason may be that since it is an animal fat. It may contain some

compounds which were catalyzing the peroxidation process and cause the increase of the formation of secondary oxidation products including the α, β-unsaturated 4-hydroxyaldehydes. This question should be further investigated in the future.

Although our objective was to measure the formation and concentration of α,β-unsaturated 4- hydroxyaldehydes in commercial oils and fats, it should be noted that the sum total of individual polar aldehydes measured by HPLC also increased and remained in the oils due to longer heating time and higher temperatures. Total nonpolar aldehydes remaining in the oil increased slightly with increased temperatures and heating time in vegetable oils but decreased in lard and beef tallow. The change of total nonpolar aldehydes which remained in the oil was related to their formation and the evaporation. The short-chain nonpolar aldehydes easily evaporated at higher temperatures and did not remain in the animal fats due to longer heating time.

Since the α,β-unsaturated 4- hydroxyaldehydes are very reactive toxic compounds and they have been shown to relate to a number of diseases, it is important to understand their formation in commercial oils and fats which are used mostly at high temperatures especially in commercial frying. Based on the results of this study, the oils or fats that have low level of unsaturated fatty acids which produce less toxic 4- hydroxyaldehydes should be used especially for commercial frying. To minimize the formation of α, β-

unsaturated 4-hydroxyaldehydes in high linoleic and linolenic acids containing oils in food, the lowest possible temperature and shortest heating time should be used.

Since HNE is readily absorbed from the diet, its toxicity is well established in the literature, and it is related to a number of pathological conditions, frequent consumption of heated oils that contain considerable HNE, a toxic aldehyede, may be of public health concern.

References

1. Liu, Q., A.K. Raina, M.A. Smith, L.M. Sayre, and G. Perry, Hydroxynonenal, toxic carbonyls, and Alzheimer disease, *Mol Aspects Med* 24:305-313 (2003).

2. Reed, T.T., Lipid peroxidation and neurodegenerative disease, *Free Radical Bio Med* 51:1302-1319 (2011).

3. Uchida, K., S. Toyokuni, K. Nishikawa, S. Kawakishi, H. Oda, H. Hiai, and E.R. Stadtman, Michael Addition-Type 4-Hydroxy-2-Nonenal Adducts in Modified Low-Density Lipoproteins - Markers for Atherosclerosis, *Biochemistry-Us* 33:12487-12494 (1994).

4. Yoritaka, A., N. Hattori, K. Uchida, M. Tanaka, E.R. Stadtman, and Y. Mizuno, Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease, *P Natl Acad Sci USA* 93:2696-2701 (1996).

5. Grootveld, M., M.D. Atherton, A.N. Sheerin, J. Hawkes, D.R. Blake, T.E. Richens, C.J. Silwood, E. Lynch, and A.W. Claxson, In vivo absorption, metabolism, and urinary excretion of alpha,beta-unsaturated aldehydes in experimental animals. Relevance to the development of cardiovascular diseases by the dietary ingestion of thermally stressed polyunsaturate-rich culinary oils, *J Clin Invest* 101:1210-1218 (1998).

6. Kruman, I., A.J. BruceKeller, D. Bredesen, G. Waeg, and M.P. Mattson, Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis, *J Neurosci* 17:5089-5100 (1997).

7. Mark, R.J., M.A. Lovell, W.R. Markesbery, K. Uchida, and M.P. Mattson, A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide, *J Neurochem* 68:255-264 (1997).

8. Seppanen, C.M., and A.S. Csallany, Incorporation of the toxic aldehyde 4-hydroxy-2-trans-nonenal into food fried in thermally oxidized soybean oil, *J Am Oil Chem Soc* 81:1137-1141 (2004).

9. LaFond, S.I., J.P. Jerrell, K.R. Cadwallader, and W.E. Artz, Formation of 4-Hydroxy-2-(E)-Nonenal in a Corn-Soy Oil Blend: a Controlled Heating Study Using a French Fried Potato Model, *J Am Oil Chem Soc* 88:763-772 (2011).

10. Seppanen, C.M., and A.S. Csallany, Simultaneous determination of lipophilic aldehydes by high-performance liquid chromatography in vegetable oil, *J Am Oil Chem Soc* 78:1253-1260 (2001).

11. Seppanen, C.M., and A.S. Csallany, The effect of intermittent and continuous heating of soybean oil at frying temperature on the formation of

4-hydroxy-2-trans-nonenal and other alpha-, beta-unsaturated hydroxyaldehydes, *J Am Oil Chem Soc* 83:121-127 (2006).

12. Surh, J., and H. Kwon, Estimation of daily exposure to 4-hydroxy-2-alkenals in Korean foods containing n-3 and n-6 polyunsaturated fatty acids, *Food Addit Contam* 22:701-708 (2005).

13. Lang, J., C. Celotto, and H. Esterbauer, Quantitative-Determination of the Lipid-Peroxidation Product 4-Hydroxynonenal by High-Performance Liquid-Chromatography, *Anal Biochem* 150:369-378 (1985).

14. Boskou, G., F.N. Salta, A. Chiou, E. Troullidou, and N.K. Andrikopoulos, Content of trans,trans-2,4-decadienal in deep-fried and pan-fried potatoes, *European Journal of Lipid Science and Technology* 108:109-115 (2006).

15. Choe, E., and D.B. Min, Chemistry of deep-fat frying oils, *J Food Sci* 72:R77-R86 (2007).

16. Barrera-Arellano, D., V. Ruiz-Mendez, J. Velasco, G. Marquez-Ruiz, and C. Dobarganes, Loss of tocopherols and formation of degradation compounds at frying temperatures in oils differing in degree of unsaturation and natural antioxidant content, *J Sci Food Agr* 82:1696-1702 (2002).

17. Zeb, A., Effects of beta-carotene on the thermal oxidation of fatty acids, *Afr J Biotechnol* 10:15346-15352 (2011).

18. Tyagi, V.K., and A.K. Vasishtha, Changes in the characteristics and composition of oils during deep-fat frying, *J Am Oil Chem Soc* 73:499-506 (1996).

19. Fennema, O.R., Food chemistry, Marcel Dekker, New York, 1996.

20. Chan, H.W.S., and G. Levett, Autoxidation of Methyl Linoleate, Separation and Analysis of Isomeric Mixtures of Methyl Linoleate Hydroperoxides and Methyl Hydroxylinoleates, *Lipids* 12:99-104 (1977).

