4-HYDROXY-2-TRANS-NONENAL (HNE), A TOXIC DEGRADATION PRODUCT OF LIPID OXIDATION, IN FRIED CHICKEN AND DONUT

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Abstract

Alpha, beta-unsaturated-4-hydroxyaldehydes, a group of secondary lipid oxidation products, are highly interesting due to their high reactivity to various biological compounds including amino acids and DNA. 4-Hydroxy-2-trans-Nonenal (HNE), one of the most abundant and toxic compound in this group, was measured in commercial fried chicken breasts, chicken thighs, chicken nuggets and raised glazed donuts from different fast food stores and supermarkets. Samples were analyzed for fat content, fatty acids distribution and HNE concentration.

Fried chickens and donuts were selected because they are very common fast food and they are deep-frying at high temperatures (160-190 °C). Preliminary experiments were conducted using the thiobarbituric acid (TBARS) assay to obtain the secondary lipid oxidation products such as aldehydes, ketones and related carbonyl compounds. HNE concentration was measured as 2,4-dinitrophenylhydrazone derivatives using an HPLC method.

HNE concentrations in fried chickens from 3 different sources are ranging from 11.25 and 13.88 μg HNE/100 g breast and between 23.15 and 30.36 μg HNE/100 g thigh. For chicken nuggets and popcorn chicken, HNE concentrations are between 9.00 and 47.93 μg HNE/100 g sample. For donut samples from 3 different brands, HNE concentrations were between 18.55 and 21.71 μg HNE/100 g donut.

Measured HNE amount in chicken thighs samples is greater than chicken breasts samples, suggesting the heme iron content existed in dark meat possibly act as a catalyst to accelerate the lipid oxidation in the meat.

These results indicated that HNE is incorporated into the fried chickens and glazed donuts samples from frying oils and might pose a public health concern for long-term consumption given the toxicity of HNE.

Table of Contents

List of Tables

List of Figures

Introduction

Lipid oxidation causes chemical changes, quality issues and nutritional loss in many foods and biological systems in the presence of free radicals and oxygen. Its degradation products lead to a variety of secondary lipid oxidation products produced by decomposition of hydroperoxides, such as ketones, carboxylic acids and aldehydes including alkanals, 2-alkenals, 2,4- dienals and 4-hydroxyaldehydes. Among these aldehydes, the group of α, β-unsaturated-4-hydroxyaldehydes is of particular interests due to their high reactivity with a series compounds in biological system. The cytotoxic and mutagenic α, β-unsaturated-4-hydroxy-2-trans-nonenal (HNE)[1], is the most studied and toxic one and proved be formed due to the oxidation of w-6 fatty acids such as linoleic acid[2,3]. Numerous literatures have indicated the highly reactive HNE, associate with a series of chronic disease such atherosclerosis[4,5], stroke[6,7], liver disease[8,9],cancer[10,11], Pakinson's disease and Alzheimer's[12–17]due to its ability to covalent reaction with protein and DNA adducts[1]. It has been demonstrated that HNE is present in various food systems[18] especially in fats and oils high in linoleic acid such as soybean oil and corn oil[19]after thermal treatment. Previous studies in present laboratory have evidenced that HNE produced by oxidation of frying oil subjected to heat treatment could be incorporated from the frying oil into the potato chips under laboratory condition [20] and it has also shown in French fries from various fast food restaurants[21] and pointed out that long term consumption of these fried food might pose a health concern to public.

Tremendous investigations of HNE formation in frying oils are in the literature, however there is little information on HNE formation in fried chickens and donuts. The objective of this project is to measure the concentration of HNE that has

incorporated into fried chickens and glazed donuts from the frying oils. The samples for analysis obtained from fast food restaurants and local grocery stores.

Part I. Literature Review

Lipid oxidation and its mechanism

Unsaturated fatty acids would react with free radicals in the presence of oxygen and produce primary hydroperoxides and further oxidation products including alkanals, 2-alkenals, 4-hydroxydldehydes, ketones and related carbonyl compounds and polymers. And this process is called lipid oxidation, or thermal oxidation when oils and fats are subjected to heat. In general, the mechanism of lipid oxidation or autoxidation involves three stages: Initiation, propagation and termination. Figure 1 shows this mechanism:

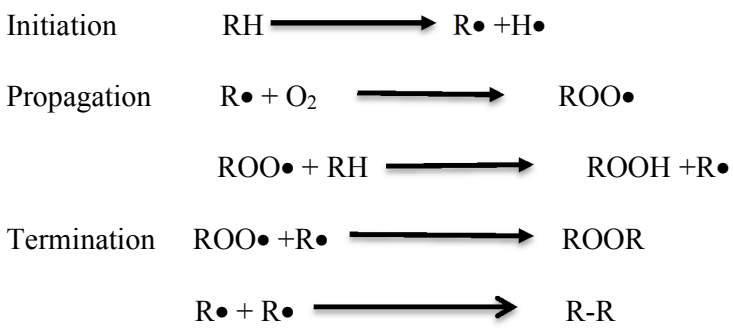


Figure 1. Mechanism of chain reaction of autoxidation

RH: Fatty acid R•: Lipid free radical ROO •: Peroxy free radical

ROOH: Hydroperoxide R-R: Polymers and dimers

In the initiation reaction, initiator like free radicals will attack the methylene hydrogen next to the double bond and abstract its hydrogen since this hydrogen-carbon bond is the weakest and requires least energy to form radical state[22]. In the propagation phase, this lipid free radical would react with oxygen molecule to form lipid peroxy radical and it will attack another free unsaturated fatty acid to produce lipid hydroperoxide

and generate a new lipid free radical. Similar reactions are repeated and form a chain reaction. And such chain reaction greatly accelerates this auto-oxidation. The termination is the hydrogen donation of antioxidant to lipid peroxy radical resulting in the formation of non-radical products such as dimers and polymers. Many factors would affect lipid peroxidation in food matrixes including fatty acid composition, degree saturation of fatty acids, oxygen concentration, temperature, time, light, catalysts such as iron and antioxidants[23]. Unsaturated fatty acids, especially polyunsaturated fatty acids are susceptible to oxidation than saturated fatty acids since they have higher degree of unsaturation. And higher temperature accelerates thermal oxidation, causing more secondary oxidation and polymerization products.

Secondary oxidation products in thermal oxidation

Primary oxidation products like lipid hydroperoxides are not stable and easily decompose to lower weight molecules including aldehydes, ketones and other short chain carbonyl compounds. And Figure 2 shows the general scheme of lipid oxidation[1]

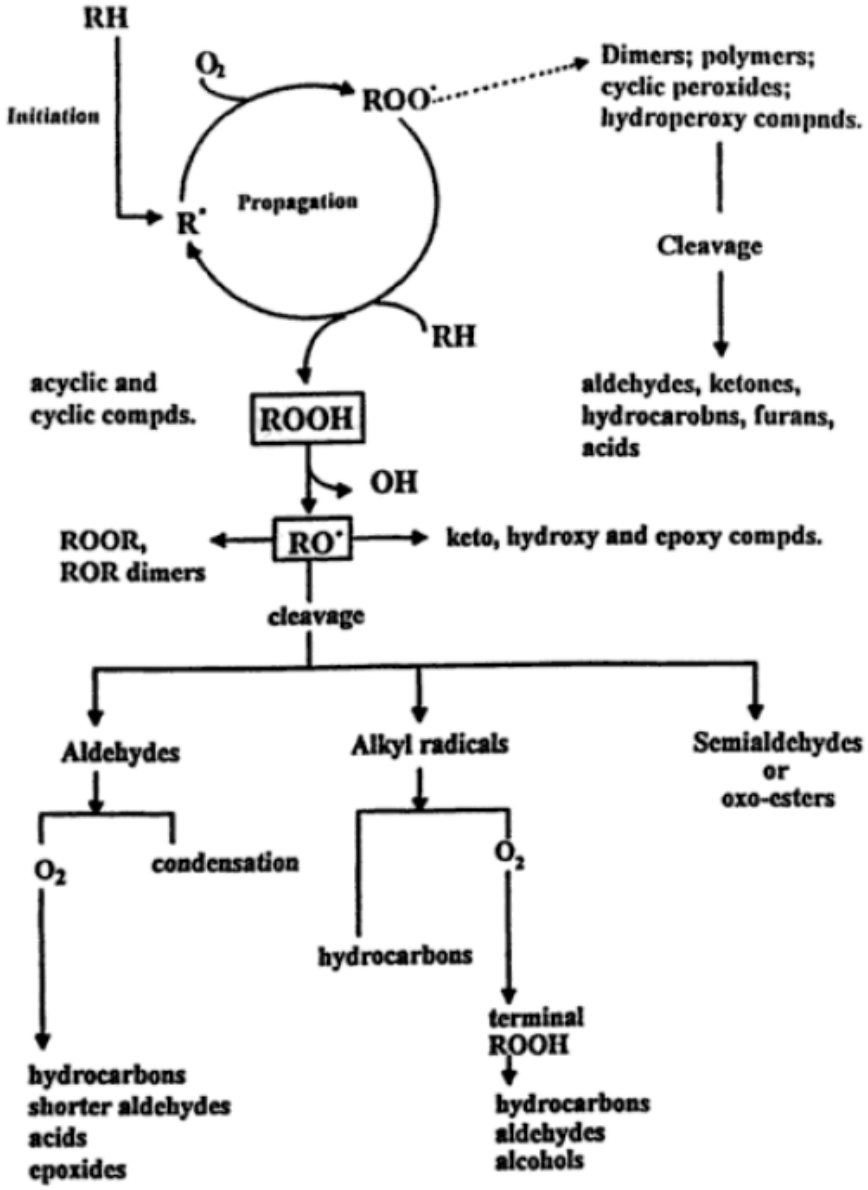


Figure 2. General scheme for lipid oxidation (Ref 61)

At first, the lipid hydroperoxide easily decompose to a hydroxyl radical (OH•) and an alcoxy radical (RO•) through hemolytic cleavage. Then the alcoxy radical will break down to alkyl radicals through β-scission. Then after a series of cleavage and rearrangement, numerous short chain secondary oxidation products such as alkanals, 2-alkenals, 2,4-alkadienals, 4-hydroxyaldehydes and ketones and other carbonyl

compounds are produced. It's a complicated process. Figure 3 shows the general mechanism of hydroperoxides decomposition and formation of secondary oxidation products[24].

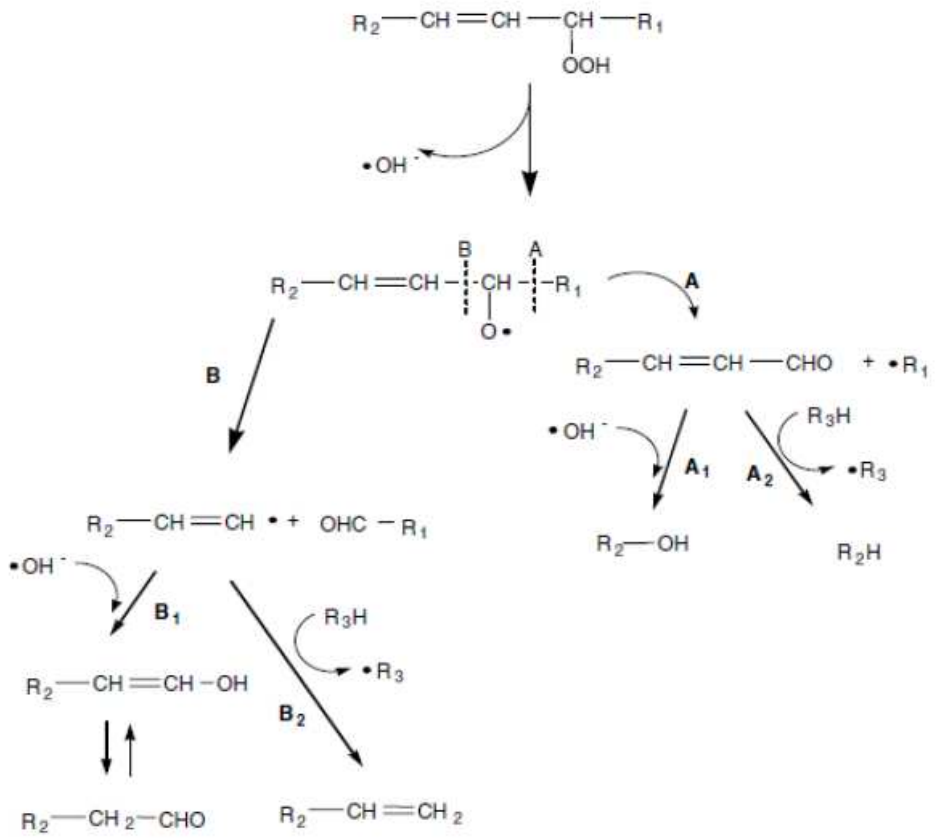


Figure 3. Mechanism of hydroperoxide decomposition to secondary oxidation products (Ref 24)

Based on their structures features, the reactive short-chain aldehydes generated from hydroperoxides can be mainly classified into three categories: 2-alkenals, 4-hydroxy-2-alkenals and ketoaldehydes (Figure 4)[3].

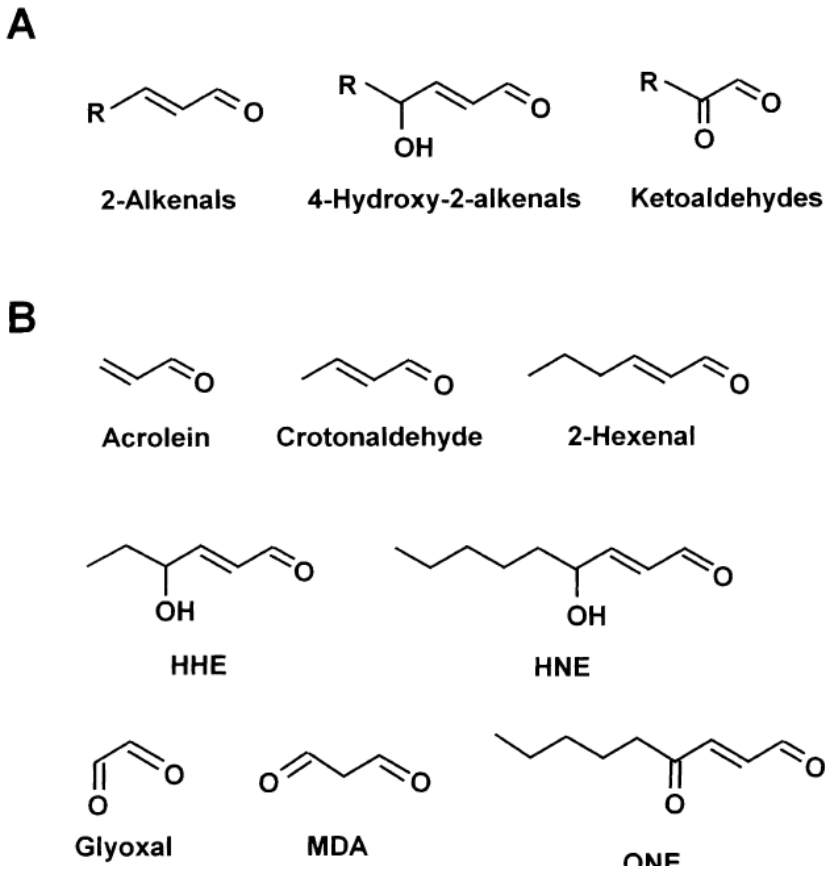
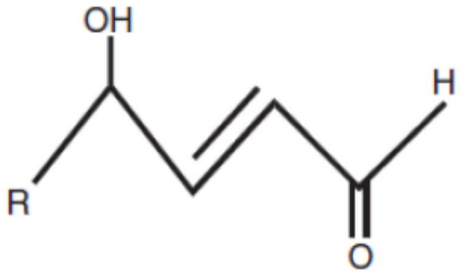


Figure 4. General structures of A. Some reactive aldehydes and B. Some important lipid peroxidation specific aldehydes (Ref 3)

Among these main short-chain aldehydes, the most interesting group is α, β-unsaturated-4-hydroxyaldehyde that was discovered by Esterbauer and his colleagues[1]. Four important types of this cytotoxic group compounds: 4-hydroxy-2-trans-hexenal (HHE), 4-hydroxy-2-trans-nonenal (HNE), 4-hydroxy-2-trans-octenal (HOE), 4-hydroxy-2-trans-decenal (HDE). The only difference between them is the chain length and Figure 5 shows their general structures. These conjugated aldehydes have three functional groups: a carbonyl group (C=O), a trans double bond (C=C) in position two and a hydroxyl group (-OH) in position four. The combination of these functional groups makes this group highly active.



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, HDE

Figure 5. Chemical structure of four α, β-unsaturated-4-hydroxyaldehydes

Formation of 4-Hydroxy-2-Nonenal (HNE) and its Toxicity

Within this group, HNE is the one of best recognized and most studied because it has been shown to be toxic. HNE is considered from oxidation of ω-6 fatty acid including linoleic acid and arachidonic acid and the main process leading to the HNE likely involves β-cleavage of lipid alkoxy radicals. It was suggested by Grein et al. that HNE came from linoleic acid through the formation and decomposition of 2,4-decadienal[25]. While previous studies in my lab reported that 2,4-decadienal was not founded as intermediate in the formation of HNE in thermally induced oxidation of fatty acids methyl esters. During the oxidation of linoleic acid, free radical would first abstract the methylene hydrogen on carbon 11 because this carbon-hydrogen bond is the weakest and requires least energy to break down to become radical[22]. Then such lipid free radical will be rearranged due to resonance and react with oxygen to form to 9- and 13-hydroperoxyoctadecadienoic acid (9-HPODE and 13-HPODE) as shown in Figure 6. Heat, light, metals and oxygen would facilitate such process. Schneider and Tallman[26] used 9-HPODE and 13-HPODE of linoleic acid as starting material and experimentally provided evidence for mechanisms leading to formation of 4-hydroperoxy-HNE undergoing allylic hock cleavage. Hock cleavage occurs easily for hydroperoxides that have unsaturated unit attached to carbon bearing hydroperoxide group and leads to cleavage of C-C bond and formation of two carbonyl compounds. Figure 7 shows the two distinctive mechanisms of HNE formation starting from 13-HPODE and 9-HPODE separately. There are also other proposed mechanism of HNE formation And Figure 8 shows the formation of HNE from another ω-6 polyunsaturated fatty acid-arachidonic acid[16]. In the presence of transition metal Fe, hydroperoxides converted into HNE after a series of β session, further oxidation and hydration reaction.

