Trans Fat Formation and Lipid Oxidation in Palm Olein during Prolonged Thermal Treatments

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***Abstract*—*Trans* Fat formations of palm olein, currently the most common edible oil in the world, during different oxidation conditions resulted from prolonged thermal treatments were elucidated. When palm olein was heated at 180 °C and exposed to air and daylight up to 16 days, *trans* fat increased from 0.22% to 0.36% in the first 5 days of heat treatment and then fluctuated around a value of 0.33%. Under these conditions, peroxide value (PV) increased to a maximum value (4.98 mequiv O_2/kg) on the first day. The anisidine value (AV) increased gradually from the second day to the sixteenth day. As palm olein was heated at 180 °C and limited to the exposure of air and daylight, *trans* fat increased from 0.21% up to 0.29% in the first 4 days of heat treatment and then fluctuated around a value of 0.25%. However, in these conditions, PV decreased rapidly on the first day of heat treatment and little change was observed on the AV.**

***Index Terms*—*Trans* fat, oxidation, palm olein, FTIR, peroxide value.**

I. Introduction

The concern about *trans* fat has increased in the last decade due to negative effects of *trans* fat on human health. van Tol, Zock, Gent, Scheek, and Katan [1] and Stampfer, Sacks, Salvini, Willett, and Hennekens [2] reported that *trans* fat intake increased a risk of coronary heart disease. Other studies also revealed that *trans* fat raised levels of triglycerides and lipoprotein [3], reduced triglyceride uptake as well as esterification of newly synthesized cholesterol, and raised production of free fatty acids [4].

Typical foods containing *trans* fat are fast or frozen foods, packaged snacks, bakery products, margarines, and butters [5]. Due to very high contents of *trans* fat in fast food, it is possible to consume 10 to 25 g *trans* fat in one day. Customers who have a habit of consuming fast food every day have a daily intake of *trans* fat about 5 g. This level of *trans* fat daily intake is associated with 25 percent increase in the risk of ischemic heart disease [6].

In the view of negative impacts of *trans* fat on human health, the knowledge on *trans* fat formation in food processing is very important and needed to be clarified. Although significant amounts of *trans* fat were found in processed foods, previous studies done in laboratory often showed low levels of the *trans* fat formation when vegetable oils were subjected to heat treatments [7], [8]. The cause of these observations could be that such heat treatments might be not long enough to observe changes of *trans* fat level. The present study focuses on the effects of prolonged heat treatments of palm olein on the *trans* fat formation along with the lipid oxidation. .

Two mechanisms leading to *trans* fat formation in heat treatment are singlet oxygen induced *trans* fat formation and free radical induced isomerization. Singlet oxygen reacts with *cis* double bond and alters *cis* double bond into *trans* configuration [9]. In addition, a free radical can be added reversibly to a double bond to form a radical adduct. When a double bond is reconstructed, *trans* configuration is favored because a *trans* double bond is more thermodynamically stable [10]. Singlet oxygen and free radical are known as the key initiators in lipid oxidations.

During thermal treatment, both lipid oxidation and *trans* fat formation occur simultaneously; however, *trans* fat formation has never been reported along with the lipid oxidation. Tsuzuki, Matsuoka, and Ushida [7] and Tsuzuki [11] studied the formation of *trans* fat in various kinds of edible oil by monitoring the fatty acid composition and the antioxidant contents. Liu, Stephen Inbaraj, and Chen [8] and Mölleken [12] studied the *trans* fat formation in vegetable oil, focusing on different temperatures and varied time lengths.

This research aims to investigate *trans* fat formation during different lipid oxidation conditions resulted from prolonged thermal treatments. Palm olein was used as a representative oil in this study because palm olein has been reported as the major source of vegetable oil in the world [13]. Negative Second Derivative Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (FTIR) method, established by Mossoba et al. [14], is a method of choice for *trans* fat analysis. This FTIR method is rapid and is able to differentiate all of the isolated double bonds, regardless of position of the *trans* double bond in lipid and the lipid molecular weight.

II. Material and Method

A. Reagents

Palm olein was purchased from a local supermarket. Acetic acid and isooctane were purchased from Merck KGaA (Darmstadt, Germany). Chloroform was purchased from RCI Labscan Limited (Bangkok, Thailand).

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Potassium iodine and sodium thiosulfate were purchased from Ajax Finechem Pty Ltd (Taren Point, New South Wales, Australia). p-Anisidine was purchased from Aldrich (Buchs, Switzerland). Glyceryl tripalmitate was purchased from Sigma (Spruce, Saint Louis, USA). Glyceryl trielaidate was purchased from Tokyo Chemical Industry Co., LTD (Tokyo, Japan).