21. Haywood, R.M., A.W.D. Claxson, G.E. Hawkes, D.P. Richardson, D.P. Naughton, G. Coumbarides, J. Hawkes, E.J. Lynch, and M.C. Grootveld, Detection of Aldehydes and Their Conjugated Hydroperoxydiene Precursors in Thermally-Stressed Culinary Oils and Fats - Investigations Using High-Resolution Proton Nmr-Spectroscopy, *Free Radical Res* 22:441-482 (1995).

22. Choe, E., and D.B. Min, Mechanisms and factors for edible oil oxidation, *Compr Rev Food Sci F* 5:169-186 (2006).

23. Esterbauer, H., Aldehydic products of lipid peroxidation, *Free radicals, lipid peroxidation and cancer*:101-128 (1982).

24. Guillén, M.D., and A. Ruiz, Monitoring of heat-induced degradation of edible oils by proton NMR, *European Journal of Lipid Science and Technology* 110:52-60 (2008).

25. Esterbauer, H., R.J. Schaur, and H. Zollner, Chemistry and Biochemistry of 4-Hydroxynonenal, Malonaldehyde and Related Aldehydes, *Free Radical Bio Med* 11:81-128 (1991).

26. Coscione, A.R., and W.E. Artz, Vegetable Oil Stability at Elevated Temperatures in the Presence of Ferric Stearate and Ferrous Octanoate, *Journal of Agricultural and Food Chemistry* 53:2088-2094 (2005).

27. Aladedunye, F.A., and R. Przybylski, Degradation and Nutritional Quality Changes of Oil During Frying, *Journal of the American Oil Chemists' Society* 86:149-156 (2008).

28. Houhoula, D.P., V. Oreopoulou, and C. Tzia, A kinetic study of oil deterioration during frying and a comparison with heating, *J Am Oil Chem Soc* 79:133-137 (2002).

29. Chung, T.Y., J.P. Eiserich, and T. Shibamoto, Volatile Compounds Identified in Headspace Samples of Peanut Oil Heated under Temperatures Ranging from 50-Degrees-C to 200-Degrees-C, *Journal of Agricultural and Food Chemistry* 41:1467-1470 (1993).

30. Goicoechea, E., and M.D. Guillen, Analysis of Hydroperoxides, Aldehydes and Epoxides by (1)H Nuclear Magnetic Resonance in Sunflower Oil Oxidized at 70 and 100 degrees C, *J Agr Food Chem* 58:6234-6245 (2010).

31. Paul, S., and G.S. Mittal, Regulating the use of degraded oil/fat in deep-fat/oil food frying, *Crit Rev Food Sci* 37:635-662 (1997).

32. Gere, A., Study of Some Factors Affecting Frying Fat Deterioration, *Fett Wiss Technol* 85:18-23 (1983).

33. Claxson, A.W.D., G.E. Hawkes, D.P. Richardson, D.P. Naughton, R.M. Haywood, C.L. Chander, M. Atherton, E.J. Lynch, and M.C. Grootveld, Generation of Lipid-Peroxidation Products in Culinary Oils and Fats during Episodes of Thermal Stressing - a High-Field H-1-Nmr Study, *Febs Lett* 355:81-90 (1994).

34. Han, I.H., and A.S. Csallany, Formation of Toxic alpha,beta-Unsaturated 4-Hydroxy-Aldehydes in Thermally Oxidized Fatty Acid Methyl Esters, *J Am Oil Chem Soc* 86:253-260 (2009).

35. Grein, B., M. Huffer, G. Scheller, and P. Schreier, 4-Hydroxy-2-Alkenals and Other Products Formed by Water-Mediated Oxidative Decomposition of Alpha,Beta-Unsaturated Aldehydes, *J Agr Food Chem* 41:2385-2390 (1993).

36. Schneider, C., K.A. Tallman, N.A. Porter, and A.R. Brash, Two distinct pathways of formation of 4-hydroxynonenal. Mechanisms of non-enzymatic transformation of the 9- and 13-hydroperoxides of linoleic acid to 4-hydroxyalkenals. (vol 276, pg 20831, 2001), *Journal of Biological Chemistry* 276:32392-32392 (2001).

37. Schneider, C., K.A. Tallman, N.A. Porter, and A.R. Brash, Two distinct pathways of formation of 4-hydroxynonenal - Mechanisms of nonenzymatic transformation of the

9-and 13-hydroperoxides of linoleic acid to 4-hydroxyalkenals, *J. Biol. Chem.* 276:20831-20838 (2001).

38. Fullana, A., A.A. Carbonell-Barrachina, and S. Sidhu, Volatile aldehyde emissions from heated cooking oils, *J Sci Food Agr* 84:2015-2021 (2004).

39. Zhu, X.D., K.X. Wang, J.L. Zhu, and M.R. Koga, Analysis of cooking oil fumes by ultraviolet spectrometry and gas chromatography-mass spectrometry, *J Agr Food Chem* 49:4790-4794 (2001).

40. Barrera-Arellano, D., V. Ruiz-M&#65533;ndez, J.n. Velasco, G. M&#65533;rquez-Ruiz, and C. Dobarganes, Loss of tocopherols and formation of degradation compounds at frying temperatures in oils differing in degree of unsaturation and natural antioxidant content, *Journal of the Science of Food and Agriculture* 82:1696-1702 (2002).

41. Esterbauer, H., Cytotoxicity and Genotoxicity of Lipid-Oxidation Products, *Am J Clin Nutr* 57:S779-S786 (1993).

42. Gardner, D.R., R.A. Sanders, D.E. Henry, D.H. Tallmadge, and H.W. Wharton, Characterization of Used Frying Oils .1. Isolation and Identification of Compound Classes, *J Am Oil Chem Soc* 69:499-508 (1992).

43. Becalski, A., B.P.Y. Lau, D. Lewis, and S.W. Seaman, Acrylamide in foods: Occurrence, sources, and modeling, *Journal of Agricultural and Food Chemistry* 51:802-808 (2003).

44. International Agency for Research on Cancer. IARC Monographs on the EValuation of Carcinogenic Risks to Humans; Lyon, France, 1994; Vol. 60, pp 389-433.

45. Erkkila, A., V.D.F. de Mello, U. Riserus, and D.E. Laaksonen, Dietary fatty acids and cardiovascular disease: An epidemiological approach, *Prog Lipid Res* 47:172-187 (2008).