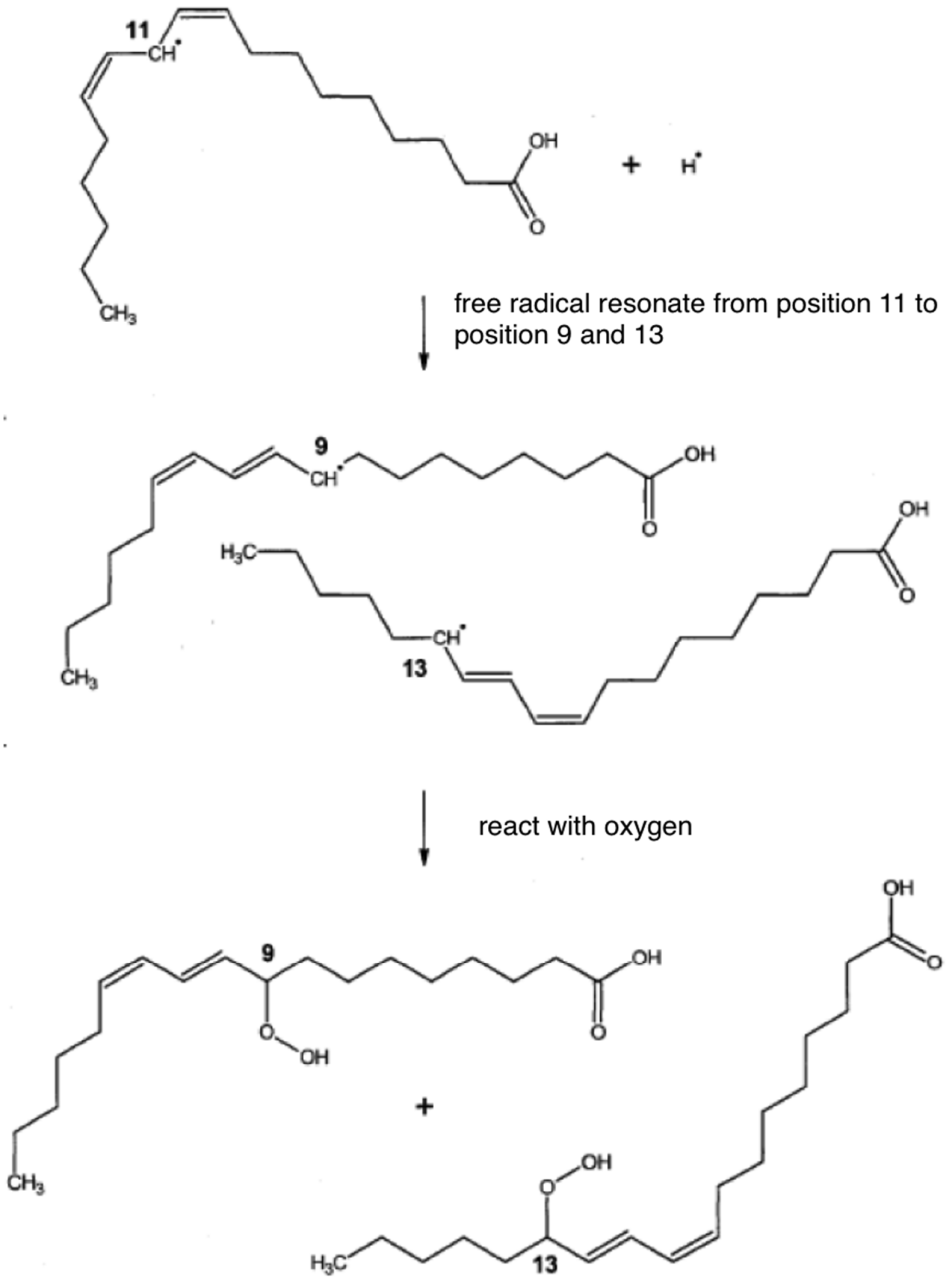
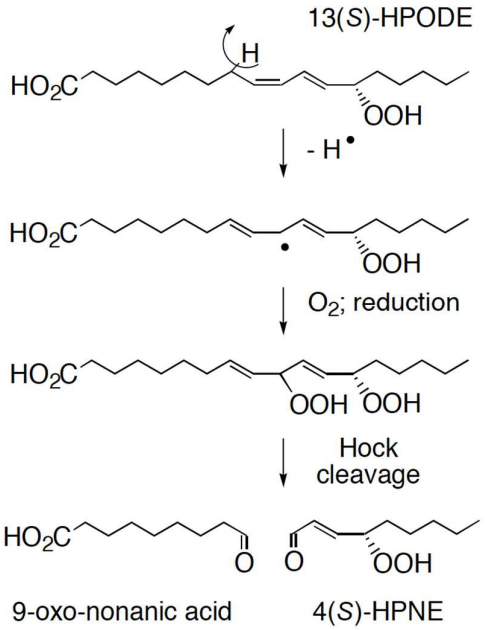


Figure 6. Formation of 9- and 13-hydroperoxyoctadecadienoic from linoleic acid

A



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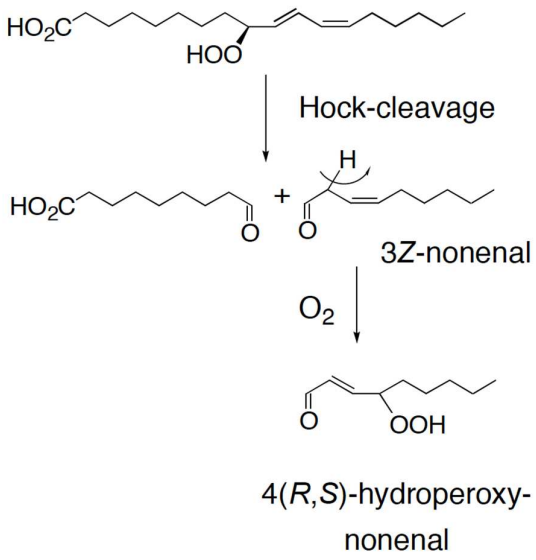


Figure 7. Mechanism of formation of HNE from A. 13(S)-HPODE and B.9(R)-HPODE (Ref 26)

*R and S means right and left configuration of chiral hydroperoxides and Z means a isomer of nonenal

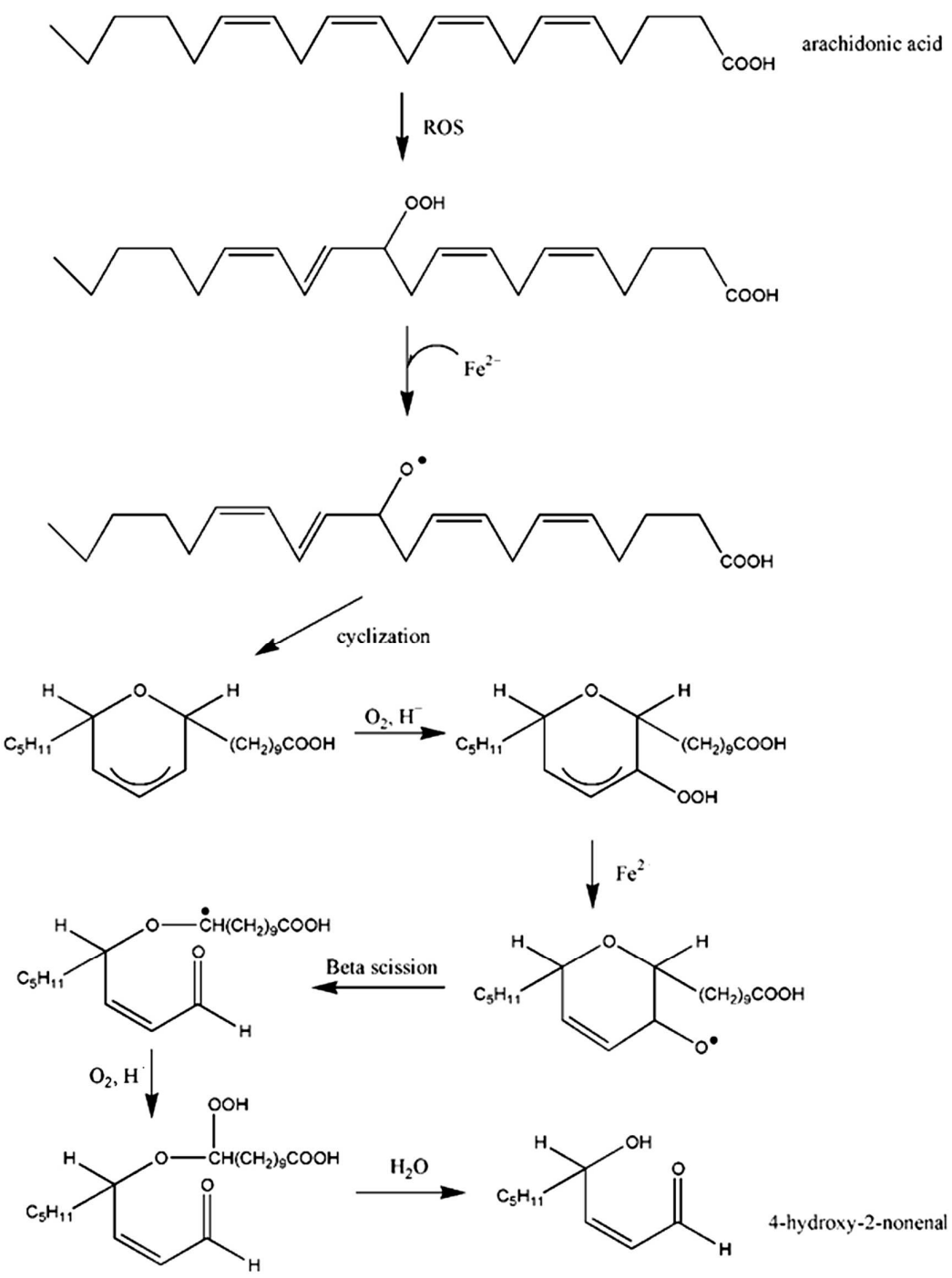


Figure 8. Formation of 4-hydroxy-nonenal from arachidonic acid (Ref 16)

Once formed, HNE can covalently attach to protein by forming stable adducts through Michael addition with thiol group through its conjugated double bonds, which can alter protein structure and cause a loss of protein function and activity[27]. The nucleophilic thiol compound attack carbon 3 and followed by the intramolecular hemiacetal reaction of 4-hydroxy and group and carbonyl group as Figure 9 showed. According to some kinetic investigation, reactions of thiols with HNE are easily and straightforward. Figure 10 shows HNE adducts with cysteine, histidine and lysine amino acid residues[3].

It has been reported HNE Michael adducts represent more than 99% of the protein modifications detected and could serve as one of the most useful biomarkers for the extent of oxidative stress. In addition to double bond activity, its aldehydes group is able to react with amino groups ($-NH_2$) in amino acids, phospholipids and proteins to produce Schiff bases like the HNE-lysine adduct.

HNE could form endogenously upon enzymatic peroxidation of cellular ω-6 fatty acids such as the triglycerides and phospholipid in the membrane. It's reported that physiological HNE level in plasma is about 0.3-0.7 μM[28]. Esterbauer[1] and his colleagues indicated that concentration 1-20 μM in disease states result in inhibition DNA and protein synthesis. And a variety of enzymes have been sown to susceptible to inactivation from HNE adduction including glutathione-s-transferase, glutathione reductase and so on, wile detailed mechanism of such medication of enzyme is not well understood yet.

HNE toxicity is considered to associate with atherosclerosis, liver disease and LDL oxidation, Parkinson's, Alzheimer's and Hunting's according to many researches. Except free HNE, it is more frequently existed as HNE adduct to proteins or glutathione (GSH). And recent development of specific antibodies against protein-

HNE adduct, mainly using anti-HNE KLH polyclonal[8] or anti HNE—histidine monoclonal antibodies[29], has made it possible to obtain high probable evidence for the occurrence of oxidative stress in vivo.

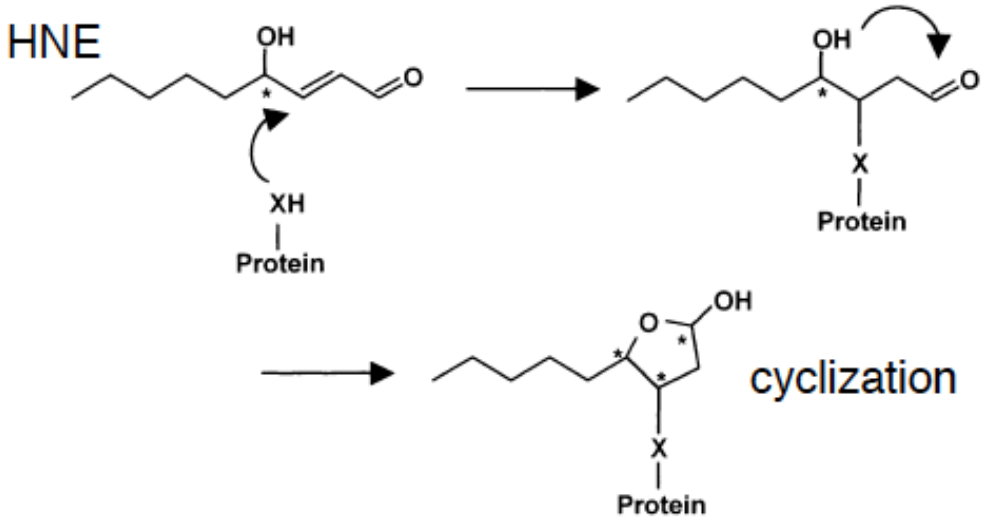


Figure 9. Michael-type addition of HNE to protein. XH-: Sulfhydryl group of cysteine, imidazole group of histidine and amino group of lysine (Ref 3)

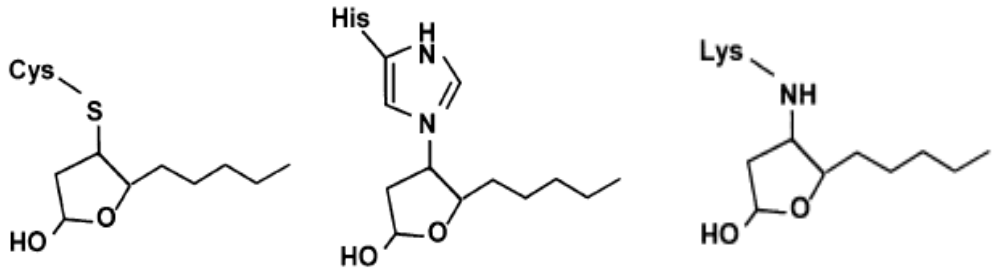


Figure 10. Michael addition reaction and HNE adduct products of cysteine, histidine and Schiff base with lysine (Ref 3)

Chemical Reactions in Frying Oils

Deep-frying is one of the oldest and most common cooking methods both at home cooking and food processing in the industry. This way offers food desired flavor, crispy texture and golden yellow color that attract more people. In American, the economy of commercial deep-frying, mainly fast foods is estimated to 83 billion dollars per year, which is more than twice the amount for the rest of the world. The most common frying temperatures usually range between 170 to 190 °C[30]. Frying temperatures and time, frying oil profiles, food surface area, moistures content of food, types of breading, frying medium, number of frying would affect the hydrolysis, oxidation and polymerization of oil during frying[22].

a) Hydrolysis of oil

Moisture existed in foods forms steam during the frying, which evaporates to bubble and decrease along with frying time. Such steam would attack ester linkage of triglycerides and hydrolyze them to free fatty acids, mono- and- diacylglycerols. Free fatty acids value in frying oils increase with the number of frying[31] thus it's used to monitor frying oil quality. And frequent replenishment of fresh oil could slow down hydrolysis of oil[32] while sodium hydroxide and other alkalis containing cleaner would facilitate hydrolysis of oil[33]. These free fatty acids and their further oxidation products cause off-flavor of oils and quality deterioration of frying oils.

b) Polymerization of oil

The major decomposition products of frying oils are nonvolatile polar compounds and triglycerides dimers and polymers. . Dimers and polymers are large molecule weight compounds and formed based on the reactions between –C-C, -C-O-C, and –C-O-O-C

bonds. Frying oil profiles, frying temperature and number of frying affect the formation of dimer and polymers. It's been reported that amount of polymers increase with the higher frying temperature and increasing number of frying.

c) Oxidation of oil

The mechanism of thermal oxidation is principally the same as the autoxidation mechanism and its oxidation rate is much faster due to the heat treatment, while the detailed scientific information remained unknown largely. The hydroperixdes formed during the frying are not stable during deep-frying, and would decompose alkoxy radicals and hydroxyl radicals by homolysis of peroxide bond.

And the secondary lipid oxidation products are mainly aldehydes, which are considered to be responsible for the toxicity of frying oils. The nature and concentration of these decomposed toxic compounds depend on the fatty acids distribution of frying oils, frying temperature and time, metals such as iron. Therefore, heating unsaturated oils such soybean oil or sunflower oils will produce cytotoxic aldehydes including 4-hydroxy-2-alkenals (HNE) and 4,5-epoxy-2-alkenals that are considered to relate to degenerative diseases such as Alzheimer and Parkinson[34,35]. Because these aldehydes are major components of total polar compounds in frying oils, thus the level of total polar compounds in frying oils and fried foods is an indicator of potential health risk. For public health, the frying oil regulation is established limiting total polar compounds less than 25%[36].

Figure 11 shows the general chemical reactions in frying oils in the presence of oxygen and heat.

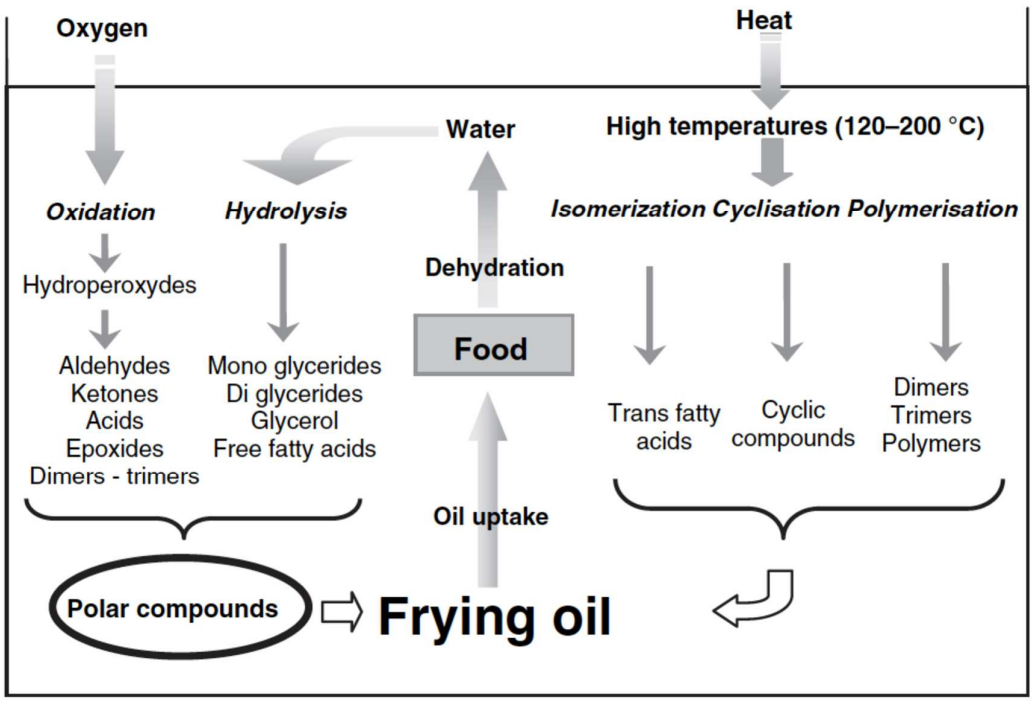


Figure 11. Outline of the oil degradation and other reactions during frying and the consequences on the product quality (Ref 22)

Health concern of frying oils and recent studies of HNE formation in various food matrixes

In a typical American diet, around 1/3 daily energy intake comes from added fat and oils and half of these added fat intake is from thermally oxidized oils from baked and fried foods, which is equivalent to daily 35 g intake[57]. Due to the importance of frying oils related to human health, many studies on deep-frying oils have been carried out. Maria[35] and his group proposed that the nature and lipid content of the fried food and its ability to absorb the frying medium both affect its composition changes, and they proved that lipid composition of foods with a low original content in lipids and a great capacity to absorb lipid, would change significantly in the deep-frying process like fried dough. For foods with significant high lipid content, a less ability for absorption would undergo less lipid composition change in their experiment condition. Csallany[19]and her group conducted a series of HNE analysis produced in soybean oils that are high in linoleic acid heated at frying temperature (185°C) for different hours (2,4,6, 8 and 10 h). And their results showed that soybean oils would produce HNE under heating at frying temperature and HNE formation increased greatly with increasing heating time. The HNE would form considerably at 2 hours and continued to increase to reach the maximum until 6 hours. After 6 hours, they would decrease due to possible thermal decomposition. Also, they found HNE formation was temperature dependent after they compared HNE formation three different test oils[37]: soybean oil, corn oil and butter oil heated at two frying temperature 190 °C and 218 °C. The concentration of HNE at 218 °C was large greater for all the three oils than that at 190 °C. And their study of HNE formation in fatty acid methyl esters (FAMEs) of stearic oleic, linoleic and linolenic acids heated at 185 °C for 0 and 6 hours found that HNE was only detected in methyl linoleate. And methyl stearate and

methyl oleate did not produce any α,β-unsaturated -4-hydroxyalkenals after thermal treatment[38]. Additionally, they also investigated the effects of intermittent heating and continuous heating on HNE formation[39]. They found that concentration of these α,β-unsaturated -4-hydroxyalkenals increased similarly both under intermittent and continuous heating conditions over a total 5 hour heating time and such results demonstrated that formation of HNE and other α,β-unsaturated -4-hydroxyalkenals produced at frying temperature is a cumulative result of peroxidation process over time.

The same group has also reported the incorporation of HNE from the frying oils into fried foods. They simulated the deep-frying French Fries at 185 °C for 5 hours under laboratory condition using soybean oil as frying oil[20]. Their results showed that concentration of HNE in the oil after frying was similar with that in the oil extracted from the fried potato. These results indicated that HNE is produced in the frying oils due to heat treatment and then easily incorporated into fried foods.

Later, same researchers also investigated the incorporation of HNE from French Fries in various fast food restaurants[21]. And the results again confirmed that HNE formed in frying oils subjected to heat treatment, was incorporated into French Fries.

Other researches also concerned about the risk of exposure to considerable HNE and other aldehydes absorbed from the diet. Antonios Papastergiadis[40] and his group analyzed HNE, HHE and MDA of sixteen food categories through Belgian with and found that consumption of fry nuts, fried snacks, French Fires and minced meat products were observed to contribute most to the intake of MDA and HNE.

There also researchers investigated the influences of antioxidants on formation of HNE in fats and oils due to heat treatment. Pamela [41]and his group investigated the influence of polydimethylsiloxanen (PDMS), which is used as antifoaming agent

during frying in the industry, on the formation of HNE in soybean oil at frying temperature and found the certain concentration addition PDMS could prevent the formation of HNE effectively. Eunok[42] and his group also found that egg yolk powder added fried flour dough could act as antioxidant in the oil oxidation during frying at 180°C by decreasing the formation of conjugated linolenic acids and polar compounds.

Recent studies on HNE metabolites after oral administration and its toxicity

In addition to its endogenous formation, HNE can forms non-enzymatic oxidation of dietary PUFAs during food processing or storage including fried nuts, fried snacks, French fries, minced meat products and frozen fish[18,40,43]and could be incorporated into fried foods from frying oils.