46. Yashodhara, B.M., S. Umakanth, J.M. Pappachan, S.K. Bhat, R. Kamath, and B.H. Choo, Omega-3 fatty acids: a comprehensive review of their role in health and disease, *Postgrad Med J* 85:84-90 (2009).

47. Engler, M.M., and M.B. Engler, Omega-3 fatty acids: role in cardiovascular health and disease, *J Cardiovasc Nurs* 21:17-24, quiz 25-16 (2006).

48. Grootveld, M., C.J.L. Silwood, and A.W.D. Claxson, Warning: thermally-stressed polyunsaturates are damaging to health, *Food Chem* 67:211-213 (1999).

49. Kim, S.S., D.D. Gallaher, and A.S. Csallany, Lipophilic aldehydes and related carbonyl compounds in rat and human urine, *Lipids* 34:489-496 (1999).

50. Ramsden, C.E., D. Zamora, B. Leelarthaepin, S.F. Majchrzak-Hong, K.R. Faurot, C.M. Suchindran, A. Ringel, J.M. Davis, and J.R. Hibbeln, Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis, *Bmj* 346:e8707-e8707 (2013).

51. Uchida, K., 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress, *Prog Lipid Res* 42:318-343 (2003).

52. Schaur, R.J., Basic aspects of the biochemical reactivity of 4-hydroxynonenal, *Mol Aspects Med* 24:149-159 (2003).

53. Zarkovic, K., 4-hydroxynonenal and neurodegenerative diseases, *Mol Aspects Med* 24:293-303 (2003).

54. Lynch, M.P., C. Faustman, L.K. Silbart, D. Rood, and H.C. Furr, Detection of lipid-derived aldehydes and aldehyde : protein adducts in vitro and in beef, *J Food Sci* 66:1093-1099 (2001).

55. Uchida, K., and E.R. Stadtman, Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra- and intermolecular cross-linking reaction, *J Biol Chem* 268:6388-6393 (1993).

56. Witz, G., Biological interactions of alpha,beta-unsaturated aldehydes, *Free Radic Biol Med* 7:333-349 (1989).

57. Gardner, H.W., and M.J. Grove, Soybean lipoxygenase-1 oxidizes 3Z-nonenal. A route to 4s-hydroperoxy-2e-nonenal and related products, *Plant Physiol* 116:1359-1366 (1998).

58. Eckl, P.M., A. Ortner, and H. Esterbauer, Genotoxic properties of 4-hydroxyalkenals and analogous aldehydes, *Mutat Res* 290:183-192 (1993).

59. Hu, W., Z. Feng, J. Eveleigh, G. Iyer, J. Pan, S. Amin, F.L. Chung, and M.S. Tang, The major lipid peroxidation product, trans-4-hydroxy-2-nonenal, preferentially forms DNA adducts at codon 249 of human p53 gene, a unique mutational hotspot in hepatocellular carcinoma, *Carcinogene*sis 23:1781-1789 (2002).

60. Zarkovic, N., 4-hydroxynonenal as a bioactive marker of pathophysiological processes, *Mol Aspects Med* 24:281-291 (2003).

61. Seppanen, C.M., and A.S. Csallany, Formation of 4-hydroxynonenal, a toxic aldehyde, in soybean oil at frying temperature, *J Am Oil Chem Soc* 79:1033-1038 (2002).

62. Han, I.H., and A.S. Csallany, Temperature dependence of HNE formation in vegetable oils and butter oil, *J Am Oil Chem Soc* 85:777-782 (2008).

63. Gerde, J.A., E.G. Hammond, and P.J. White, Influence of Polydimethylsiloxane on the Formation of 4-Hydroxynonenal in Soybean Oil at Frying Temperature, *J Am Oil Chem Soc* 88:1503-1510 (2011).

64. Official Methods and Recommended Practices of the American Oil Chemists' Society, 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1997, Method Cd 8-53.

65. Metcalfe, L.D., Citation Classic - the Rapid Preparation of Fatty-Acid Esters for Gas-Chromatographic Analysis, *Cc/Life Sci*:16-16 (1981).

66. Ulu, H., Evaluation of three 2-thiobarbituric acid methods for the measurement of lipid oxidation in various meats and meat products, *Meat Science* 67:683-687 (2004).

67. Frankel, E.N., In Search of Better Methods to Evaluate Natural Antioxidants and Oxidative Stability in Food Lipids, *Trends Food Sci Tech* 4:220-225 (1993).

68. Buege, J.A., and S.D. Aust, Microsomal lipid peroxidation, *Methods Enzymol* 52:302-310 (1978).

69. Marmesat, S., A. Morales, J. Velasco, M.V. Ruiz-Mendez, and M.C. Dobarganes, Relationship between changes in peroxide value and conjugated dienes during oxidation of sunflower oils with different degree of unsaturation, *Grasas Aceites* 60:155-160 (2009).

Appendix A: Statistical Analyses

HNE-corn									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.997								
165-1	0.147	0.535							
185-1	**0.040**	0.174	0.999						
145-3	0.098	0.391	1.000	1.000					
165-3	**0.000**	**0.000**	**0.006**	**0.020**	**0.008**				
185-3	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**			
145-5	0.058	0.247	1.000	1.000	1.000	**0.014**	**0.000**		
165-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	1.000	**0.000**	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.004**	**0.000**	**0.001**
HNE-soybean									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	0.727	0.975							
185-1	0.236	0.535	0.997						
145-3	0.497	0.858	1.000	1.000					
165-3	**0.007**	**0.017**	0.109	0.428	0.196				
185-3	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.008**			
145-5	**0.024**	0.063	0.378	0.899	0.593	0.999	**0.002**		
165-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.083	**0.000**	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	0.112
HNE-peanut									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.421								
165-1	**0.003**	0.110							
185-1	**0.008**	0.291	1.000						
145-3	**0.000**	**0.002**	0.237	0.087					
165-3	**0.000**	**0.000**	**0.041**	**0.015**	0.985				
185-3	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.011**			
145-5	**0.000**	**0.000**	**0.026**	**0.010**	0.919	1.000	**0.017**		
165-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.008**	**0.000**	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**

HNE-canola									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.735								
165-1	0.112	0.949							
185-1	0.077	0.835	1.000						
145-3	0.549	1.000	0.995	0.954					
165-3	**0.047**	0.727	1.000	1.000	0.905				
185-3	**0.000**	**0.004**	0.126	0.395	**0.009**	0.310			
145-5	**0.027**	0.418	0.990	1.000	0.602	1.000	0.980		
165-5	0.480	1.000	1.000	1.000	1.000	1.000	0.155	0.954	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**
HNE-lard									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	**0.023**								
165-1	0.241	0.999							
185-1	**0.000**	**0.000**	**0.000**						
145-3	**0.000**	**0.000**	**0.000**	1.000					
165-3	**0.000**	0.054	**0.004**	0.871	0.551				
185-3	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**			
145-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**		
165-5	**0.000**	**0.000**	**0.000**	0.960	0.999	0.094	**0.000**	**0.026**	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
HNE-beef tallow									
	0.000								
185-5	**0.007**								
HNE					p value				
	145-1	145-3	145-5	165-1	165-3	165-5	185-1	185-3	185-5
corn-canola	0.245	**0.001**	**0.019**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
lard-canola	**0.001**	**0.000**	**0.000**	**0.027**	**0.000**	**0.002**	**0.000**	**0.000**	**0.000**
peanut-canola	**0.037**	**0.000**	**0.002**	**0.001**	**0.000**	**0.001**	**0.009**	**0.000**	**0.000**

soybean-canola	0.963	**0.019**	**0.003**	**0.042**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**
lard-corn	0.139	0.981	0.083	**0.041**	**0.000**	**0.001**	0.408	0.651	0.109
peanut-corn	0.779	0.542	0.300	0.992	**0.000**	**0.026**	**0.012**	**0.000**	**0.001**
soybean-corn	0.648	0.390	0.461	0.134	**0.000**	0.981	0.126	**0.003**	0.501
peanut-lard	0.680	0.703	0.950	0.086	1.000	0.190	**0.001**	**0.000**	**0.010**
soybean-lard	**0.014**	0.130	0.780	0.994	**0.041**	**0.001**	**0.006**	**0.006**	0.935
soybean-peanut	0.165	**0.043**	0.994	0.244	0.081	**0.014**	0.462	**0.003**	**0.008**
canola-beef									0.357
corn-beef									**0.000**
lard-beef									**0.000**
peanut-beef									**0.000**
soybean-beef									**0.000**
HHE-corn									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	0.564	0.564							
185-1	0.586	0.586	1.000						
145-3	0.859	0.859	1.000	1.000					
165-3	0.123	0.123	0.942	0.989	0.867				
185-3	0.735	0.735	1.000	1.000	1.000	0.949			
145-5	0.738	0.738	1.000	1.000	1.000	0.948	1.000		
165-5	0.281	0.281	1.000	1.000	0.993	1.000	0.999	0.999	
185-5	0.141	0.141	0.964	0.994	0.902	1.000	0.968	0.967	1.000
HHE-soybean									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.998								

165-1	0.449	0.923							
185-1	0.329	0.820	1.000						
145-3	0.602	0.981	1.000	1.000					
165-3	**0.007**	**0.024**	0.221	0.311	0.149				
185-3	**0.000**	**0.000**	**0.001**	**0.001**	**0.000**	**0.033**			
145-5	**0.004**	**0.015**	0.139	0.200	0.093	1.000	0.053		
165-5	**0.000**	**0.000**	**0.002**	**0.003**	**0.002**	0.173	0.991	0.271	
185-5	**0.000**	**0.000**	**0.001**	**0.001**	**0.001**	0.056	1.000	0.091	1.000
HHE-peanut									
	p value								
185-5 vs 0	0.683								
HHE-canola									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.953								
165-1	**0.037**	0.402							
185-1	**0.007**	0.081	0.996						
145-3	0.112	0.785	1.000	0.866					
165-3	0.175	0.909	0.999	0.723	1.000				
185-3	**0.001**	**0.006**	0.573	0.999	0.216	0.127			
145-5	**0.044**	0.375	1.000	1.000	0.998	0.988	0.979		
165-5	0.918	1.000	0.994	0.798	1.000	1.000	0.363	0.969	
185-5	**0.000**	**0.000**	**0.003**	0.062	**0.001**	**0.000**	0.240	**0.049**	**0.010**
HHE									
	145-1	145-3	145-5	165-1	165-3	165-5	185-1	185-3	185-5
corn-canola		0.109	0.099	**0.026**	0.398	0.933	**0.016**	0.117	**0.001**
soybean-canola	0.701	0.959	0.061	0.768	**0.002**	**0.042**	0.372	**0.015**	**0.002**
soybean-corn		0.214	**0.012**	0.087	**0.002**	**0.025**	0.068	**0.004**	**0.000**
soybean-peanut									**0.000**
peanut-can									**0.000**

ola									
peanut-corn									0.755
HOE-corn									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.998								
165-1	0.800	0.999							
185-1	0.805	0.999	1.000						
145-3	0.994	1.000	1.000	1.000					
165-3	**0.002**	**0.006**	**0.019**	**0.019**	**0.007**				
185-3	**0.035**	0.142	0.447	0.443	0.173	0.562			
145-5	0.656	0.991	1.000	1.000	0.997	**0.028**	0.592		
165-5	**0.002**	**0.006**	**0.021**	**0.021**	**0.007**	1.000	0.598	**0.031**	
185-5	**0.003**	**0.012**	**0.040**	**0.040**	**0.014**	1.000	0.843	0.060	1.000
HOE-soybean									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	0.986	0.999							
185-1	0.806	0.936	1.000						
145-3	0.958	0.995	1.000	1.000					
165-3	0.061	0.100	0.328	0.646	0.416				
185-3	**0.003**	**0.005**	**0.017**	**0.041**	**0.022**	0.657			
145-5	0.377	0.549	0.951	1.000	0.983	0.973	0.131		
165-5	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.012**	0.243	**0.002**	
185-5	**0.005**	**0.007**	**0.024**	0.059	**0.031**	0.791	1.000	0.187	0.173
HOE-peanut									
185-5 vs 0	0.333								
HOE-canola									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	0.573	0.937							
185-1	**0.020**	0.066	0.351						
145-3	0.998	1.000	1.000	0.449					