Many researches were conducted to explore the metabolism fate and its toxicity of HNE after intravenous administration. In body, HNE can be metabolized mainly to water soluble and less cytotoxic compounds and excreted in feces and urine[44] very quickly. However, an estimation of 1%-8% HNE remains to bind to proteins including cysteine, histidine and lysine[45]. The most important detoxification of HNE is conjugation to the thiol (-SH) group of the antioxidant-glutathione (GSH) in the presence of glutathione-S-transferases (GST) and followed by reduction to 1,4-dihydroxynonene-GSH by aldose reductase[46]. Another pathway involves the aldehyde dehydrogenase (ALDH) that is able to oxidize HNE to 4-hydroxynonenoic acid (HNA), which is then metabolized by cytochrome P450 (CYP) to form 9-hydroxy-HNA. HNE may also be metabolized by alcohol dehydrogenase (ADH) to produce 1,4-dihydroxy-2-nonene (DHN). Figure 12 shows the general metabolism pathways of HNE[47]. It's reported that main component of metabolites of HNE in rats urine that

was treated intravenous administration found to be 1,4-dihydroxynonane-mercapturic acid (DHN-MA)[48].

Several studies have evidenced the oral toxicity of HNE. Oarada[1] et .al reported that mice that orally treated with HNE –rich fraction of oxidized linoleic acid would undergo sever lymphocyte necrosis in the thymus 24 h later. And they concluded that the LD50 of HNE is 68.6mg/kg/body weight for mice if given intraperitoneally. Nishikawa[49] et.al reported that HNE (10-1000mg/kg body weight)'s acute toxicity after single dose by gavage. All treated animals suffered diffusive liver cell necrosis after 14 days treatment and rats died few hours after 1000mg/kg dosing presented kidney tubular necrosis. And Kang [50]et.al reported nephron and hepatotoxicity of HNE after 4 weeks of daily dose ranging from 0 to 12.5mg/kg body weight. And they concluded a no-adverse-effect-level (NOAEL) below 0.5mg/kg/day for HNE. Grootveld[4] et. al demonstrated the typical trans-2-alkenal compounds are absorbed from the gut into the systemic circulation in vivo, metabolized and excreted in the urine as C-3 mercapturate conjugates in rats, and indicated the dietary ingestion of thermally stressed PUFA-rich culinary oils promote the induction and development of cardiovascular diseases. And Indart[51] et.al reported the administration of lipid oxidation products (including n-alkanals, trans-2-alkenals) rich thermally stressed culinary oils increase the rate of neural tube defects in experimental rats model. And Julia Keller[52] and his group revealed the dietary HNE metabolism fate through analyzing urine metabolites of rats that are treated by gavage with water containing 13-C and 3-H labeled HNE. Their results showed 48% administrated radioactivity excreted in urine and 15% in the feces. And 3% of the administered radioactivity was detected in intestinal contents and 2% remained in major organs including liver, kidney, brain and colon. And the main HNE metabolite in the excreted urine was

identified as 9-hydroxy-nonenoic that might. Such results demonstrated that dietary HNE could be absorbed from foods, metabolized and accumulated in the body.

Most of studies mentioned above investigated the formation of HNE formed in frying oils, however, almost none information of HNE in fried foods such as chickens and donuts, which are common fried foods in the USA.

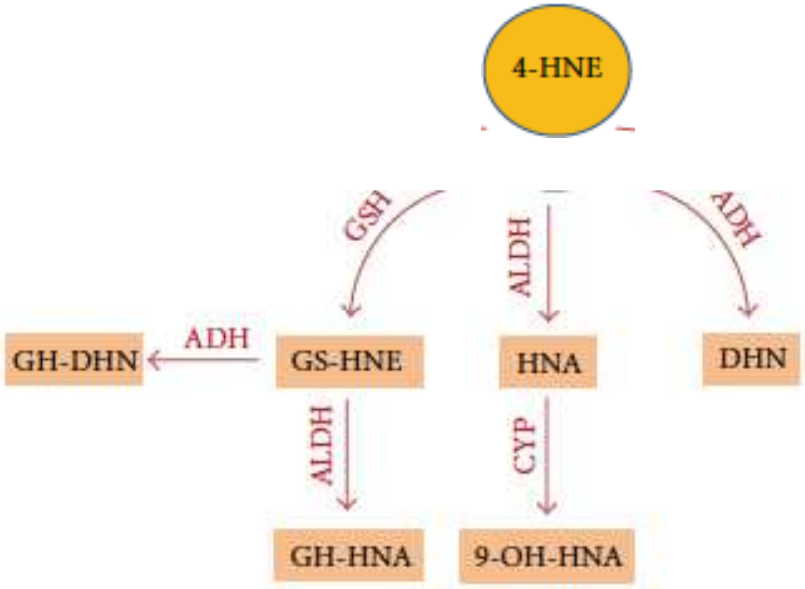


Figure 12. General Metabolites Pathway of HNE (Ref 47)

Objectives

The objective of present experiments is to measure the concentration of 4-hydroxy-2-trans-nonenal (HNE) that was incorporated into fried chickens and glazed donuts. Both of the foods are commonly available in fast food restaurants.

Part II: Experiments

Materials and Methods

Chemicals and Instruments

2.4-dinitrophenylhydrazine (DNPH) was purchased from Eastman Kodak Co. (Rochester, NY). 2-thiobarbituric acid, trichloroacetic acid, HPLC-grade methanol, HPLC-grade water, HPLC-grade dichloromethane, ACS-grade methanol and boron trifluoride-methanol solution were purchased from Sigma-Aldrich Company (St. Louis, MO), sodium thiosulfate was purchased from Fisher Scientific (Fair Lawn, NJ), hydrochloric acid was from Mallinckrodt Baker Inc (Paris, KY). HPLC-grade hexane was from EMD Chemicals,Inc (Gibbstwon, NJ).HNE standard solution was from Cayman Chemicals Co.(Ann Arbor,MI). Plates for thin layer chromatography (TLC) were purchased from EMD Millipore Corp (Billerila, MA). Syringe was from Terumo Medicare Cop (Somerset, NJ). No.1 filter paper and 0.45 um syringe filters were purchased from Whatman Ltd. (Kent, England).

The gas chromatography used the 5830A Gas Chromatography (Hewlett-Packard, Saginaw, MI) with flame ionization detection and a carbowax capillary column. The GLC reference Standard for GC was from Nu-Chek Prep, Inc (Elysian, MN)

The HPLC system consisted of a sample injector, a solvent delivery system (9050, Varian, Walnut Greek, CA) and a UV detector (Spectra SERIES UV 150). The HPLC column was Ultrasphere ODS (5μ, 4.6mm×25 cm. Hichrom, Berkshire, UK). Detailed operating parameters are provided later in the methods section.

Fried chicken breasts and chicken thighs, chicken nuggets and raised glazed donuts were purchased between 2-3 pm from 3 different local stores (Saint Paul, MN) separately.

Fat Extraction of Fried Chickens and Glazed Donuts

For fried chicken breast and thigh samples, skin and meat were separately analyzed and bones discarded for the fat extraction. ~50 g skin or meat samples were mixed with 100 mL hexane in a blender for 1 min, then filter and collect the filtrate. Add another 100 mL hexane and repeated this procedure for 2 times more and collect all the filtrate. Then add ~50 g sodium thiosulfate into the total filtrates and shake it for 10 min and repeat it 2 times. Then filter solution again to remove sodium thiosulfate and evaporate it using vacuum evaporation to remove hexane. Same protocols applied to chicken nuggets without separating skin and meat, and glazed donuts as well. All samples were stored at -70 °C freezer and all extracted fats were stored at -20 °C freezer until analyzed. Fat extraction was duplicated for each sample.

Thiobarbituric Acid (TBARS) Test

The TBARS method is the most widely test used for measuring secondary lipid oxidation products in various meats, and it's a colorimetric technique determined by the absorbance of the pink color compounds produced by the reaction between thiobarbituric acid and aldehydes and related carbonyl compounds. TBARS tests measure all aldehydes including malondialdehyde (MDA), ketones and related carbonyl compounds[53].

In order to obtain the degree of secondary oxidation of the samples, the thiobarbituric acids test was conducted to measure the formation of secondary oxidation products in these fats extracted from samples.

The TBARS test was done in the method of Buege and Aust. The standard curve was determined by combing 200µL MDA /water solution with 4mL of TBA/TCA/HCL solution (equal volumes of 15% w/v trichloroacetic acid (TCA), 0.375% w/v 2-

thiobarbituruc acid (TBA) and 0.25N HCL) MDA concentration was showed below in Table 1. The mixture of MDA and TCA/TBA/HCL solution was heated for 15 min in a boiling water bath. And absorbance was measured at 535nm with UV spectrophotometer. The standard curve is shown in Figure 13.

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	50	150	0.043
3	100	100	0.086
4	150	50	0.129
5	**200**	**0**	**0.172**

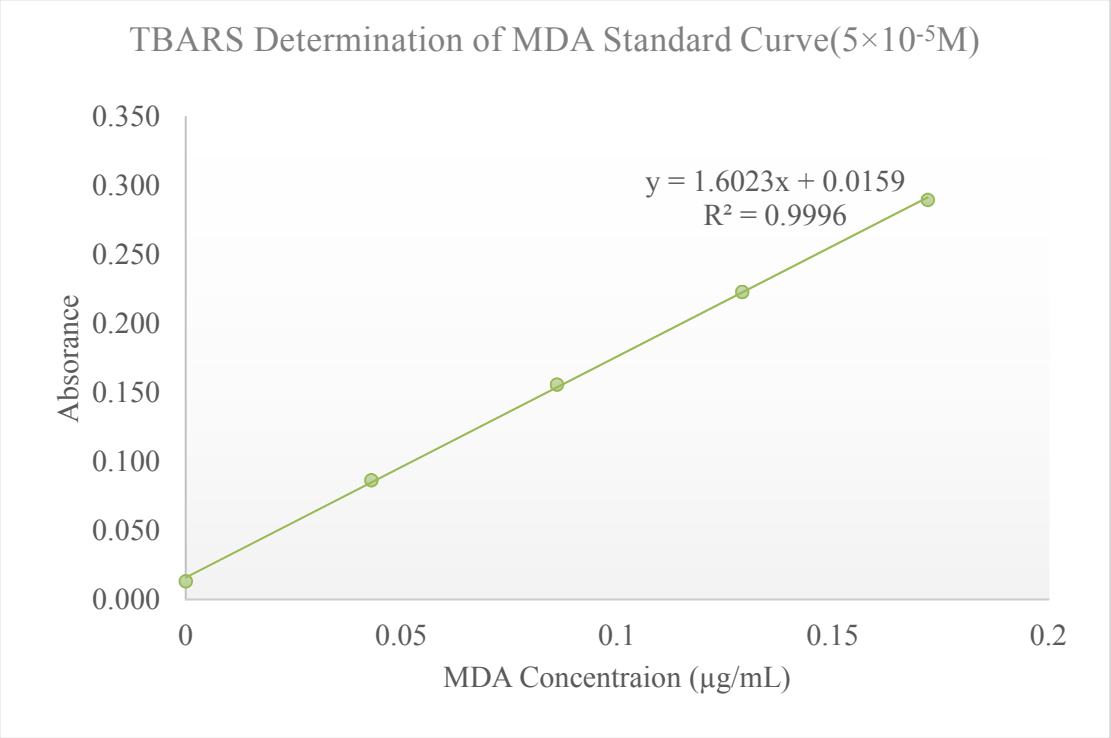


Figure 13. MDA concentration standard curve

For samples, 0.1875g(±0.0005g) (which is the weight of 200μL) of extracted fat was mixed with 4mL of same TBA/TCA/HCL solution and then heated for 15 mins in boiling water bath. And absorbance of the samples was measured at 535nm with UV spectrophotometer after cooling down.

Calculations of TBARS results (μg MDA equivalence /g extracted fat)

Reaction solution volume: 4mL (TBA+TCA+HCL)+200 μL=4.2mL

Containing fat amount: 0.1875g

Fat concentration: 0.1875g/4.2mL=0.0446 g/mL

MDA standard curve: Absorbance=Concentration *1.6023+0.0159

$$\mu g \text{ MDA equi/ g extracted fat} = \frac{\mu g \text{ MDA equi/mL solution}}{0.0446 g/mL}$$

Fatty Acid Distribution by Gas Chromatography

Fatty acid distribution of extracted fats from all fried chicken samples and glazed donuts were measured by gas chromatography. 2 drops of fats was added into 3 mL of BF_3-Methanol (14% BF_3 in methanol) in a 20 mL test tube in duplicate. The test tubes were capped and shaken vigorously. Then these tubes were placed in boiling water bath for 1 h. after cooling, 2 mL distilled water and 10 mL hexane was added and then mixture was shaken for 10 mins. Collect the top hexane layer and the hexane layer were dried with the addition of ~2g of sodium sulfate. 10 µL of dry hexane layer sample was injected into GC and fatty acids distribution was identified and measured based on the retention time of fatty acids standards.

Determination of HNE by High Performance Liquid Chromatography

This method is developed by Seppanen and Csallany[54]. The principle of this method was based on the nucleophilic addition reaction between aldehyde and ketone's carbon-oxygen double bond and 2,4-dinitrophenylhydrazine (DNPH) to form DNPH derivatives that could be detected by UV light at 375nm. Thus HNE could be detected and measured as its DNPH derivative form. Typical examples of HPLC chromatogram are in the appendix.

Outline of HPLC methods for HNE:

1. Preparation of DNPH recrystallization and DNPH reagent
2. Reaction of extracted fats samples with DNPH
3. Extraction of DNPH derivatives with 75:25/methanol: water and 100% dichloromethane and evaporation into 0.5mL
4. Separation of polar and non-polar compounds on TLC plates
5. Extraction from TLC plates with methanol and evaporated into exactly 1mL

6. Injection of 50 μL sample into HPLC using mobile phase ratio of 50:50/methanol: water for polar compounds analysis and ratio of 75:25/methanol: water for non-polar analysis at 375 nm in UV detection

Details are described as following:

I. Recrystallization of DNPH

2 g DNPH was dissolved in 200 mL methanol and heated at 60 °C for 1h for complete dissolve. The dissolved DNPH was then placed in ice bath for 24 h for crystallization. The crystallized DNPH was then filtered by No.1 filter paper and re-dissolved in 200 mL methanol again and same protocols was repeated for 3 times more and the final recrystallized DNPH was stored in a desiccator for 3 days for drying.

II. Preparation of DNPH Reagent

DNPH reagent was prepared freshly for each analysis. 10 mg recrystallized DNPH was dissolved in 20 mL 1N hydrochloride acid and heated with stirring at 50°C for about 1h. Then DNPH reagent was washed with 10 mL hexane for 4 times using separating funnel to remove impurities and hexane layer was discarded.

III. Preparation of the DNPH reagent blank and acetone-DNPH standard

DNPH reagent blank standard was prepared by combination of 5 mL HPLC grade water with 5 mL fresh DNPH reagent, and then the mixture was shaken slightly in the dark overnight at room temperature. After incubation, the DNPH derivatives were extracted with 10 mL dichloromethane for 3 times. The collect dichloromethane extract was then evaporated under N_2 gas until 0.5 mL. The preparation of acetone-DNPH standard was the same procedures as DNPH reagent blank using 5 mL acetone with 5 mL fresh DNPH reagent.

IV. Preparation of HNE-DNPH standard

100 μL HNE (5 mg/500 μL ethanol) was added into 10 mL fresh DNPH reagent and the mixture was shaken slightly in the dark overnight at room temperature. Then the HNE-DHPH was extracted by dichloromethane for 3 times and the collect dichloromethane was evaporated under N2 gas to about 1.5 mL. Then apply the concentrated HNE-DNPH to 2 Thin Layer Chromatography (TLC) plates. Then collect the polar region and extracted with 10 mL methanol for 3 times. The combined methanol extract was evaporated under N_2 gas to the extract volume of 10 mL.

V. Reaction of DNPH derivatives with Fat Samples and Separation

1 g extracted fat (in duplicate) was reacted with 5 mL freshly prepared DNPH reagent in a 50 mL Erlenmeyer flask and then was incubated at room temperature overnight in the dark with slight shaking. The DNPH derivatives were firstly extracted with 10 mL 75:25/methanol: water for 3 times and separated by centrifugation for 10 mins. Then the combined methanol extracts were extracted with 10 mL dichloromethane for 3 times and separated by separating funnel. Collect the bottom dichloromethane layer and then the combined dichloromethane extracts was evaporated under N_2 gas until the volume decreased to about 0.5 mL.

VI. Separation of DNPH Derivatives by Thin Layer Chromatography (TLC)

The concentrated dichloromethane extracts was applied to 2 silica gel thin-layer chromatographic plates. Total sample solution was transferred to the bottom of plate through a 250 μL micropipette as a very thin line. Extra dichloromethane was used to rinse the test tube and also applied to the plates. The DNPH reagent blank and acetone -DNPH standard were spotted next to the sample on the plate to help identify the position of the polar and nonpolar aldehyde-DNPH in samples based on the polarity. Then the plates were developed in the shallow pool of HPLC-grade dichloromethane

solution for 45 mins. Figure 14 shows a typical TLC plate visualization. The polar and nonpolar 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 original sample line and DNPH reagent blank band. Nonpolar carbonyl compounds (NPC), such as alkanals, alkenals and ketones were located between the acetone-DNPH band and solvent front. Then collect polar part and non-polar part separately and extract them with 10 mL methanol for 3 times. The combined methanol extracts were evaporated under N_2 gas to the exact 1mL in a volumetric flask and stored -20°C in amber vials tightly covered with Para film until HPLC analysis.

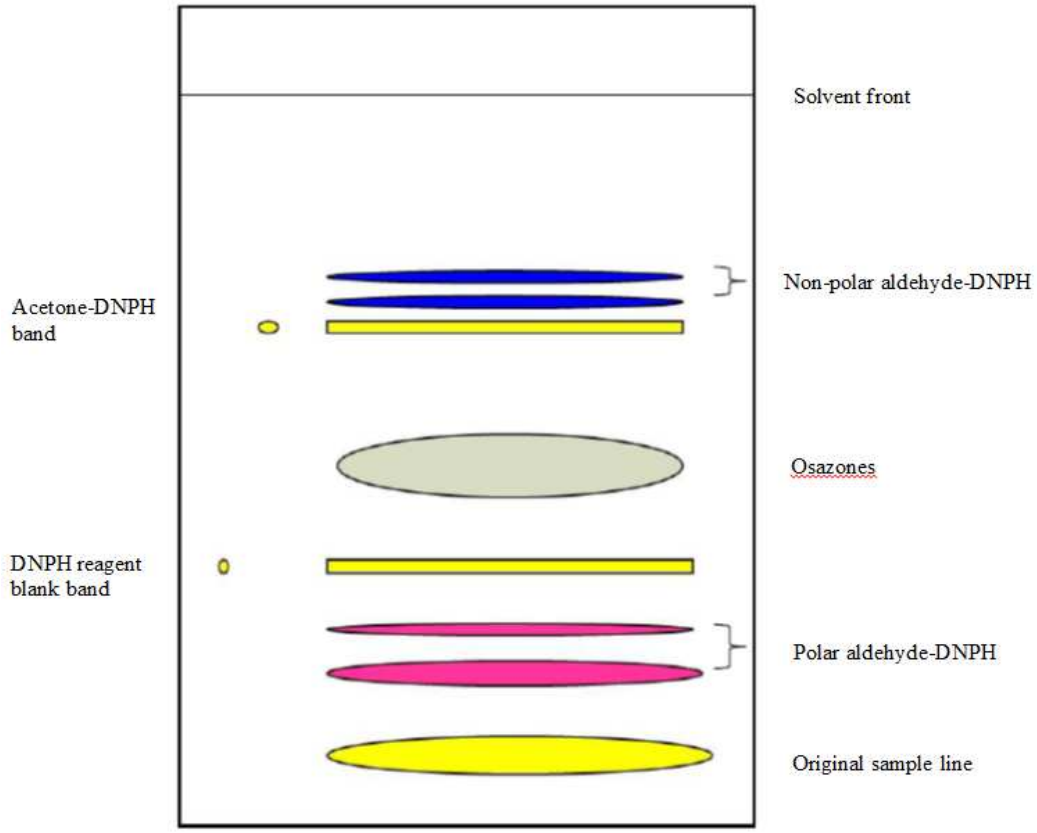


Figure 14. Visualization of polar and non-polar aldehydes-DNPH derivatives after development in TLC plate

VII. Identification of DNPH derivatives of polar (PC) and non-polar (NPC) lipophilic aldehydes and related carbonyl compounds by HPLC

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

a) For polar compounds (PC), protocols consist of 10 mins of isocratic elution of 50:50/methanol: water, then followed by 20 mins of linear gradient to 100% methanol and additional 10 mins of 100% methanol at a flow rate of 0.8mL/min. Absorbance was measured at 375nm.
b) For non-polar compounds (NPC), the protocols consist of 10 mins of isocratic elution of 75:25/methanol: water then followed by 20 mins of linear gradient to 100% methanol and additional 10 mins of 100% methanol with the same flow rate of 0.8mL/min as polar compounds protocols. Absorbance was also measured as 375nm.

The HNE-DNPH standard was injected into the HPLC before each analysis of samples injection to check the reproducibility of HPLC system. Figure 14 shows the typical peak shape and retention time of pure HNE-DNPH standard in the PC solvent system.

Identification of the HNE-DNPH derivatives and other polar and non-polar aldehydes from fats are accomplished by combination of comparison of retention time of standard peak and sample peaks and Co-chromatography. Co-chromatography was determined by spiking the known amount of HNE standard with unknown sample to see how much increase for the peak area. The recovery is calculated and found to be generally between 98% and 105%.