165-3	0.288	0.694	1.000	0.655	0.998				
185-3	**0.007**	**0.030**	0.183	1.000	0.382	0.472			
145-5	**0.044**	0.138	0.623	1.000	0.650	0.899	1.000		
165-5	0.301	0.652	0.999	0.949	0.988	1.000	0.926	0.996	
185-5	**0.000**	**0.000**	**0.000**	0.146	**0.003**	**0.000**	**0.033**	0.065	**0.006**
HOE									
	145-1	145-3	145-5	165-1	165-3	165-5	185-1	185-3	185-5
corn-canola	0.270	0.939	1.000	**0.007**	**0.000**	0.181	0.770	0.165	0.061
soybean-canola	0.882	0.608	0.418	**0.013**	**0.001**	**0.023**	0.852	**0.002**	**0.003**
soybean-corn	0.420	0.689	0.428	0.853	0.326	0.102	0.496	**0.023**	0.068
soybean-peanut									**0.000**
peanut-canola									**0.023**
peanut-corn									**0.003**
HDE-corn									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	0.997	1.000							
185-1	0.886	0.997	1.000						
145-3	0.992	1.000	1.000	1.000					
165-3	0.094	0.226	0.378	0.708	0.424				
185-3	**0.028**	0.070	0.126	0.294	0.144	1.000			
145-5	0.949	1.000	1.000	1.000	1.000	0.592	0.224		
165-5	**0.001**	**0.003**	**0.004**	**0.010**	**0.005**	0.174	0.491	**0.007**	
185-5	**0.001**	**0.002**	**0.004**	**0.008**	**0.004**	0.143	0.420	**0.006**	1.000
HDE-soybean									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	1.000	1.000							

185-1	0.994	1.000	1.000						
145-3	0.999	1.000	1.000	1.000					
165-3	0.830	0.982	0.993	1.000	0.999				
185-3	0.106	0.331	0.256	0.418	0.332	0.780			
145-5	0.971	0.999	1.000	1.000	1.000	1.000	0.537		
165-5	**0.007**	**0.033**	**0.015**	**0.026**	**0.020**	0.066	0.647	**0.036**	
185-5	0.121	0.363	0.288	0.464	0.372	0.826	1.000	0.588	0.595
HDE-peanut									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	1.000	1.000							
185-1	0.994	1.000	1.000						
145-3	0.999	1.000	1.000	1.000					
165-3	0.830	0.982	0.993	1.000	0.999				
185-3	0.106	0.331	0.256	0.418	0.332	0.780			
145-5	0.971	0.999	1.000	1.000	1.000	1.000	0.537		
165-5	**0.007**	**0.033**	**0.015**	**0.026**	**0.020**	0.066	0.647	**0.036**	
185-5	0.121	0.363	0.288	0.464	0.372	0.826	1.000	0.588	0.595
HDE-canola									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	0.100	0.365							
185-1	0.342	0.769	1.000						
145-3	0.999	1.000	0.346	0.805					
165-3	0.257	0.685	1.000	1.000	0.706				
185-3	**0.001**	**0.007**	0.474	0.241	**0.004**	0.174			
145-5	0.249	0.607	1.000	1.000	0.641	1.000	0.718		
165-5	**0.023**	0.087	0.980	0.844	0.077	0.813	1.000	0.992	
185-5	**0.000**	**0.000**	**0.003**	**0.002**	**0.000**	**0.001**	0.278	**0.015**	0.219
HDE									
	145-1	145-3	145-5	165-1	165-3	165-5	185-1	185-3	185-5
corn-canola	0.113	0.148	0.083	0.213	**0.000**	0.097	**0.042**	**0.000**	**0.016**
soybean-c	0.791	0.303	0.136	0.998	**0.003**	0.166	0.321	**0.000**	0.388

anola									
soybean-corn	0.240	0.812	0.801	0.307	**0.001**	0.792	0.293	0.180	0.113
soybean-peanut							0.991	0.293	0.180
peanut-canola							0.616	**0.001**	**0.024**
peanut-corn							0.324	**0.025**	0.971
HHE, HNE, HOD and HDE in corn									
	corn	soybean	peanut	canola					
HHE-HDE	0.199	0.884	**0.014**	**0.038**					
HNE-HDE	**0.000**	**0.000**	**0.000**	**0.000**					
HOE-HDE	0.840	0.748	**0.017**	0.086					
HNE-HHE	**0.000**	**0.000**	**0.000**	**0.001**					
HOE-HHE	0.439	0.998	0.994	0.933					
HOE-HNE	**0.000**	**0.000**	**0.000**	**0.000**					
185-5									
	HHE	HOE	HNE	HDE					
corn-canola	**0.001**	**0.234**	**0.000**	**0.007**					
peanut-canola	**0.000**	**0.108**	**0.000**	**0.012**					
soybean-canola	**0.002**	**0.028**	**0.000**	**0.185**					
peanut-corn	0.751	**0.015**	**0.002**	0.965					
soybean-c	**0.000**	0.541	**0.240**	**0.070**					

orn									
soybean-peanut	**0.000**	**0.003**	**0.008**	**0.129**					
Total polar-corn									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.372								
165-1	**0.031**	0.836							
185-1	**0.011**	0.426	1.000						
145-3	**0.035**	0.870	1.000	1.000					
165-3	**0.000**	**0.001**	**0.005**	**0.016**	**0.005**				
185-3	**0.000**	**0.000**	**0.001**	**0.002**	**0.001**	0.910			
145-5	**0.004**	0.155	0.921	1.000	0.894	**0.048**	**0.005**		
165-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.113	0.743	**0.000**	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.006**	0.054	**0.000**	0.667
Total polar-soybean									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	0.415	0.838							
185-1	0.144	0.410	1.000						
145-3	0.264	0.647	1.000	1.000					
165-3	**0.002**	**0.005**	0.060	0.191	0.102				
185-3	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	0.053			
145-5	**0.003**	**0.008**	0.098	0.299	0.164	1.000	**0.033**		
165-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	0.110	**0.000**	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	0.328	**0.001**	1.000
Total polar-peanut									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	**0.007**								
165-1	**0.000**	0.104							
185-1	**0.000**	0.237	1.000						
145-3	**0.000**	**0.000**	**0.001**	**0.001**					
165-3	**0.000**	**0.000**	**0.008**	**0.012**	0.910				
185-3	**0.000**	**0.000**	**0.000**	**0.000**	1.000	0.650			
145-5	**0.000**	**0.000**	**0.000**	**0.000**	0.754	0.096	0.848		