VIII. Calculation of the concentration of HNE in the extracted fats

Aldehyde concentration was expressed as ng hexanal equivalent /g extracted fat by calculating peak area of hexanal-DNPH that was synthesized by known amount hexanal, which turned out the area equals to 1 ng hexanal equivalent was 22,182. HNE concentration was expressed as μg HNE/g extracted fat and μg HNE/100 g sample based on the conversion of molecular weight of hexanal (100) to HNE (156). Total PC-DNPH and NPC-DNPH concentration was expressed as μg hexanal equivalent /g extracted fat since the individual aldehyde and carbonyl compounds are unknown.

Statistical Analysis

ANOVA and Tusky testing was used using XLSTAT to determine whether there are significant differences between the different type chicken samples. And statistically significant differences were determined at $p< 0.05$.

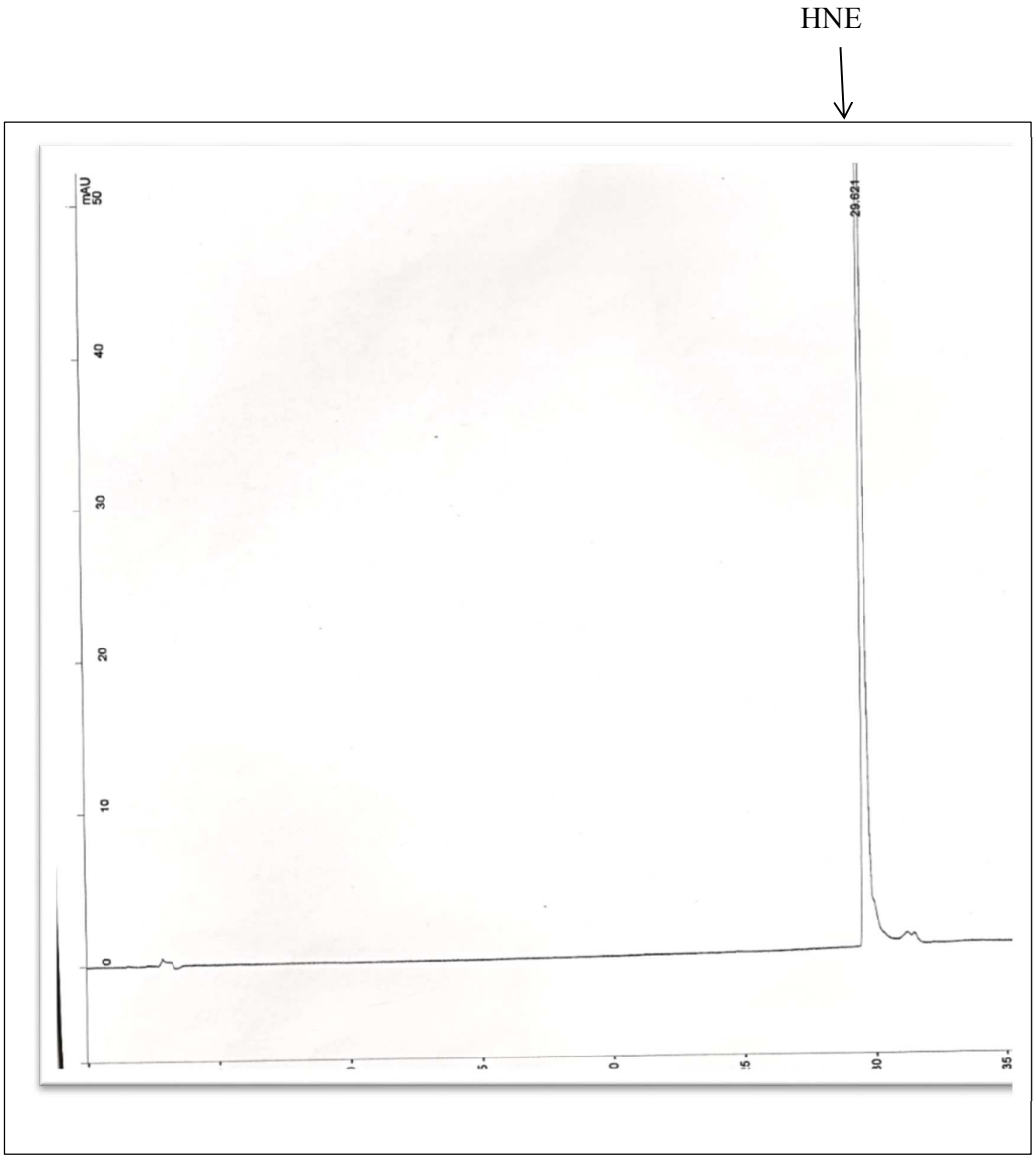


Figure 15. HNE-DNPH derivative standard curve of HPLC

Results

1) Weight of Samples and Percent of Extracted Fat

Table 2, 3 and Table 4 show weight samples and percent of extracted fat in all fried chicken breasts and chicken thighs, chicken nuggets, popcorn chickens and glazed donuts samples.

It's obviously to see that percent of fat extracted from skin is much higher than that of meat both in all chicken breast samples and chicken thigh sample. The results are predicted because most fat of chicken are from skin and coating also acts as barrier to prevent further oil absorption.

And percent of fat extracted from skin of chicken thighs was similar with that of skin of chicken breast, while fat percent of meat of chicken thigh ranging from 10.04% to 12.13% is slight higher than that of meat of chicken breast ranging from 5.24% to 7.00%.

For chicken nuggets and popcorn chickens, it's interesting to note that commercial popcorn chicken size is smaller than chicken nuggets thus it's much lighter than other two brands chicken nuggets. And brand 1 chicken nuggets have largest individual weight and lowest fat percentage.

For glazed donuts, fat percent of donuts was similar ranging from 15.99% to 26.80%.

Table 2. Weight of samples and Percent of extracted fat in chicken breasts from 3 different brands

Sample	Weight of whole breast (g)	Weight of meat (g)	Percent of meat in whole breast (%)	Extracted fat from meat (g)	Percent of fat extracted from meat (%)
CBR 1	214.56	136.99	63.85	9.54	7.00
CBR2	227.32	116.52	51.26	6.11	5.24
CBR 3	249.80	126.67	50.42	7.87	6.20
		Weight of skin (g)	Percent of skin in whole breast (%)	Extracted fat from skin (g)	Percent of fat extracted from skin (%)
CBR 1		46.56	21.70	18.93	40.65
CBR 2		85.78	37.74	28.08	32.73
CBR 3		95.81	38.28	32.20	33.69

Table 3. Weight of samples and Percent of extracted fat in chicken thighs from 3 different brands

Sample	Weight of whole thigh (g)	Weight of meat (g)	Percent of meat in whole thigh (%)	Extracted fat from meat (g)	Percent of fat extracted from meat (%)
CTH 1	159.65	82.91	51.93	10.06	12.13
CTH 2	151.32	61.66	40.75	6.78	10.99
CTH 3	153.87	60.35	39.23	6.07	10.04

	Weight of skin (g)	Percent of skin in whole thigh (%)	Extracted fat from skin (g)	Percent of fat extracted from skin (%)
CTH 1	45.28	28.36	16.03	35.40
CTH 2	67.56	44.65	24.11	35.69
CTH 3	72.84	47.29	29.61	40.71

Table 4. Weight of samples and Percent of extracted fat in chicken nuggets and popcorn chicken

Sample	Pieces	Total weight of samples (g)	Weight of individual sample (g)	Weight of extracted fat from total sample (g)	Percent of fat (%)
CNU 1	4	43.09	10.78	9.18	21.30
CNU 2	3	48.54	16.18	9.45	19.47
PCC 3	**9**	**67.80**	**7.53**	**16.19**	**23.88**

Table 5. Weight of samples and Percent of extracted fat in raised glazed donuts of 3 different brands

Sample	Pieces	Total weight of samples (g)	Weight of individual sample (g)	Weight of extracted fat from total sample (g)	Percent of fat (%)
GDO 1	1	73.37	73.37	11.73	15.99
GDO 2	2	114.74	57.37	21.08	18.37
GDO 3	1	80.55	80.55	21.58	26.80

2) Fatty Acids Distribution

The fatty acid distribution of fat extracted from meat and skin separately of fried chicken breasts and chicken thigh, fat extracted from chicken nuggets and glazed donuts were measured to determine the percentage of linoleic acid in these fried foods samples since these fatty acids are precursors for the formation of α, β-unsaturated-4-hydroxyaldehydes.

Table 6 shows the retention times of pure palmitic, stearic oleic, linoleic and linolenic acid standards using the GC methods as described in the method section. By comparing the retention time of fatty acids between the samples and the pure standards, the fatty acid distribution of fat extracted from meat and skin of chicken breasts and chicken thighs, chicken nuggets, popcorn chickens and glazed donuts are shown in Table 7, 8, and 9.

Table 6. Retention times of pure fatty acids by GC

Fatty acid	Retention time (min)
Palmitic acid (18:0)	1.69
Stearic acid (18:1)	2.95
Oleic acid (18:2)	3.19
Linoleic acid (18:3)	**3.75**
Linolenic acid (18:4)	**4.66**

Table 7. Fatty acids distribution of fat extracted from chicken breasts (CBR) and chicken thighs (CTH) meat and skin

	Sample	Fatty acids (%)				
		Palmitic	Stearic	Oleic	Linoleic	Linolenic
CBR	Meat 1	15.35	5.37	53.43	20.44	4.46
	Skin 1	10.89	3.06	57.06	21.64	4.72
	Meat 2	12.77	4.15	56.79	20.97	3.56
	Skin 2	10.75	3.99	49.40	31.38	3.38
	Meat 3	12.14	4.46	59.42	18.17	3.98
	Skin 3	8.67	3.84	63.29	17.38	5.39
CTH	Meat 1	22.53	5.71	47.17	19.11	X
	Skin 1	14.48	4.13	53.36	21.06	4.00
	Meat 2	19.55	6.26	43.89	23.04	1.31
	Skin 2	11.37	3.98	46.82	31.80	3.51
	Meat 3	**18.21**	**5.29**	**54.16**	**16.80**	**1.98**
	Skin 3	**9.46**	**4.16**	**61.90**	**17.42**	**5.22**

X= not detected.

Table 8. Fatty acids distribution of fat extracted from chicken nuggets (CNU) and popcorn chicken (PCC)

Sample	Fatty acids (%)				
	Palmitic	Stearic	Oleic	Linoleic	Linolenic
CNU 1	15.20	5.70	39.65	35.64	2.40
CNU 2	11.60	5.35	49.83	30.68	1.22
PCC 3	**6.34**	**4.45**	**63.12**	**20.53**	**5.44**

Table 9. Fatty acids distribution of fat from glazed donuts (GDO)

Sample	Fatty acids (%)				
	Palmitic	Stearic	Oleic	Linoleic	Linolenic
GDO 1	11.72	10.82	61.26	14.80	1.13
GDO 2	13.16	11.94	61.20	12.98	0.75
GDO 3	**39.92**	**5.77**	**38.55**	**13.60**	**0.75**

From results above it showed that fat extracted from skin had similar linoleic acid percentage with fat of meat both for chicken breasts samples and chicken thighs samples, ranging from 16.80% to 21.64%, except for chicken breasts and chicken thighs from brand 2. Fat extracted from skin and meat both of chicken breasts and chicken thighs of brand 2 contained higher linoleic acid percent than other 2 brands. It is interesting to see all these fats contained the highest oleic acid ranging from 49.40% to 63.29%, which is reported by the researches it would not produce[35,38] any oxygenated α,β-unsaturated aldehydes after thermally induced lipid oxidation.

For chicken nuggets, fat extracted from chicken nuggets of brand 1 contained the highest linoleic acid 35.64% followed by brand 2 30.68%.

For glazed donuts, fat of glazed donuts from 3 different brands had similar linoleic acid percentage, and overall lower than fat from chicken nuggets, and chicken breasts and chickens thigh skin fat and meat fat.

3) Thiobabituric Acid Reactive Assays (TBARS)

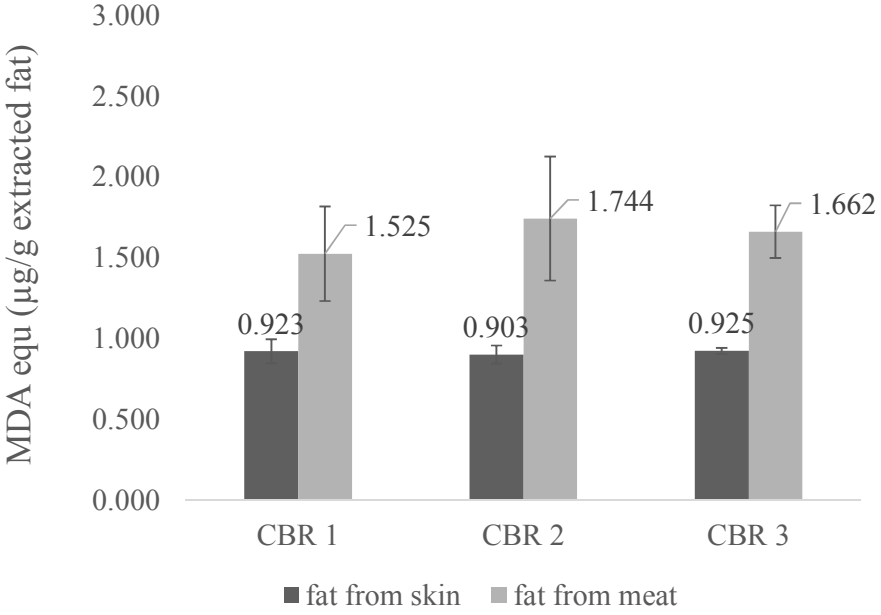
Figure 16, 17 and 18 show the results of TBARS formation in fat extracted from skin and meat of fried chicken breasts and fried chicken thighs from 3 different commercial brands, and results of fat in chicken nuggets and popcorn chickens and glazed donuts from 3 different commercial brands. TBARS results are expressed as μg MDA equi/g extracted fat.

It is interesting to see that TBARS value in fat extracted from meat is higher than that in fat extracted from skin for both chicken breast samples and chicken thighs samples, since they contained similar linoleic acid content. The possible reason could be fat from the meat might contain heme iron acted as catalysts that could accelerate lipid oxidation process. Some investigation also implicated myoglobin as a major catalyst of lipid oxidation[55]. It is expected to see fat extracted from chicken thigh meat has a higher TBARS values than that in fat extracted from chicken breast meats, because chicken thigh is dark meat that contains more myoglobin than the white meat-chicken breast. Other studies reported that the combination of free ionic iron content and heat-stable ferric ion reducing capacity (FRC) played an important role on the development of lipid oxidation in cooked meat[56].

For chicken nuggets, it is unexpected to see that fat of brand 2 chicken nuggets had the significant highest TBARS value than brand 1 while brand 1 had the highest linoleic acid percent than brand 2.

For glazed donuts, fat from 3 brands had similar low TBARS values that agreed with their linoleic acid percent.

A



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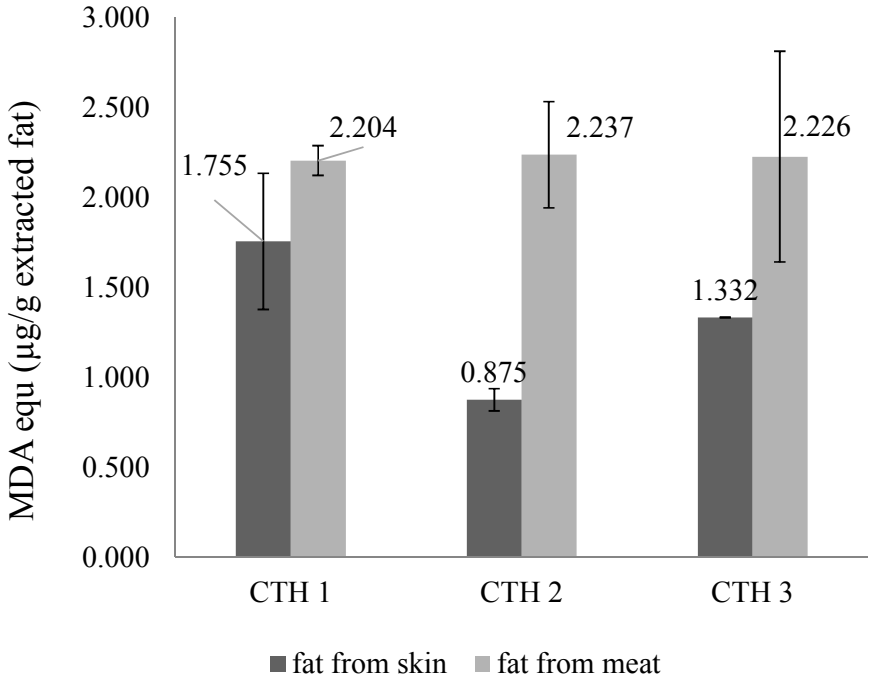

Figure 16. TBARS results of fat extracted from A. Chicken Breasts and B. Chicken Thighs

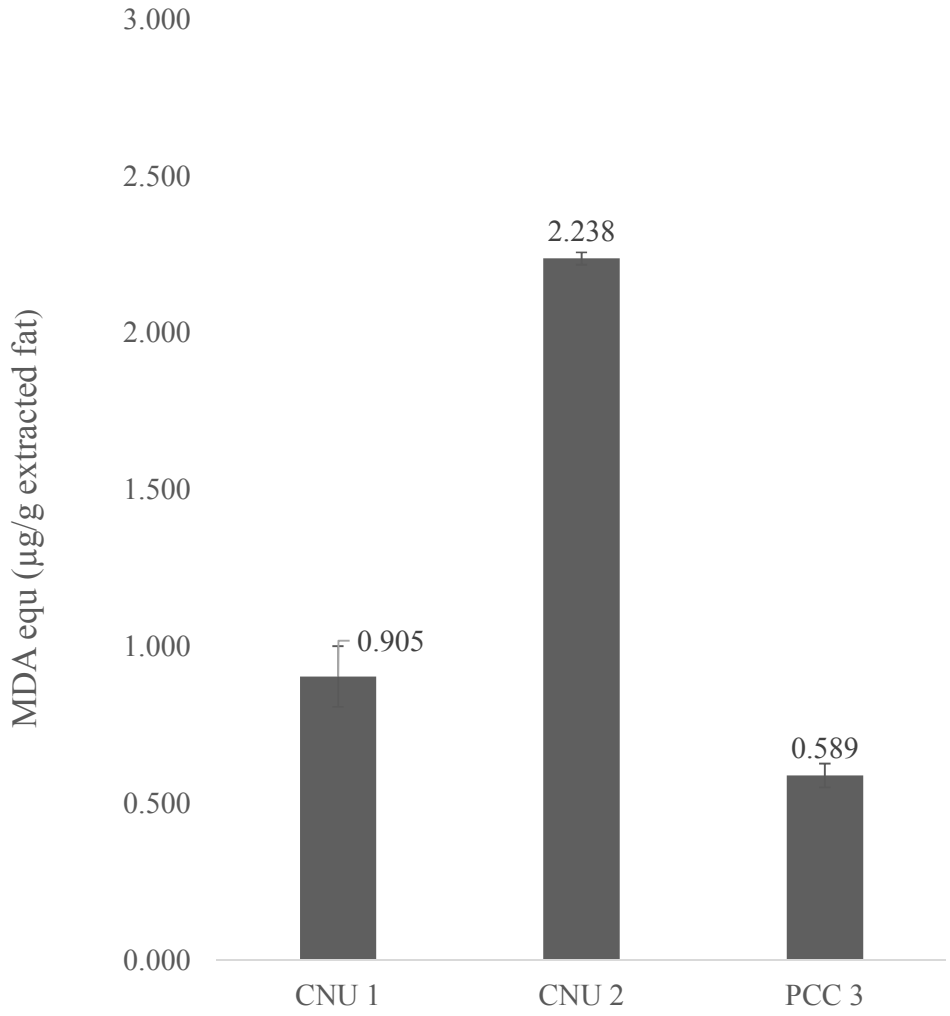

Figure 17. TBARS results of fat extracted from brand 1 and 2 Chicken Nuggets and brand 3 Popcorn Chicken

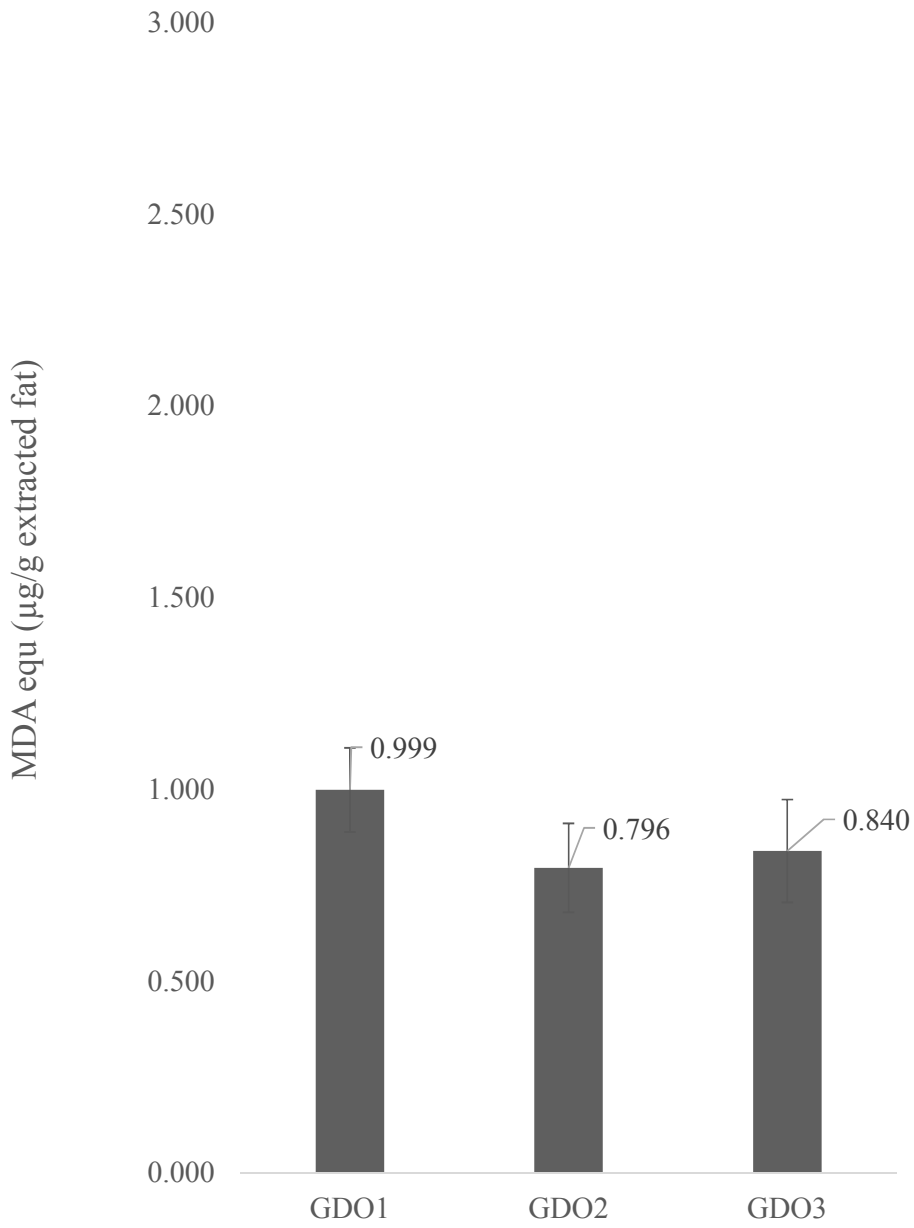

Figure 18. TBARS results of fat extracted from Glazed Donuts

4) The Formation of HNE in Commercial Fried Chicken Breast, Chicken Thighs, Chicken Nuggets, Popcorn Chickens and Glazed Donuts from 3 Different Commercial Brands

Figure 19 to 26 show the results of HNE concentration in fats extracted from fried chicken breasts, chicken thighs, chicken nuggets and glazed donuts separately. And typical examples of HPLC chromatogram are shown in the appendix.

a) Chicken Breasts

Figure 19 shows the HNE formation in fat from skin and meat of chicken breasts from 3 different commercial brands.

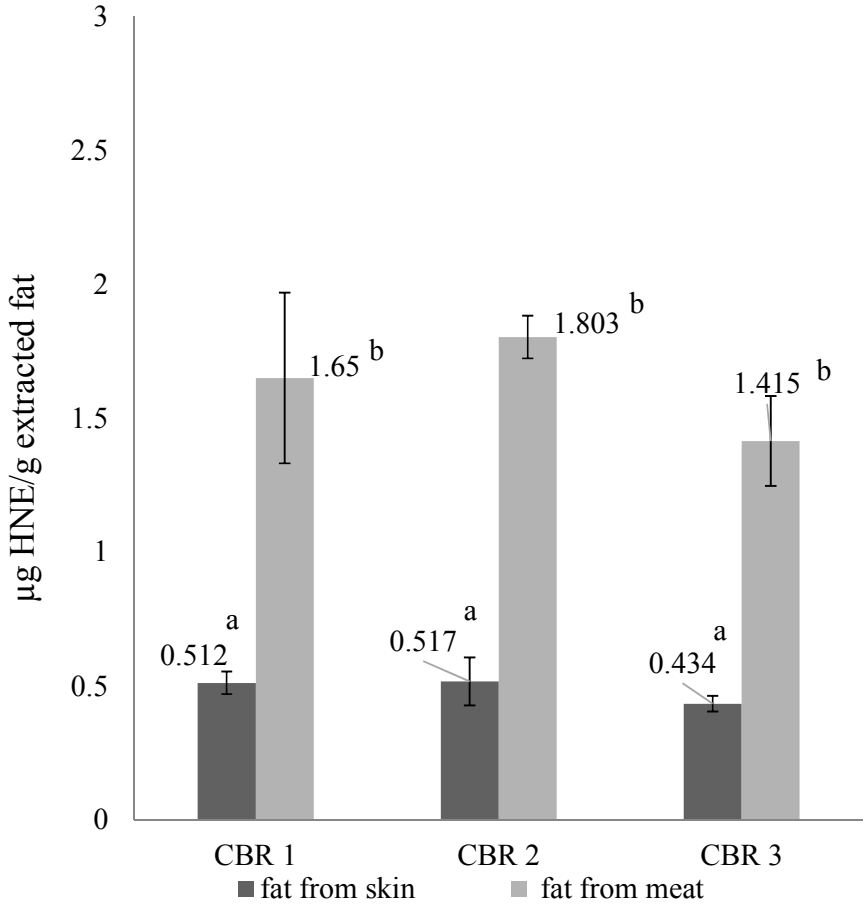

Figure 19. HNE concentration of extracted fats in fried chicken breasts meat and skin

***Group with different letters a, b means significant different and $p<0.05$

b) Chicken Thighs

Figure 20 shows the HNE formation in fat from skin and meat of fried chicken thighs from 3 different commercial brands.

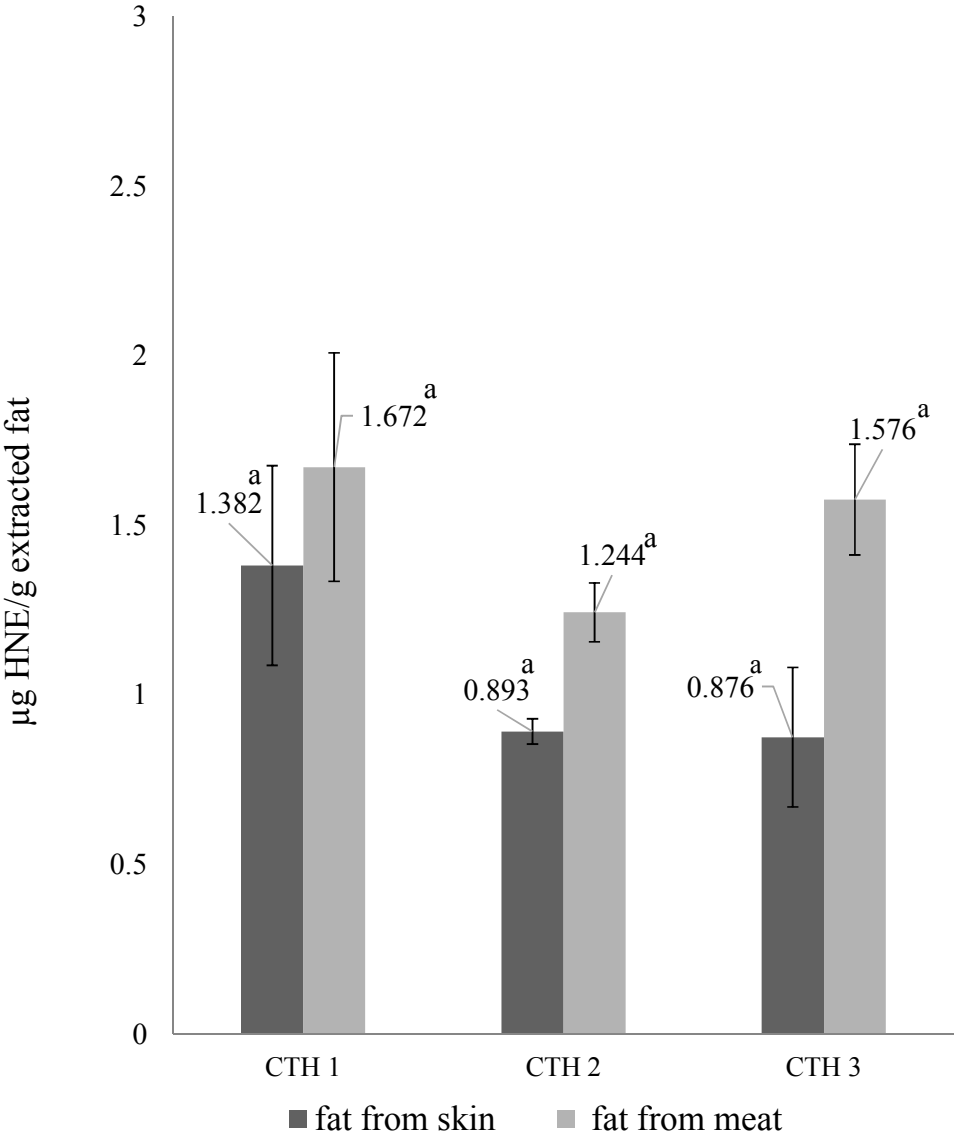

Figure 20. HNE concentration of extracted fats in fried chicken thighs meat and skin

*Groups share the same letter a means no significant difference

c) Comparison of HNE formation in Fat Extracted from Fried Chicken Breasts and Chicken Thighs

Table 10, 11 and 12 show the results of HNE concentration in skin and meat and total HNE amount in 100 g chicken breast and chicken thigh separately, and Figure 21 shows the comparison of HNE amount between meats and skins of 3 different brands.

Table 10. HNE concentration in meat fat of chicken breast and chicken thigh

	μg HNE /g extracted fat	Extracted fat from sample/g	Total HNE in meat/g
CBR1	1.65	4.47	7.38
CBR2	1.80	2.69	4.84
CBR3	1.42	3.13	4.44
CTH1	1.67	6.30	10.52
CTH2	1.24	4.50	5.58
CTH3	1.58	3.93	6.21

Table 11. HNE concentration in skin fat of chicken breast and chicken thigh

	µg HNE /g extracted fat	Extracted fat from sample/g	Total HNE in skin/g
CBR1	0.51	8.82	4.50
CBR2	0.51	12.35	6.30
CBR3	0.43	12.90	5.55
CTH1	1.38	10.04	13.86
CTH2	0.89	15.94	14.19
CTH3	0.88	19.25	16.94

Table 12. Comparison of total HNE amount in 100g chicken breast and chicken thigh

	Total weight/g (skin+meat)	HNE in total /µg	HNE amount per 100g sample (skin+meat)
CBR1	85.55	11.87	13.88
CBR2	89.00	11.14	12.52
CBR3	88.80	9.99	11.25
CTH1	80.29	24.38	30.36
CTH2	85.40	19.77	23.15
CTH3	86.52	23.15	26.76

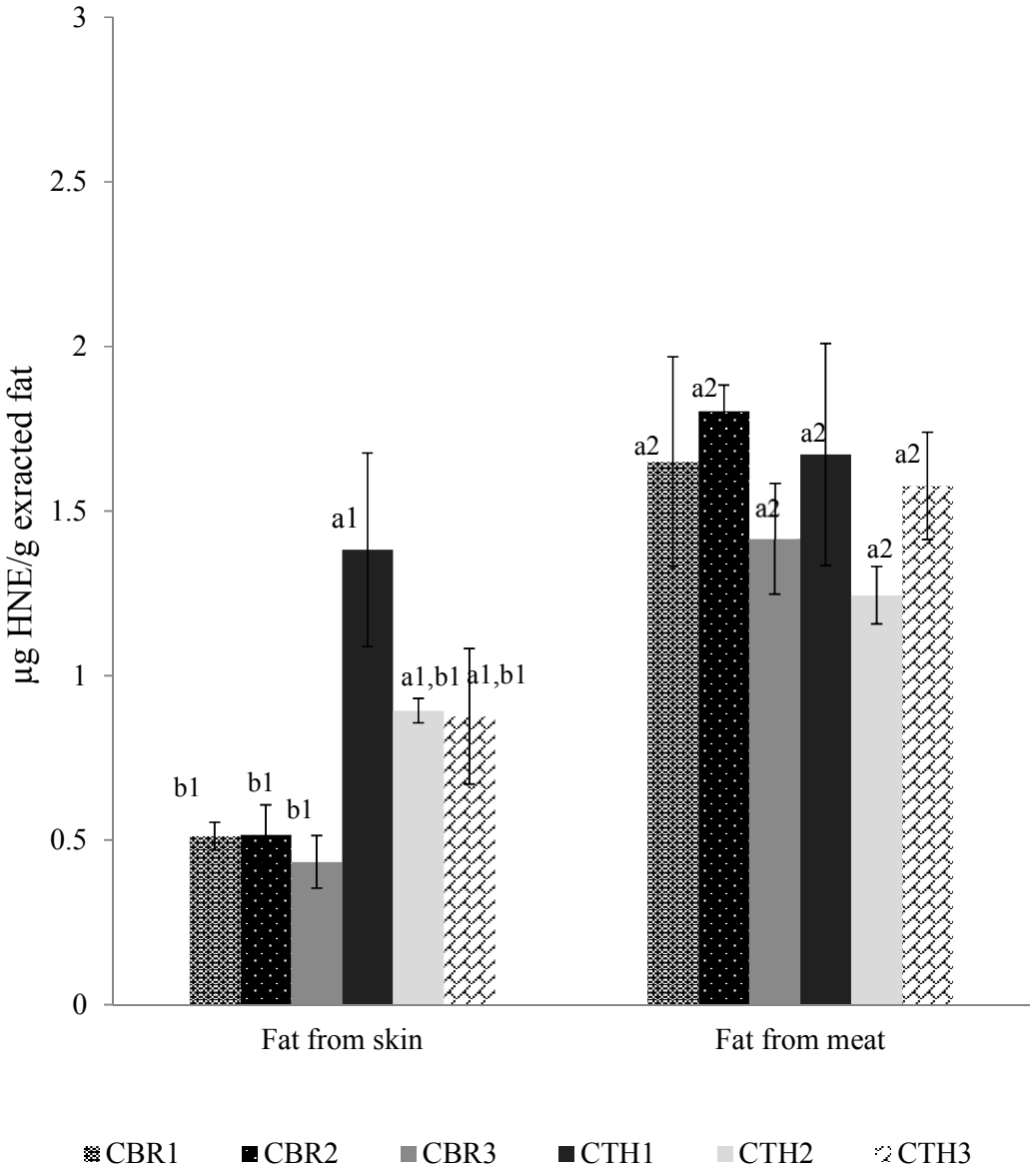

Figure 21. Comparison of HNE concentration of extracted fats in chicken breasts and thighs skins, chicken breasts and thighs meats of 3 different brands

*Letters with same number means groups doing same statistical analysis

d) Chicken Nuggets and Popcorn Chicken

Figure 23 shows the HNE concentration in fat extracted from chicken nuggets from 2 different commercial brands and popcorn chicken.

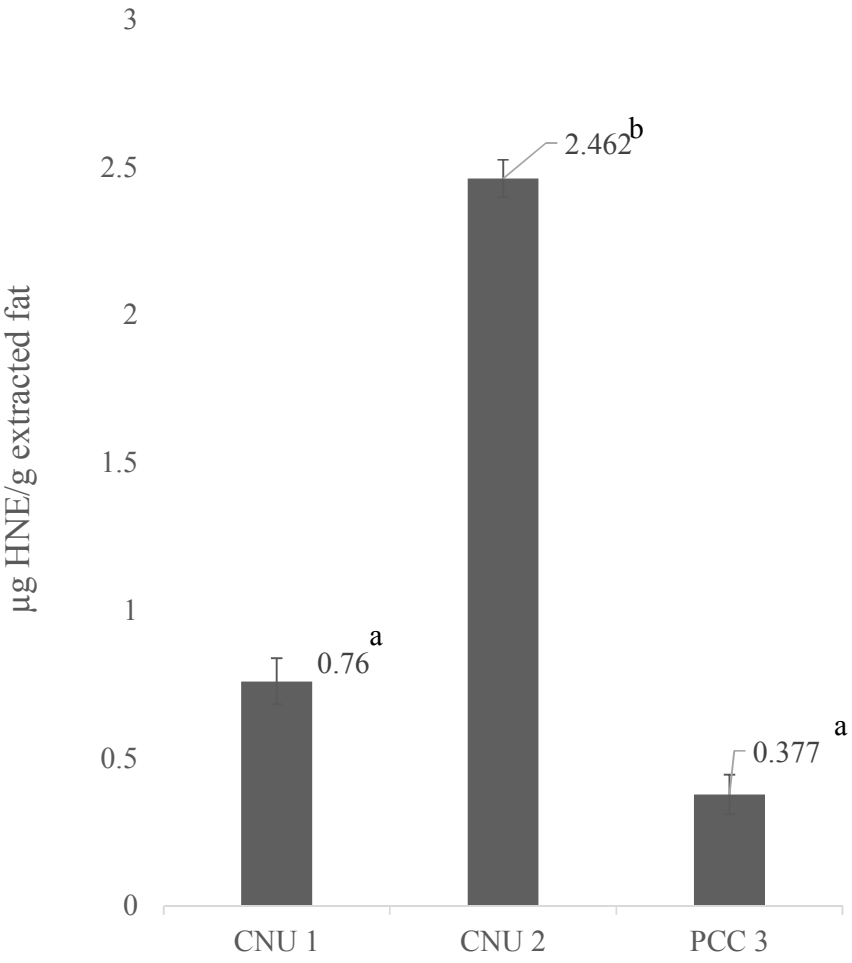

Figure 22. HNE concentration of extracted fats in fried chicken nuggets and popcorn chicken

*Group with different letters a, b means significant different and $p<0.05$

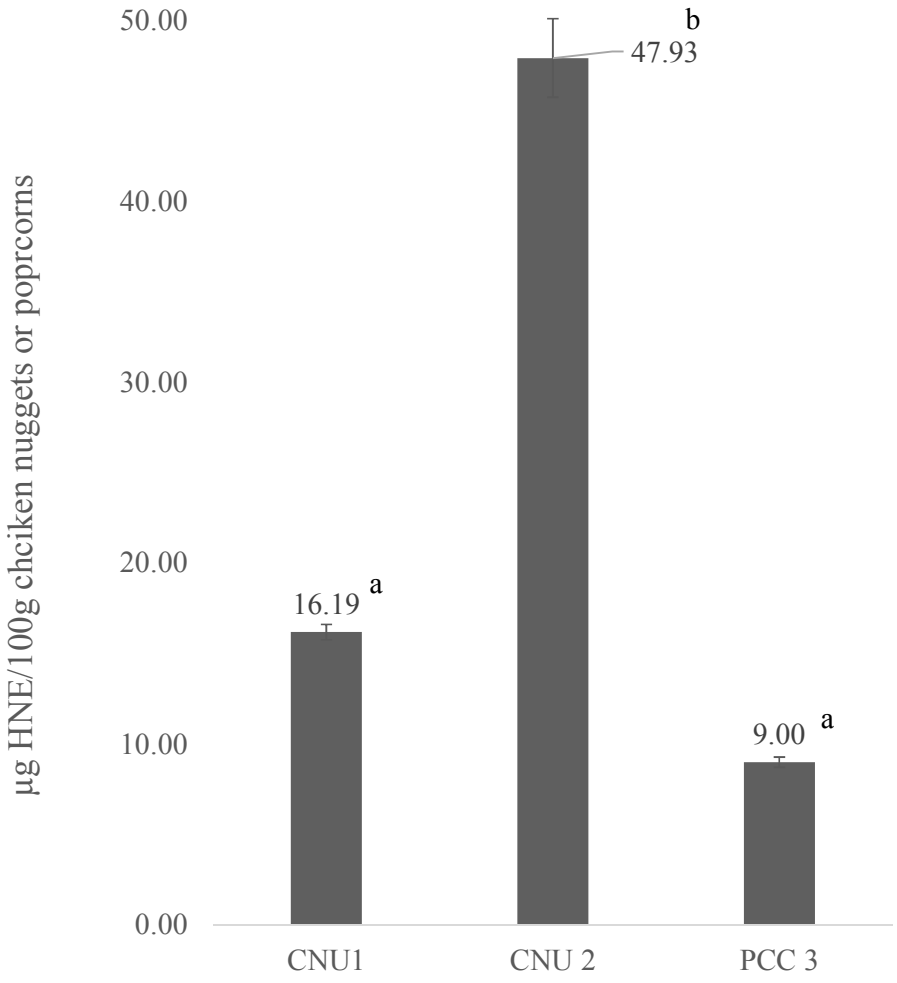

Figure 23. HNE amount in 100g chicken nuggets and popcorn chickens

*Group with different letters a, b means significant different and $p<0.05$

e) Glazed Donuts

Figure 25 and Figure 26 show results of µg HNE/g extracted fat and µg HNE/100g donut samples respectively.