165-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.012**	**0.001**	**0.011**	0.273	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.049**
Total polar-canola									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	**0.022**	**0.020**							
185-1	**0.000**	**0.000**	0.538						
145-3	0.799	0.962	0.379	**0.004**					
165-3	0.128	0.168	0.999	0.116	0.936				
185-3	**0.000**	**0.000**	**0.041**	0.996	**0.000**	**0.004**			
145-5	0.113	0.166	1.000	0.632	0.824	1.000	0.105		
165-5	0.257	0.392	1.000	0.336	0.981	1.000	**0.035**	1.000	
185-5	**0.000**	**0.000**	**0.000**	**0.044**	**0.000**	**0.000**	0.254	**0.001**	**0.000**
Total polar-lard									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.631								
165-1	1.000	0.926							
185-1	**0.001**	**0.042**	**0.001**						
145-3	**0.000**	**0.008**	**0.000**	1.000					
165-3	0.116	0.992	0.223	0.488	0.151				
185-3	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**			
145-5	**0.000**	**0.000**	**0.000**	0.652	0.965	**0.004**	**0.000**		
165-5	**0.002**	0.126	**0.002**	1.000	0.996	0.800	**0.000**	0.342	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.178	**0.000**	**0.000**
Total polar-beef									
185-5 vs 0	0.072								
Total polar									
	145-1	145-3	145-5	165-1	165-3	165-5	185-1	185-3	185-5
corn-canola	0.302	0.731	0.865	0.273	**0.029**	0.136	0.887	0.073	0.066
lard-canola	0.349	0.153	0.240	0.068	**0.004**	0.088	**0.004**	**0.000**	**0.000**
peanut-can	0.570	0.476	0.868	0.318	0.461	0.686	0.999	0.408	0.586

ola									
soybean-canola	0.622	0.323	0.308	0.150	**0.045**	**0.039**	0.909	**0.024**	**0.021**
lard-corn	0.989	0.858	0.514	0.936	0.949	1.000	**0.016**	**0.001**	**0.004**
peanut-corn	0.984	0.992	1.000	1.000	0.418	0.447	0.764	0.636	0.594
soybean-corn	0.923	0.974	0.650	0.993	0.960	0.828	1.000	0.998	0.998
peanut-lard	1.000	0.987	0.509	0.898	0.112	0.293	**0.003**	**0.000**	**0.000**
soybean-lard	0.991	0.993	0.999	0.997	0.534	0.785	**0.006**	**0.001**	**0.003**
soybean-peanut	0.999	1.000	0.644	0.983	0.680	0.103	0.783	0.367	0.308
canola-beef									0.996
corn-beef									**0.020**
lard-beef									**0.000**
peanut-beef									0.283
soybean-beef									**0.005**
NP									
	corn	soybean	peanut	canola	lard	beef			
145-0	**0.000**	0.757	0.180	**0.040**	**0.001**				
165-0	**0.000**	**0.031**	**0.031**	0.250	**0.000**				
185-0	**0.000**	0.350	0.193	0.356	**0.000**	**0.000**			
165-145	**0.010**	0.163	0.725	0.687	**0.001**				
185-145	0.972	0.879	1.000	0.539	**0.000**				
185-165	**0.020**	0.457	0.700	0.994	0.444				
NP									
	145-1	145-3	145-5	165-1	165-3	165-5	185-1	185-3	185-5
corn-canola	0.985	0.149	0.469	**0.042**	0.399	**0.000**	0.833	**0.004**	**0.017**

lard-canola	**0.000**	0.417	0.068	**0.000**	0.449	**0.000**	**0.000**	**0.004**	**0.000**
peanut-canola	0.151	0.504	0.818	0.616	0.587	**0.016**	0.057	**0.012**	0.156
soybean-canola	1.000	**0.008**	0.998	**0.036**	0.216	**0.003**	**0.014**	0.574	0.465
lard-corn	**0.000**	**0.005**	**0.003**	**0.000**	**0.021**	**0.000**	**0.001**	**0.000**	**0.000**
peanut-corn	0.262	0.911	0.970	0.448	0.997	0.111	**0.008**	0.971	0.854
soybean-corn	0.993	0.519	0.315	**0.000**	0.992	0.443	**0.002**	0.067	0.446
peanut-lard	**0.000**	**0.027**	**0.009**	**0.000**	**0.040**	**0.000**	**0.000**	**0.000**	**0.000**
soybean-lard	**0.000**	**0.000**	0.118	0.127	**0.009**	**0.000**	**0.000**	**0.000**	**0.000**
soybean-peanut	0.135	0.156	0.650	**0.002**	0.937	0.893	0.943	0.194	0.976
canola-beef									**0.000**
corn-beef									**0.000**
lard-beef									0.350
peanut-beef									**0.000**
soybean-beef									**0.000**
Total 4-hydro corn									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.999								
165-1	0.483	0.926							
185-1	0.179	0.541	1.000						
145-3	0.410	0.875	1.000	1.000					
165-3	**0.001**	**0.002**	**0.017**	0.052	**0.021**				
185-3	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.037**			
145-5	0.220	0.627	1.000	1.000	1.000	**0.041**	**0.000**		
165-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.045**	1.000	**0.000**	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.116	**0.000**	0.096

total a,b -soybean									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	1.000								
165-1	0.890	0.994							
185-1	0.507	0.800	1.000						
145-3	0.777	0.968	1.000	1.000					
165-3	**0.027**	0.058	0.274	0.632	0.376				
185-3	**0.000**	**0.000**	**0.001**	**0.003**	**0.002**	0.065			
145-5	0.084	0.181	0.661	0.966	0.795	1.000	**0.021**		
165-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	0.331	**0.001**	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.004**	0.733	**0.002**	1.000
total a,b -peanut									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.773								
165-1	**0.026**	0.374							
185-1	**0.036**	0.476	1.000						
145-3	**0.001**	**0.016**	0.586	0.472					
165-3	**0.000**	**0.005**	0.196	0.146	0.999				
185-3	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.006**			
145-5	**0.000**	**0.003**	0.139	0.103	0.990	1.000	**0.008**		
165-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.625	**0.001**	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
total a,b -canola									
	0.000	145-1	165-1	185-1	145-3	165-3	185-3	145-5	165-5
145-1	0.907								
165-1	**0.012**	0.133							
185-1	**0.004**	**0.035**	1.000						
145-3	0.371	0.996	0.715	0.283					
165-3	**0.011**	0.121	1.000	1.000	0.685				
185-3	**0.000**	**0.000**	**0.035**	0.336	**0.000**	**0.039**			
145-5	**0.002**	**0.018**	0.955	1.000	0.133	0.963	0.929		
165-5	0.155	0.798	1.000	0.978	0.999	1.000	**0.036**	0.801	
185-5	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**