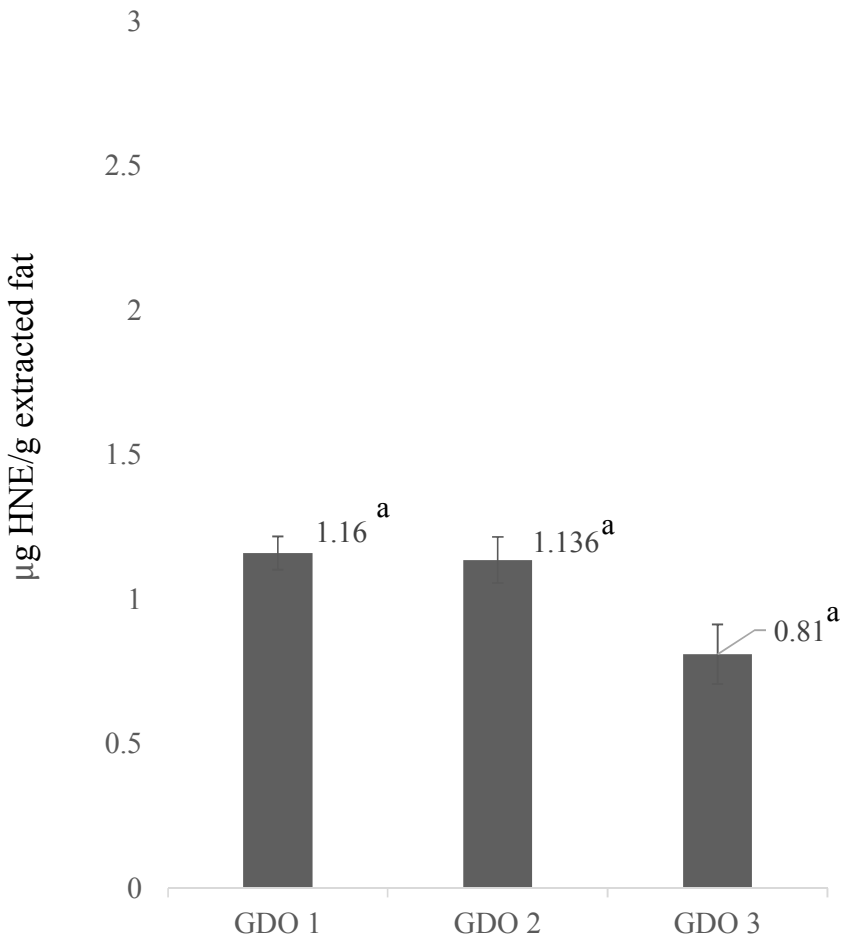

Figure 24. HNE concentration of extracted fats extracted in raised glazed donuts

*Groups share the same letter means no significant differences

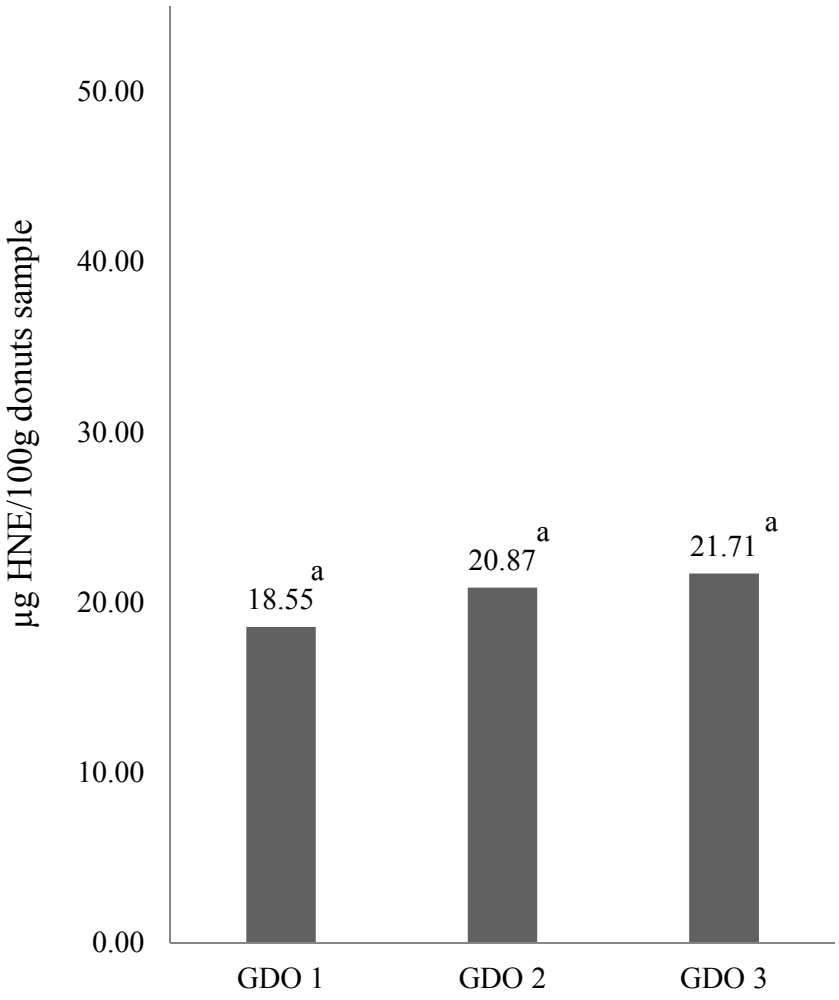

Figure 25. HNE amount in 100g raised glazed donuts

*Groups share the same letter means no significant differences

5) The Formation of the Sum Total Individual Polar Lipophilic Aldehydes in Commercial Fried Chicken Breasts, Chicken Thighs, Chicken Nuggets, Popcorn Chickens and Glazed Donuts

Figure 27 to Figure 30 shows the formation of the total individual polar lipophilic compounds in commercial fried chicken breasts, chicken thighs, chicken nuggets and glazed donuts form 3 different commercial brands respectively. HNE is a major 4-hydroxyaldehydes that belongs to polar lipophilic aldehydes, there also other α,β-unsaturated aldehydes, 2,4-alkedienals and so on that commonly existed in frying oils but are not identified and measured in this experiment. The total individual polar lipophilic compounds show the formation of all polar aldehydes and related carbonyl compounds that remained in the extracted fats from these samples.

In chicken breast samples, skin fats contained much higher total polar lipophilic compounds concentration than that in meat fats.

In chicken thigh samples, brand 1 and brand 3 skin fats containhigher total polar lipophilic compounds concentration than that in meat fats while brand 2 meat fats has higher total polar lipophilic compounds concentration than that in its skin fats.

In chicken nuggets, the concentration of total polar lipophilic compounds of 3 brands followed their TBARS values and HNE concentration trends, brand 2 that contained highest TBARS values and HNE concentration also contained the highest total polar lipophilic compounds concentration.

In glazed donuts, the concentration of total polar lipophilic compounds was similar to their TBARS results and HNE concentration trend, fat from brand 1 donuts that contained highest HNE concentration also had highest total polar lipophilic compounds concentration among 3 brands.

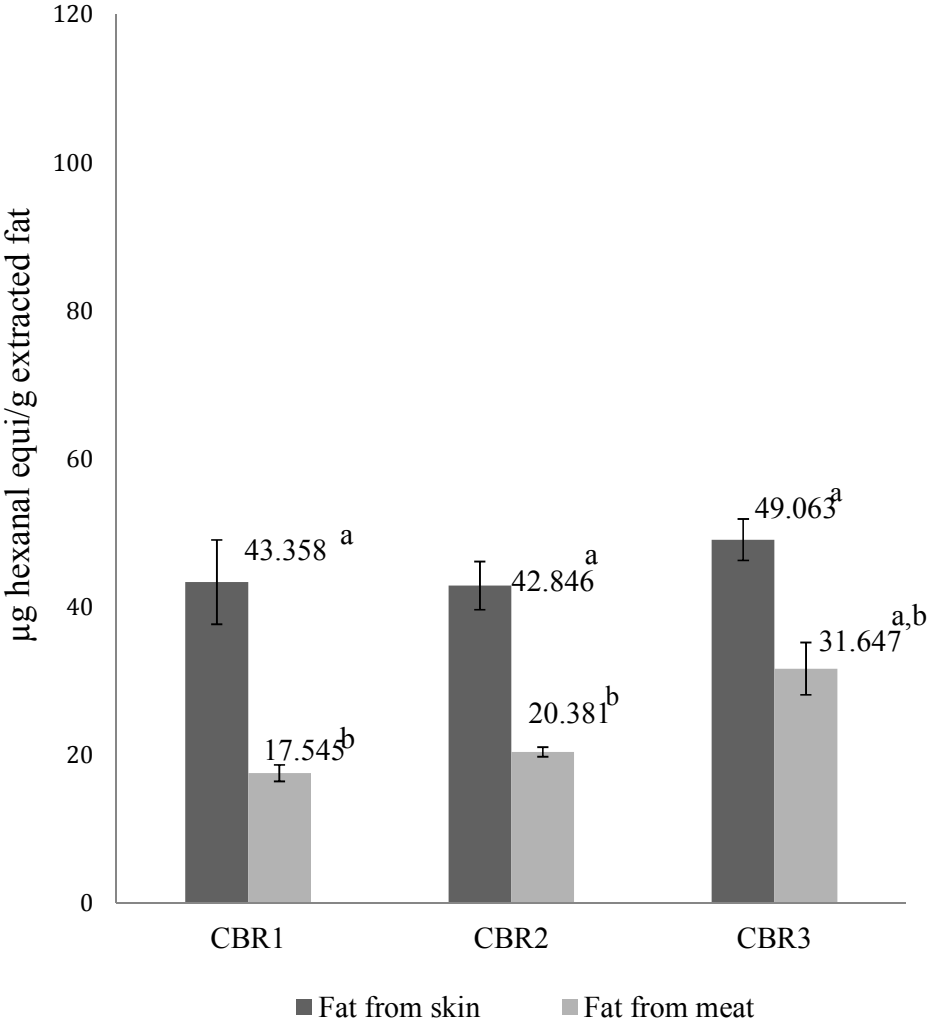

Figure 26. The concentration of individually measured (by HPLC) sum total of polar lipophilic aldehydes in extracted fats from skin and meat of fried chicken breasts

*Group with different letters a, b means significant different and $p<0.05$

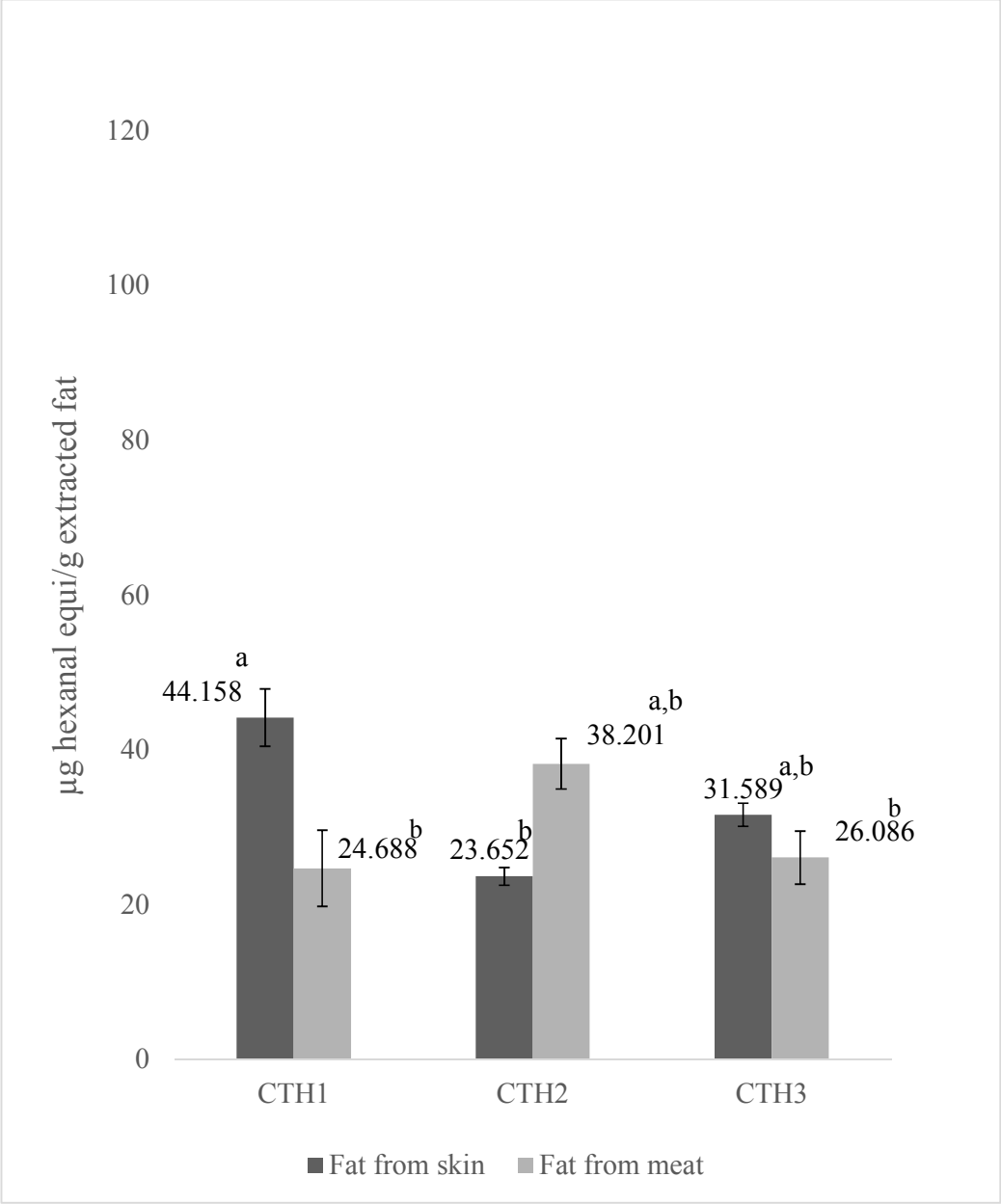

Figure 27. The concentration of individually measured (by HPLC) sum total of polar lipophilic aldehydes in extracted fats from skin and meat of fried chicken thighs

*Group with different letters means significant different and $p<0.05$

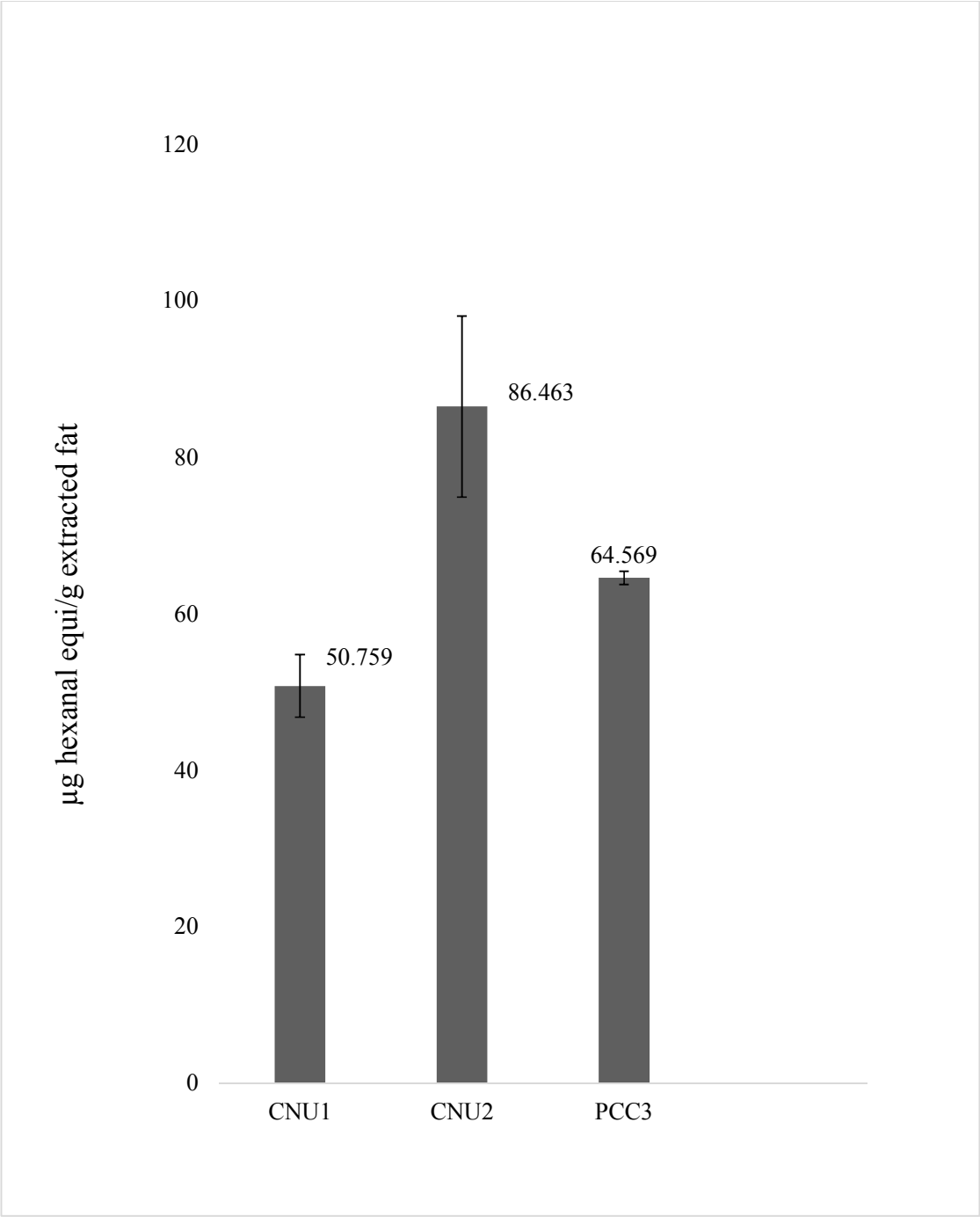

Figure 28. The concentration of individually measured (by HPLC) sum total of polar lipophilic aldehydes in extracted fats from fried 2 brands of chicken nuggets and 1 brand of popcorn chicken

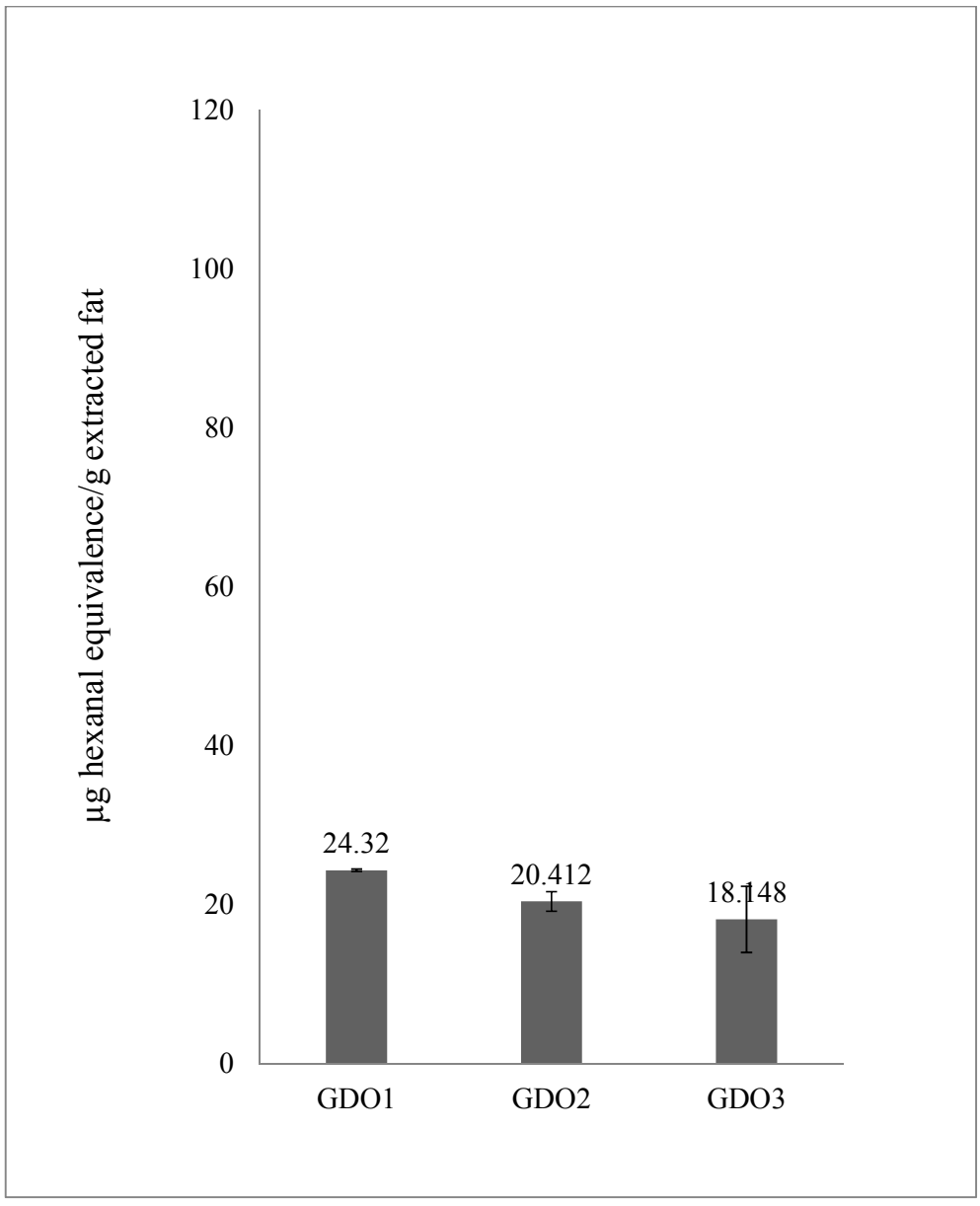

Figure 29. The concentration of individually measured (by HPLC) sum total of polar lipophilic aldehydes in extracted fats from raised glazed donuts

6) The Formation of the Sum Total of Individual Non-Polar Lipophilic Aldehydes in Commercial Fried Chicken Breasts, Chicken Thighs, Chicken Nuggets, Popcorn Chicken and Glazed Donuts

Figure 31 to Figure 34 show the formation of sum total individual non-polar lipophilic compounds in fats extracted from fried chicken breasts, chicken thighs, chicken nuggets and glazed donuts from 3 different commercial brands respectively.

In chicken breasts samples, total non-polar compounds concentration in skin fat is significantly higher than that in meat fat in all brands. Non-polar compounds were mostly volatile and likely evaporated as they were forming in the oils and fats during the heating process.

In chicken thighs samples, except brand 3, brand 1 and brand 2 samples meat fat contain higher total non-polar lipophilic compounds concentration than that in skin fat but without significant differences.

In chicken nuggets samples, it is interesting to see the fat of brand 2 chicken nuggets, which contains highest total polar compounds concentration, however contained the lowest total non-polar lipophilic compounds concentration.

In glazed donuts samples, fat from brand 1 glazed donuts that had highest total polar lipophilic compounds concentration, contained the lowest total non-polar lipophilic compounds concentration.

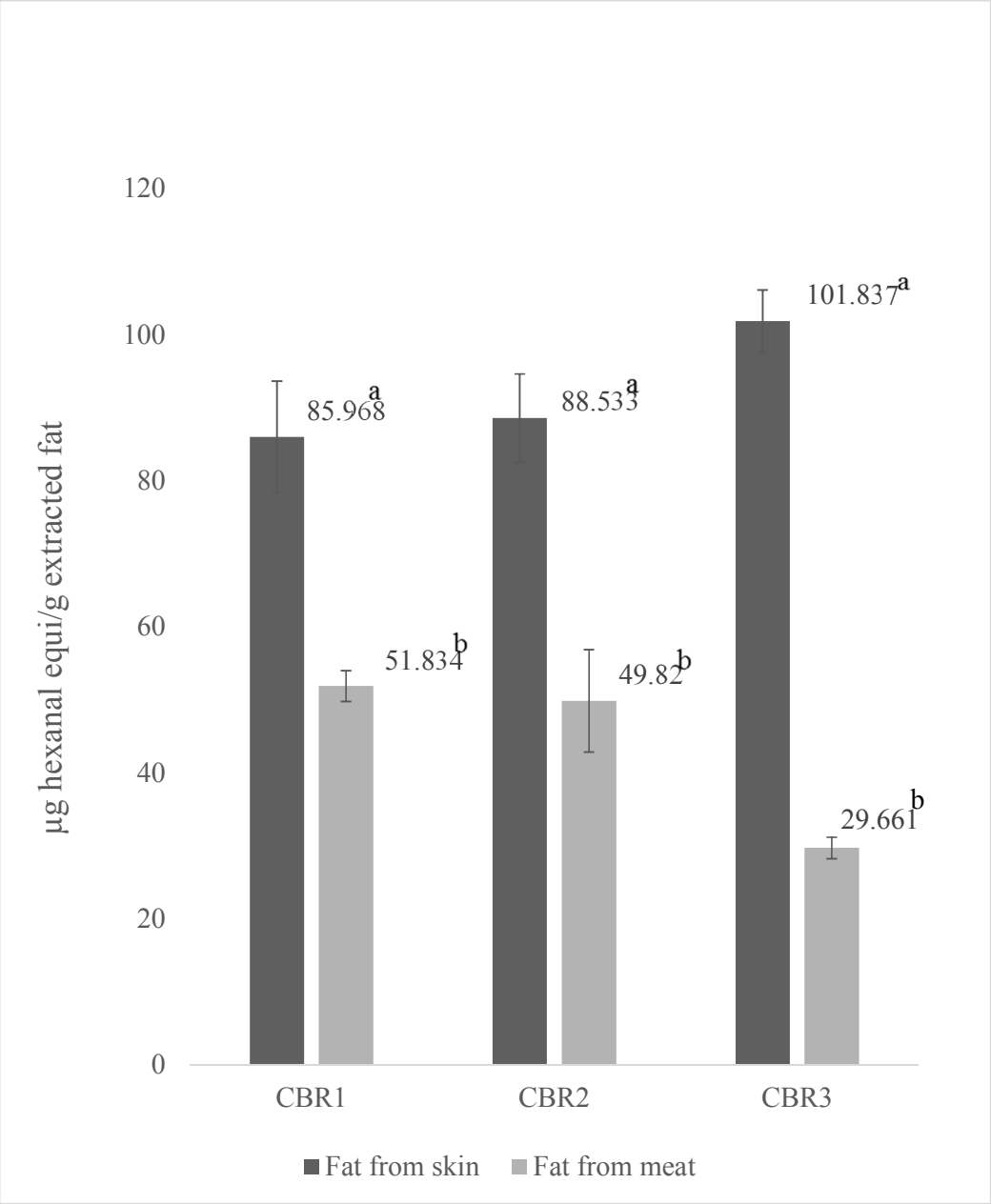

Figure 30. The concentration of individually measured (by HPLC) sum total of non-polar lipophilic aldehydes in extracted fats from skin and meat of fried chicken breasts

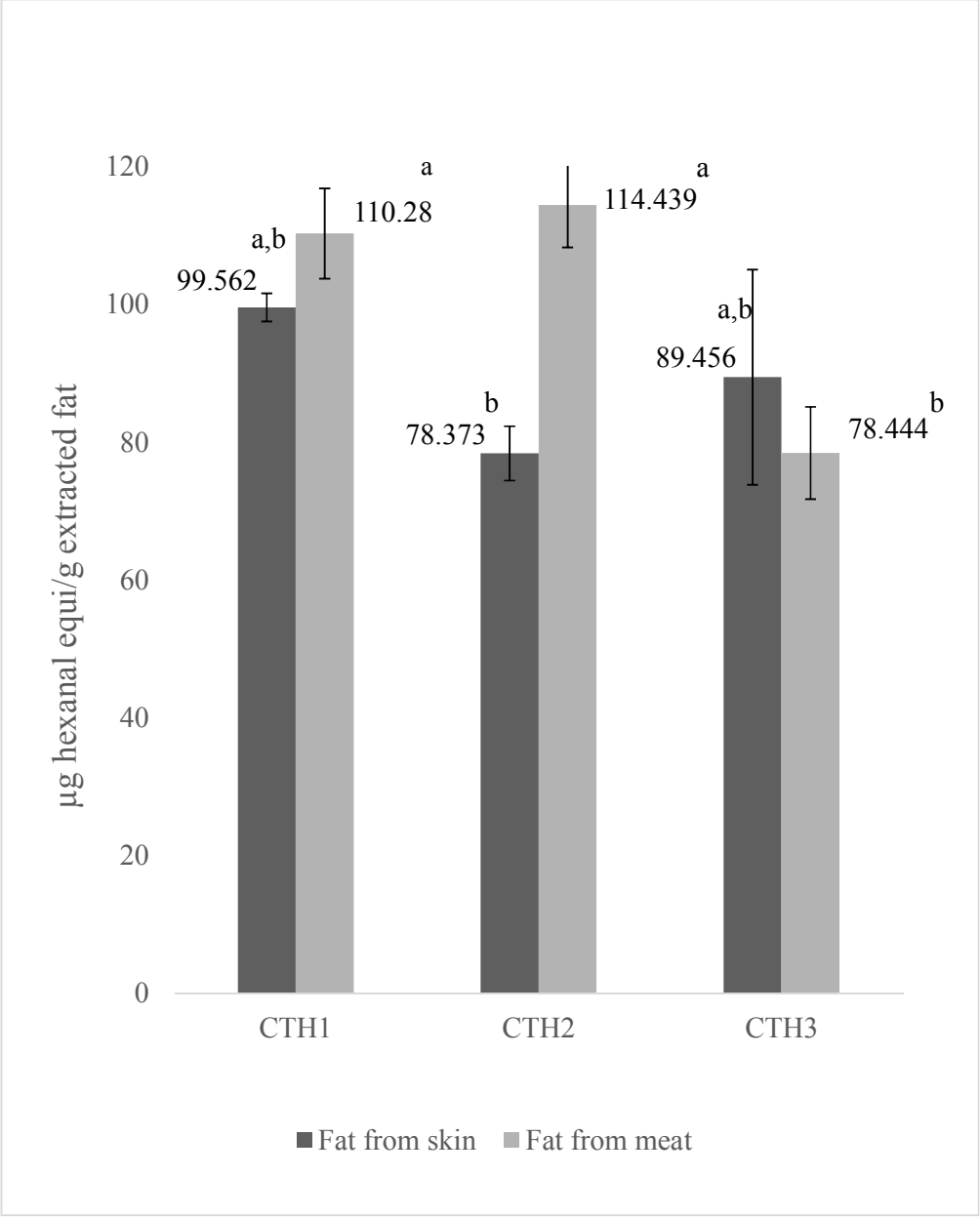

Figure 31. The concentration of individually measured (by HPLC) sum total of non-polar lipophilic aldehydes in extracted fats from skin and meat of fried chicken thighs

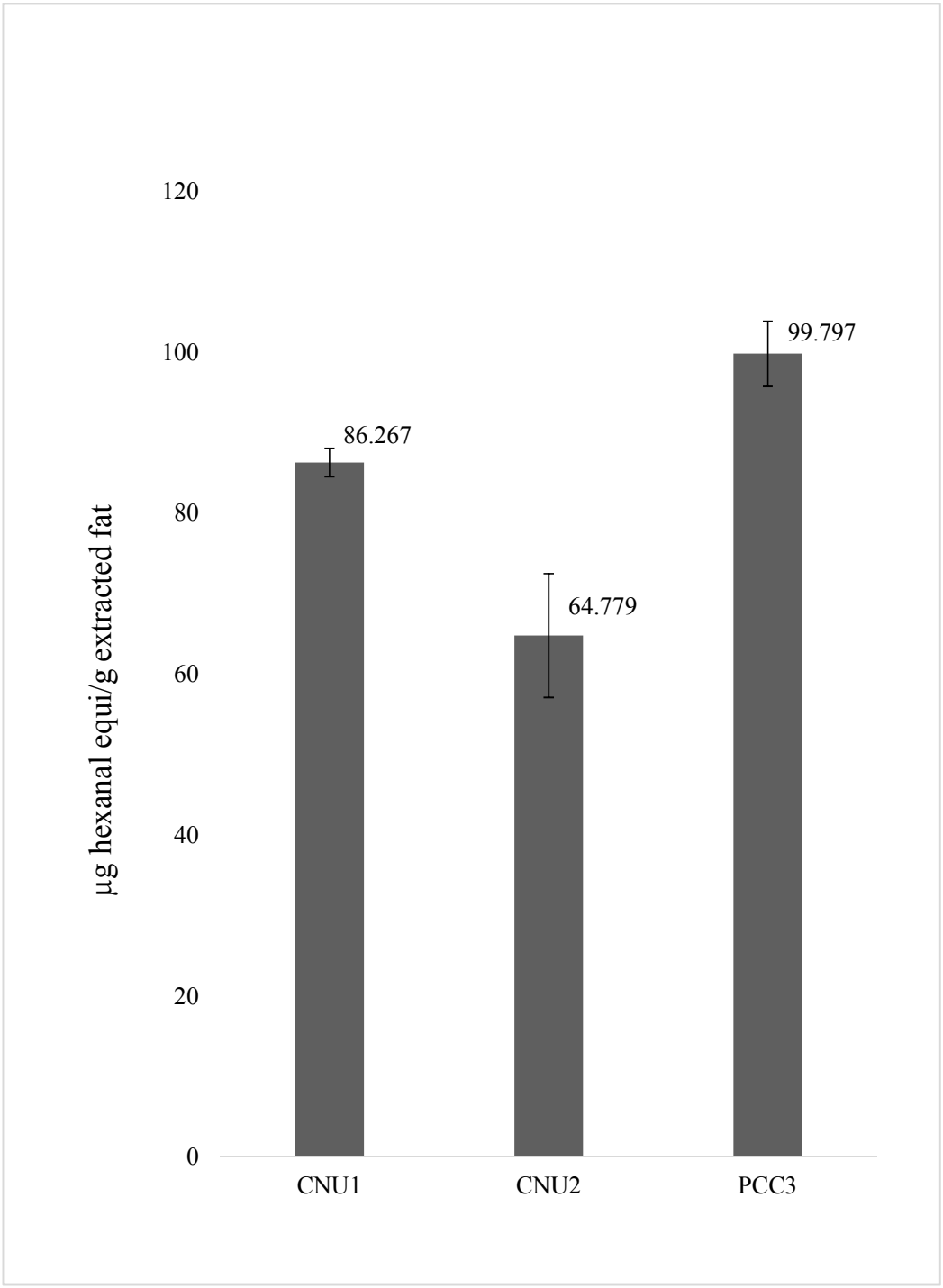

Figure 32. The concentration of individually measured (by HPLC) sum total of non-polar lipophilic aldehydes in extracted fats from 2 brands of chicken nuggets and 1 brand of popcorn chicken

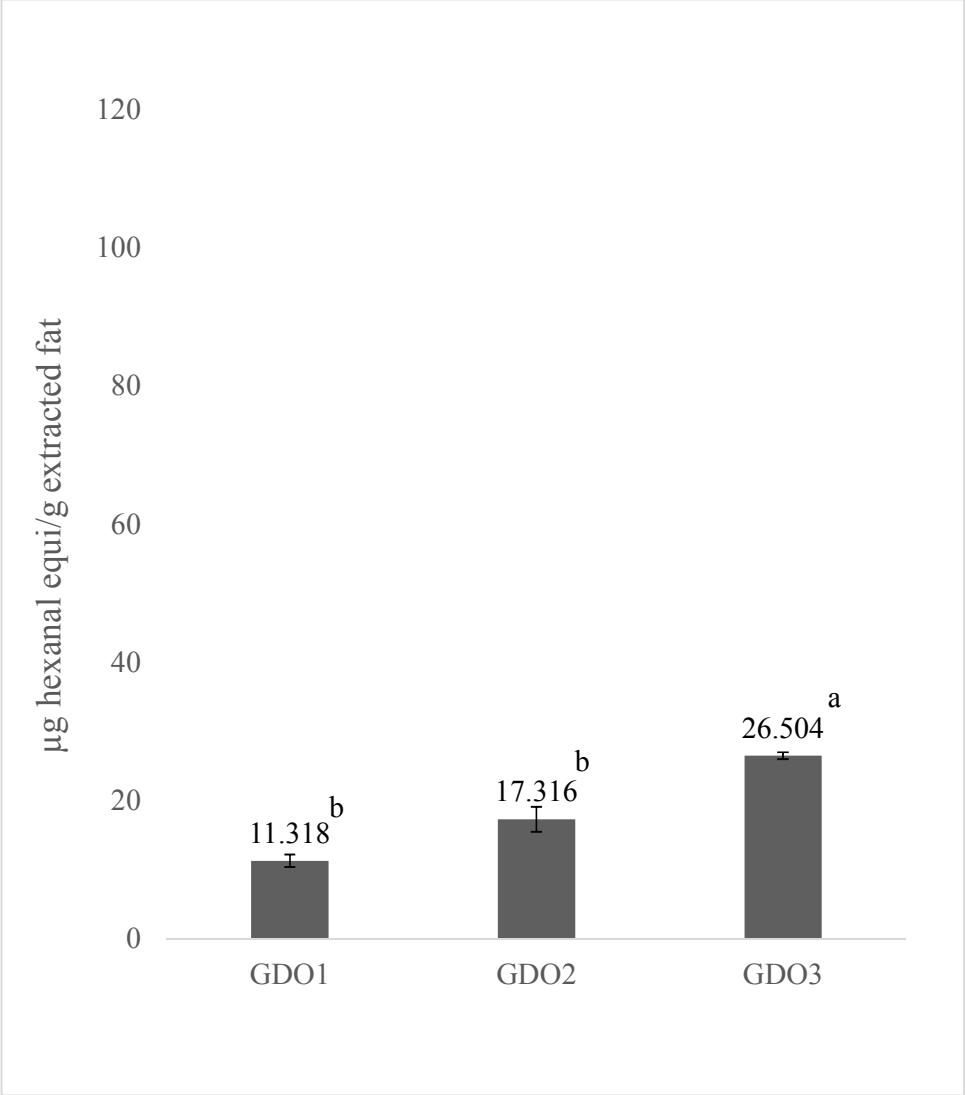

Figure 33. The concentration of individually measured (by HPLC) sum total of non-polar lipophilic aldehydes in extracted fats from raised glazed donuts

Discussion:

1) Chicken Breasts

From the Figure 19 it can be seen that there is no significant differences of HNE concentration (μg HNE/g extracted fat) in skin and meat fat among brands. Such results agreed with their fatty acids distribution since their linoleic acid percent are very similar. The However, HNE concentration in fat extracted from meat is significantly 3 times higher than from skin fat for all three brands, but their linoleic acid percent of skin fat and meat fat are similar. Possible reasons could be fat from meat might contain some iron, which is considered to promote the lipid oxidation according to some researches. A study by William E. Artz et.al showed that heme iron added into frying oil at 160, 180 and 200 °C would increase the rate of oxidation and thermal degradation including increasing of acid value and higher polymers formation with increased heating time and temperature[58]. Lombardi et.al reported that chicken breast contains 4.0 mg of Fe/Kg meat[59]. Lipid oxidation in meat is also related to storage condition including the freezing temperature, storage duration and antioxidant added into meats.

2) Chicken Thighs

It can be seen from Figure 20 that there is no significant difference of HNE concentration between skin fats and meat fats among three brands, which agree with their fatty acids distribution results since their linoleic acid percent were very similar. HNE concentration in thighs meat was higher than that in skin for all three brands, although there is no significant difference. The reason could probably be chicken thigh meat is dark meat and contained some heme iron that could release to the frying oil when the meat are subjected to deep frying and this may accumulate in frying oil

in sufficient amount to potentially accelerate oxidation[60]. The initial iron content of chicken thigh was found 7.0 mg total Fe/Kg meat, 30% of these iron are heme iron[59].

3) Comparison of HNE concentration of meat and skin per 100 g chicken breasts and chicken thighs

Table 10 to Table 12 showed that μg HNE in chicken breast skin fat is slight higher than in meat fat in 100 g breast except for brand 1 chicken breast. For chicken thigh samples, μg HNE in skin fat is much higher than in meat fat in for all three brands, because skin seems to absorb more frying oil than the meat, and more HNE present in frying oils incorporated into skin than the meat.

The total HNE amount from skin fat plus meat fat in chicken breast is much lower than the chicken thighs for all brands. Since chicken thigh is dark meat that contains higher heme iron than white meat of chicken breast, these results indicated that higher heme iron in dark meat might involves promotion of lipid oxidation therefore increased the HNE formation as previous studies indicated.[56]

4) Chicken Nuggets and Popcorn Chicken

HNE concentration of chicken nuggets and popcorn chicken appeared to have same trend with their TBARS results. Figure 23 shows brand 2 chicken nuggets that have highest TBARS values also contained the highest HNE concentration 2.462 μg HNE/g extracted fat in these 3 brands. It is significantly higher than brand 1 chicken nugget with 0.76 μg HNE/g extracted fat and popcorn chicken of brand 3 with 0.377 μg HNE/g extracted fat. It is interesting because brand 2 had 30.58% linoleic acid, somehow lower than the brand 1 35.64% linoleic acid. It's noted that brand 2 chicken nugget pieces has the largest size and thus contained more heme that might accelerate

lipid oxidation. When calculated back to μg HNE amount in 100 g chicken nuggets samples as Figure 24 shows, brand 2 chicken nuggets contained the highest HNE amount of 47.93 μg HNE/100 g chicken nuggets.

5) Glazed Donuts

It can be seen in Figure 25 that brand 1 glazed donuts has the highest 1.16 μg HNE/g extracted fat and brand 3 had the lowest 0.81 μg HNE/g extracted fat concentration but without significant difference. Such results agree with their fatty acid distribution. Fat from brand 1 donut contained the highest 14.80% linoleic acid; fat from brand 3 had second highest 13.60% linoleic acid but also very high palmitic acid content of 39.92%, which is saturated fatty acid and would not produce any α, β-unsaturated 4-hydroxyaldehydes as a consequence of thermal treatment, resulting in comparatively lower HNE concentration of fat from brand 3 donuts. There is a limited literature research finding of influence of frying oil type on doughnuts. One investigator detected the existence of HNE in the fats extracted from doughnuts and the concentration highly depends on the level of degradation of frying oils[35]. Calculations of μg HNE in 100 g donut samples are in Figure 26, it can be seen that brand 3 has the highest HNE amount because it has a highest fat percentage 26.80% among these brands thus leading a highest HNE amount of 21.71 μg /100g sample.