Appendix B: Figures

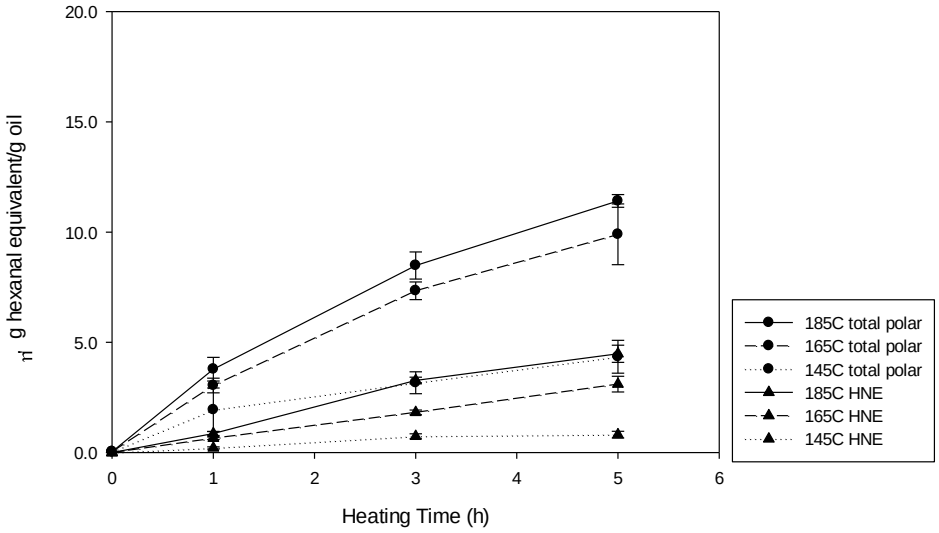


Figure 60. Comparison of the sum total of polar aldehydes and HNE formation in commercial corn oil heated for 1, 3 and 5 hours at 145, 165 and 185 ℃

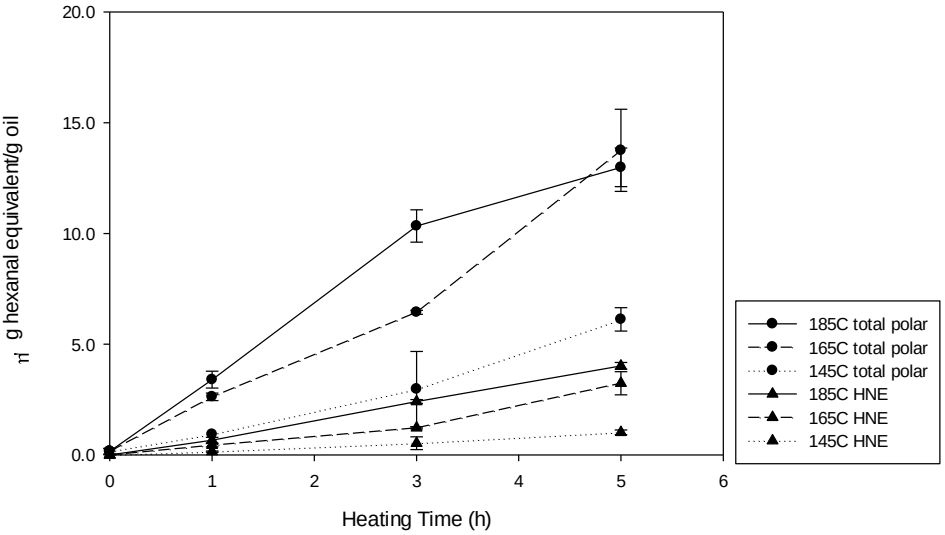


Figure 61. Comparison of the sum total of polar aldehydes and HNE formation in commercial soybean oil heated for 1, 3 and 5 hours at 145, 165 and 185 ℃

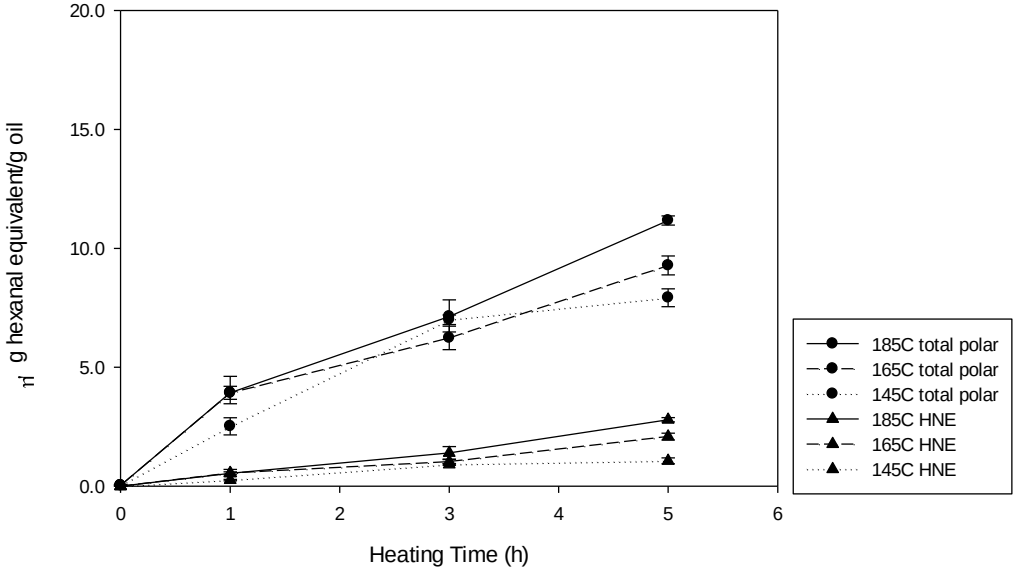


Figure 62. Comparison of the sum total of polar aldehydes and HNE formation in commercial peanut oil heated for 1, 3 and 5 hours at 145, 165 and 185 ℃

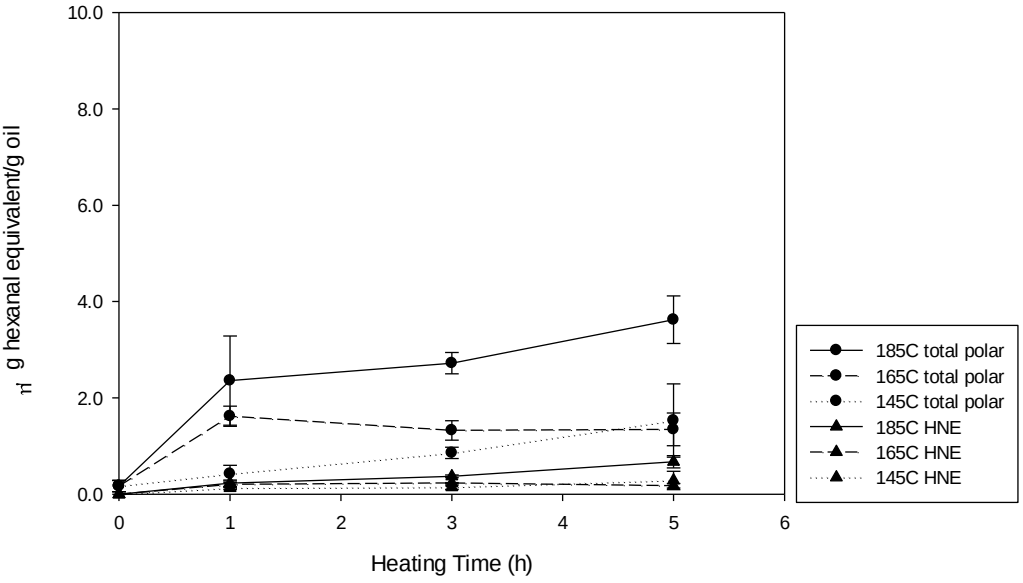


Figure 63. Comparison of the sum total of polar aldehydes and HNE formation in commercial canola oil heated for 1, 3 and 5 hours at 145, 165 and 185 ℃

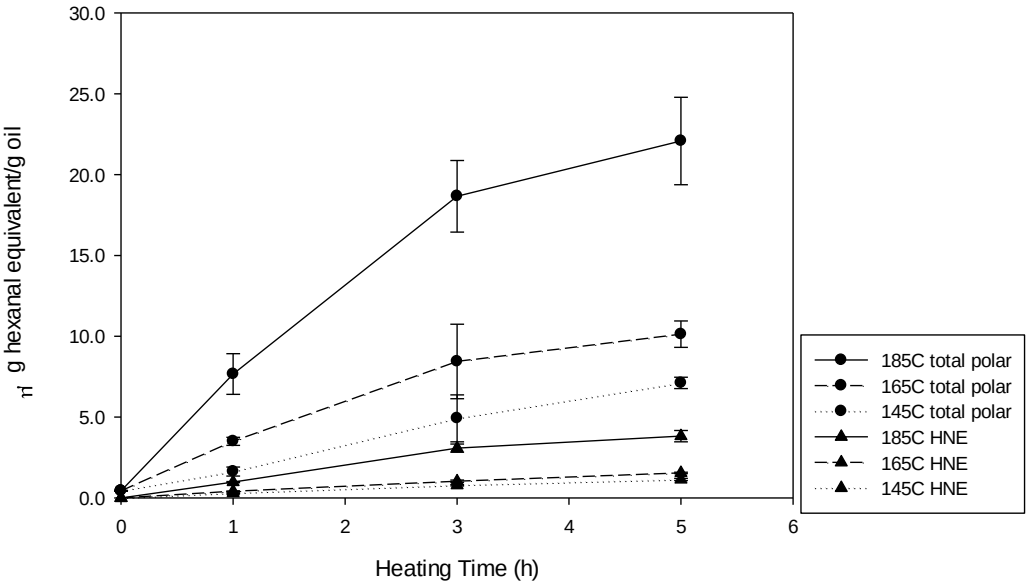


Figure 64. Comparison of the sum total of polar aldehydes and HNE formation in lard heated for 1, 3 and 5 hours at 145, 165 and 185 ℃

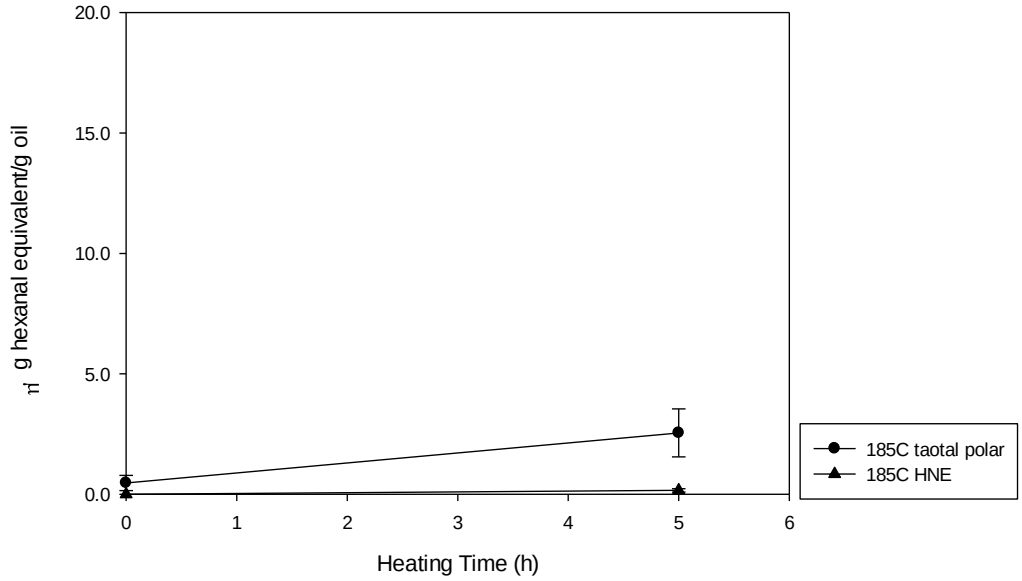


Figure 65. Comparison of the sum total of polar aldehydes and HNE formation in beef heated for 5 hours at 185 ℃