The present experiments show the incorporation of HNE, a toxic compound formed in unsaturated oils due to heat treatment and oxidation, into fried chickens and donuts. The higher the incorporation of oil into the food, the higher was HNE concentration.

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Appendix: Figures of Typical HPLC Chromatogram

A

B

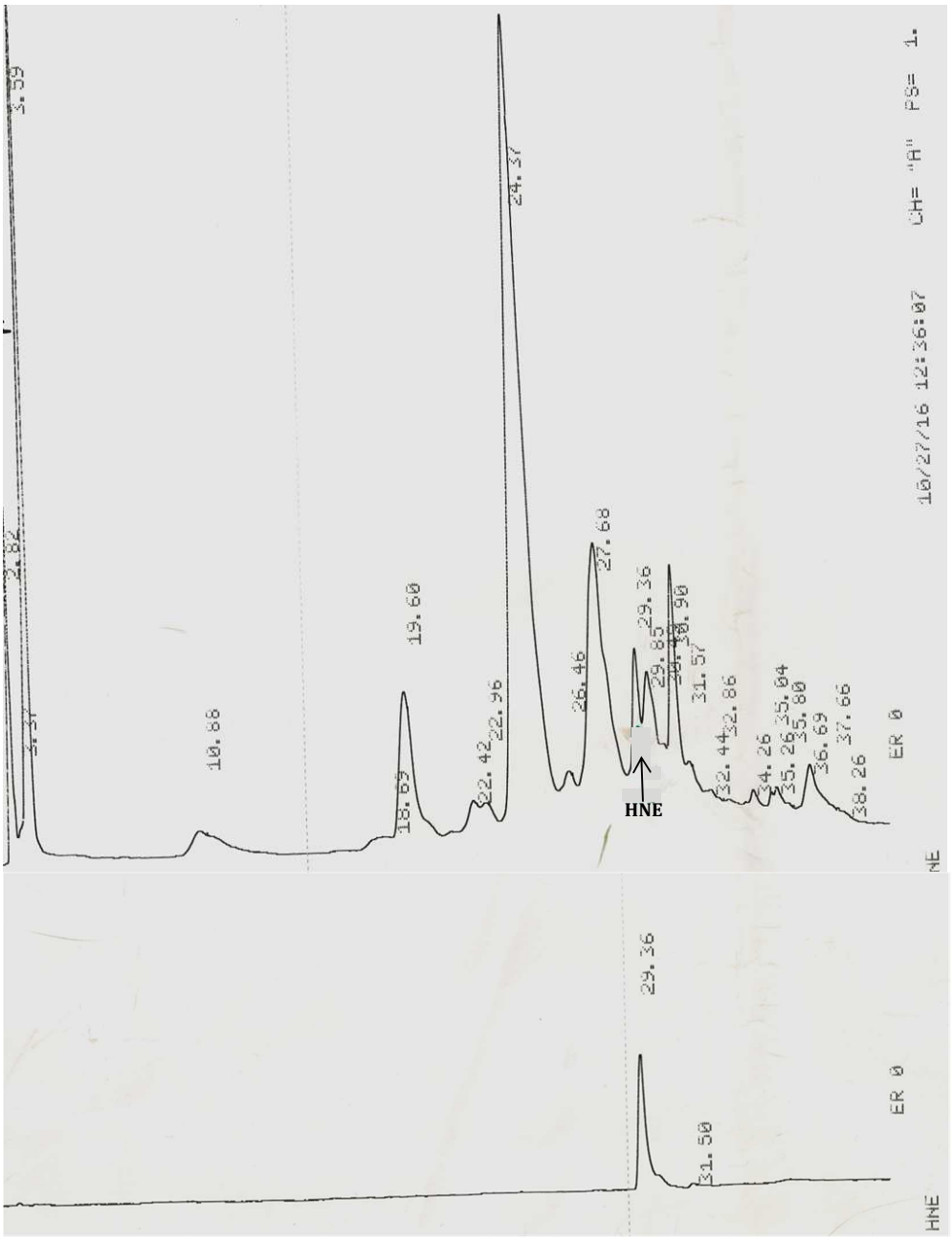
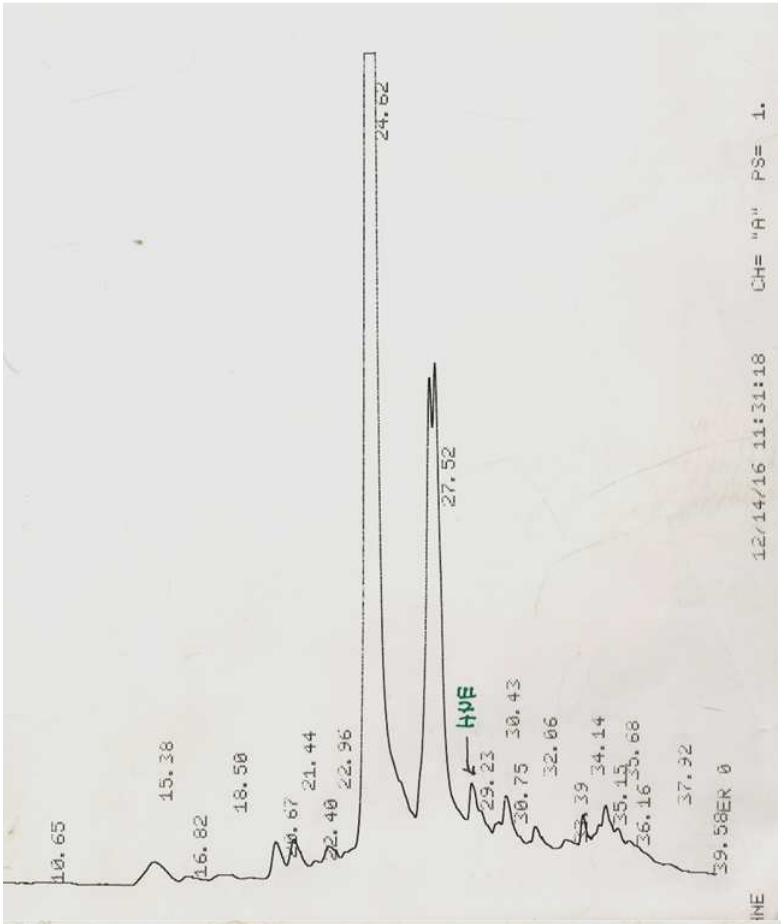


Figure 34. Typical HPLC chromatogram of A. Chicken breast meat fat B. HNE standard

A



B

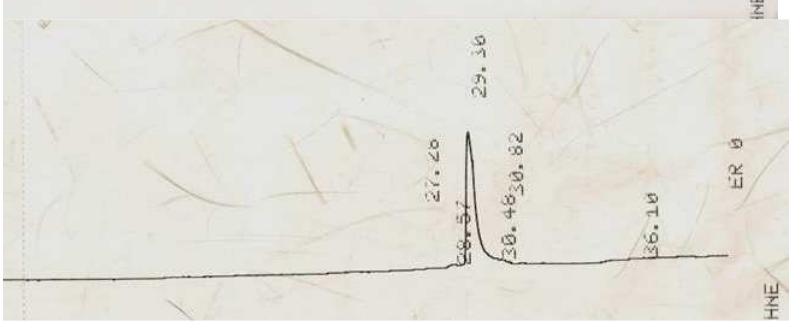
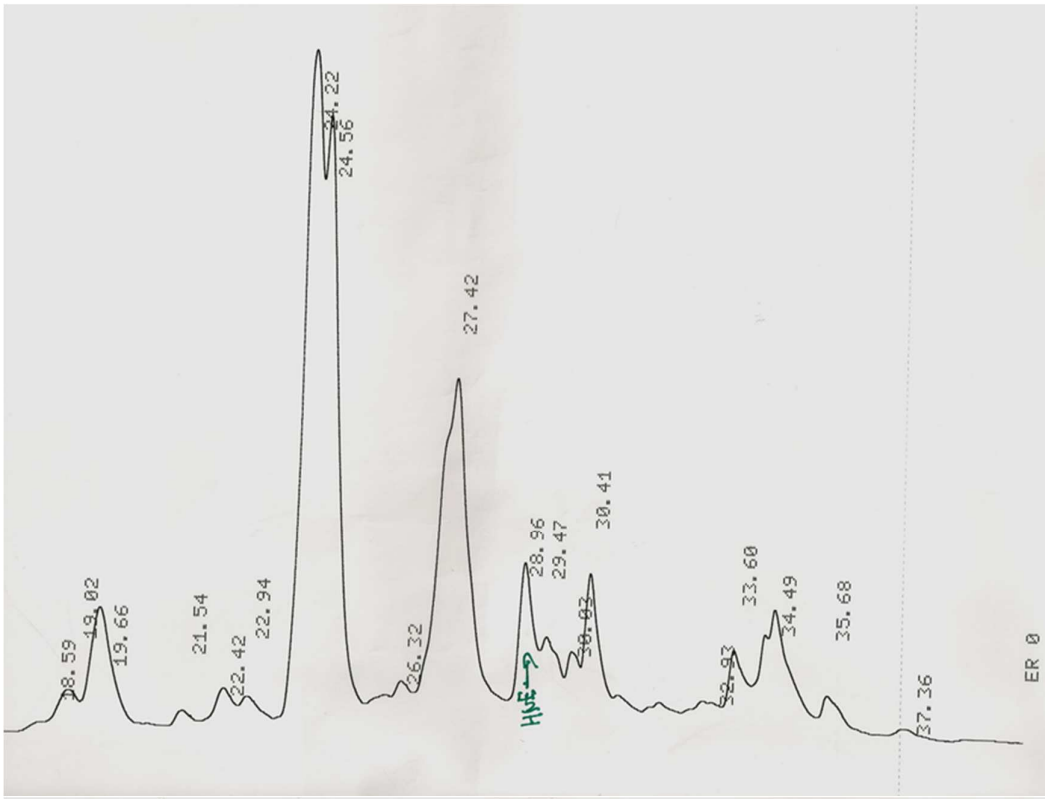


Figure 35. Typical HPLC chromatogram of A. Chicken breast skin fat B. HNE standard

A



B

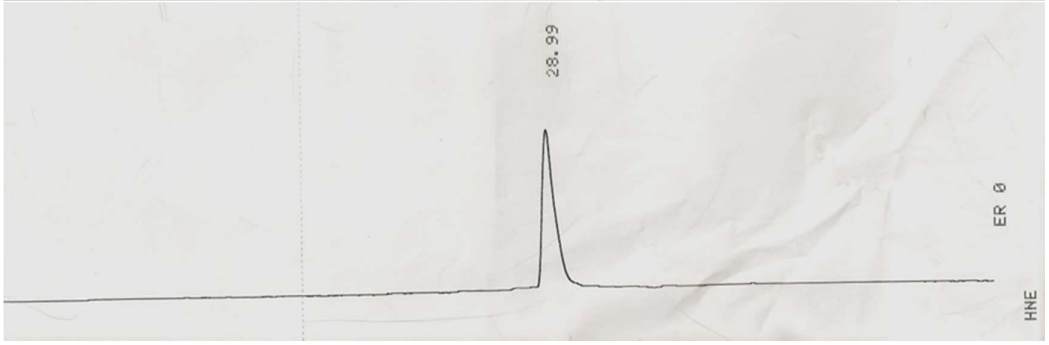
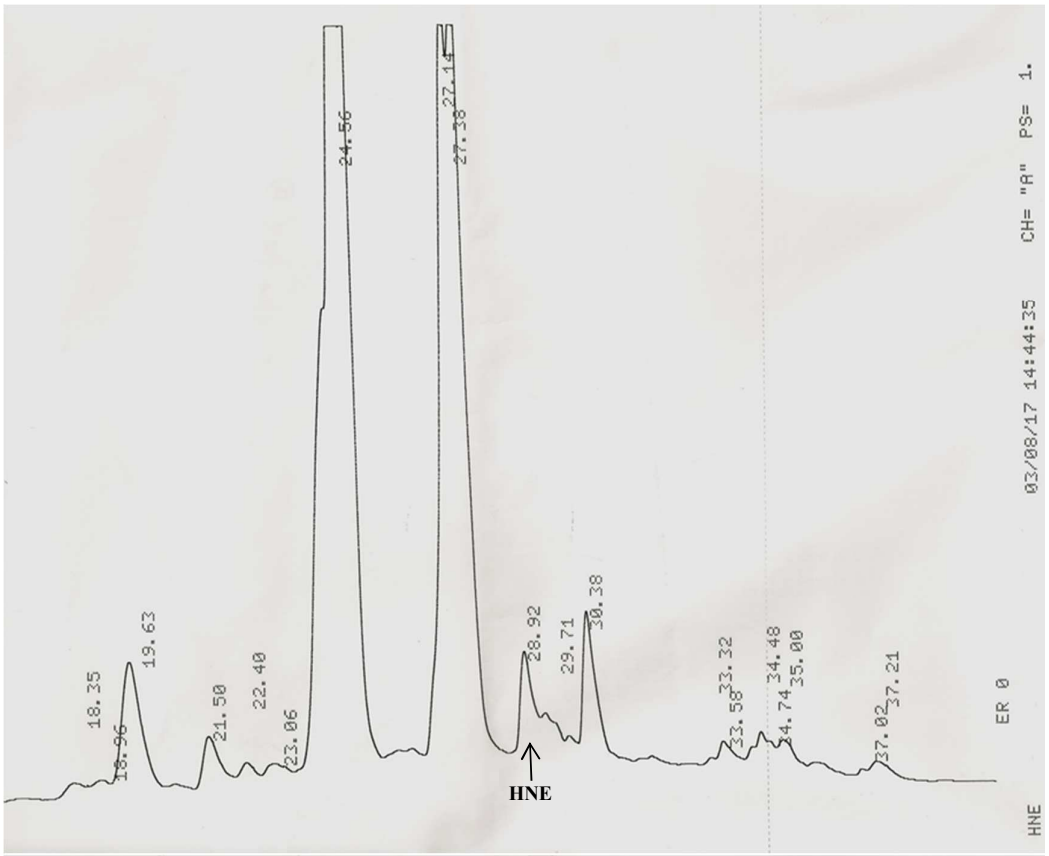


Figure 36. Typical HPLC chromatogram of A. Chicken thigh meat fat B. HNE standard

A



B

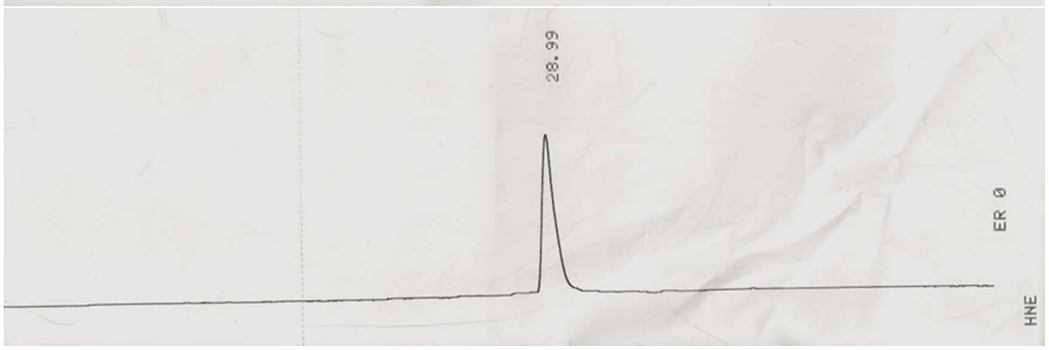
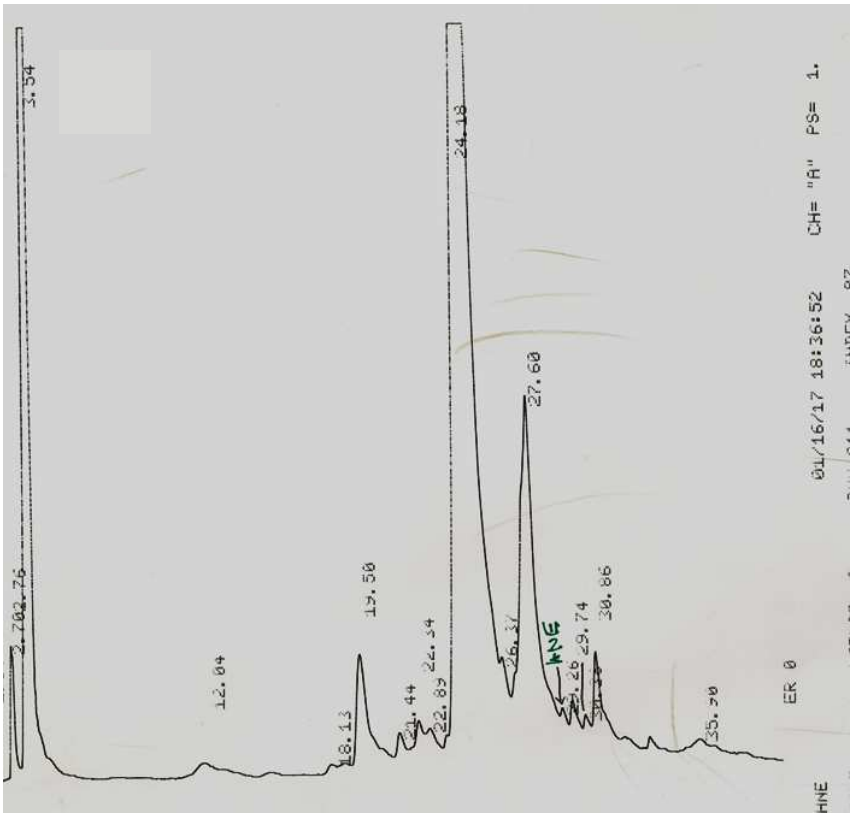


Figure 37. Typical HPLC chromatogram of A. Chicken thigh skin fat B. HNE standard

A



B

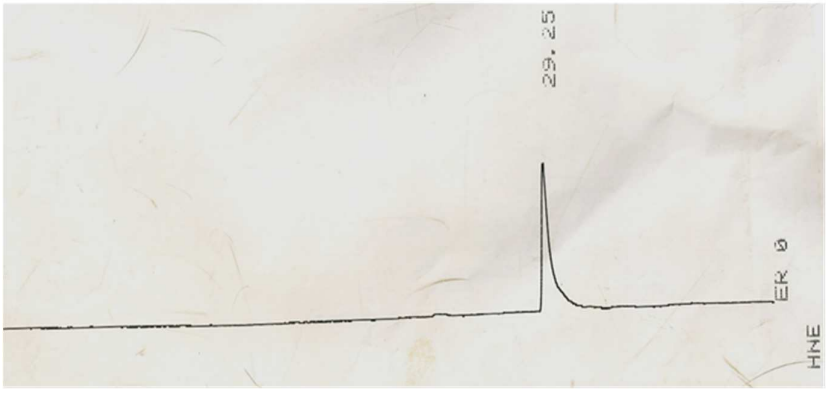
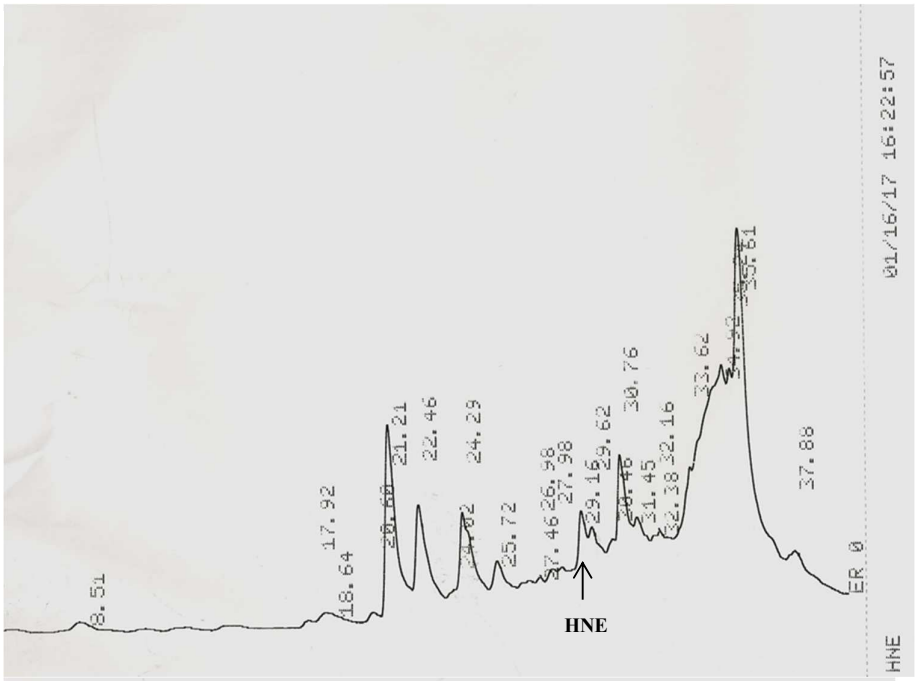


Figure 38. Typical HPLC chromatogram of A. chicken nugget fat B. HNE standard

A



B

Figure 39. Typical HPLC chromatogram of A. Raised glazed donut fat B. HNE standard