B. Determination of Peroxide Value (PV) and Anisidine Value (AV)

Samples were subjected to analyses of peroxide value (PV) by 965.33 AOAC iodometric method [15]. This method is based on the reaction of hydroperoxides (ROOH) with iodine ion (I^-). 5.00 ± 0.05 g sample was dissolved in 30 mL acetic acid : chloroform solution (3 : 2 v/v). The mixture was then reacted with 0.5 mL saturated KI solution. The I_2 product was titrated against standardized sodium thiosulfate solution using starch as indicator. The peroxide value (milliequiv O_2/kg sample) equals to

$$PV = S \times N \times 1000 / m \quad (1)$$

where:

S: The volume of sodium thiosulfate used, in milliliter.

N: The normality of sodium thiosulfate solution.

m: Weigh of sample, in gram.

The anisidine value (AV) was measured by 2.504 IUPAC method [16] using a Thermal Scientific Genesys 10 UV scanning spectrophotometer (USA). This method is based on a reaction of sample with acetic acid solution of p-anisidine. p-Anisidine reagent is a solution of 0.125 g p-anisidine in 50 mL of glacial acetic acid. Test solution was prepared by dissolving sample in isooctane. 5 mL of test solution was mixed with 1 mL of p-anisidine reagent to make reacted test solution. Another 5 mL of test solution was mixed with 1 mL of glacial acetic acid to make unreacted test solution. Blank is a mixture of 5 mL of isooctane and 1 mL of p-anisidine reagent. All of unreacted test solution, reacted test solution, and blank were kept in the dark for 8 minutes before measuring absorbance at 350 nm. The AV of the sample equals to:

$$AV = 100QV \times 1.2 \times (A_1 - A_2 - A_0) / m \quad (2)$$

where:

V: The volume in which the test sample is dissolved, in milliliter

m: The mass of test portion, in grams

Q: The sample content of the measured solution based on which the AV is expressed, in gram per milliliter (Q = 0.01 g/mL)

A_0: The absorbance of the unreacted test solution

A_1: The absorbance of the reacted test solution

A_2: The absorbance of the blank

1.2: The correction factor for the dilution of the test solution with 1 mL of the p-anisidine reagent or glacial acetic acid

C. Determination of trans Fat Level by Fourier Transform Infrared Spectroscopy (FTIR)

trans Fat determination was performed as suggested by Mossoba et al. [14]. For calibration standards, the mixtures of trielaidin (TE, *trans*-18:1) and tripalmitin (TP, 16:0) were used [17]. Fourier Transform Infrared (FT-IR) Spectroscopy was measured on a Thermo Scientific Nicolet 6700 FT-IR spectrometer (Madison, USA) operated under OMNIC software. The attenuated total reflection (ATR) mode was applied. The optical system comprises of a Vectra Plus Michelson Gold interferometer, an air bearing with dynamically alignment moving mirror, a potassium bromide (KBr) substrate beam splitter, and a deuterated L-alanine doped triglycine sulfate (DlaTGS) detector. A single reflection diamond type IIA internal reflection cell with capacity of 1 mL was used. TE was added gravimetrically to TP at the following concentration 0.64, 1.45, 3.28, 4.99, 7.49, 10.6, and 11.98%. The spectral wavenumber range was 4000 cm^{-1} to 400 cm^{-1} at a resolution of 4 cm^{-1}. Air was used as the reference background. The number of scans was 256 and the heights of the second derivative absorption bands were collected. An oil sample of 5 μL was injected each time for analysis.

D. Procedure

Palm olein was treated in 2 different conditions, namely *extended oil life* and *limited oxidation*. The different extents of oxidation resulted from the levels of air and daylight exposure. The experiments were conducted 8 hours a day, for 16 days. PV was analyzed after samples were collected and left to cool down at room temperature. Three mL of each sample was kept at -20 °C for further AV and *trans* fat analysis. The measurements of PV, AV, and *trans* fat were done in triplicate.

Extended oil life: Palm olein was heated under daylight with the initial surface to volume ratio of 0.31 cm^{-1} and the air exposure area of 314 cm^2. The oil life was prolonged by replacing 40 mL of a taken sample by fresh palm olein with an equal amount (40 mL) every 2 hours. By extending oil life, the exposure of air and daylight is increased. Thermal treatment was performed at 180 °C ± 2 °C by a C-MAG HS 7 IKAMAG hot plate magnetic stirrer and an ETS-D5 contact thermometer (IKA, India).

Limited oxidation: Lipid oxidation was limited by filling 30 mL palm olein into a test tube with a screw cap (20 mm in diameter x 150 mm in length) covered with aluminum foil to prevent light exposure. Test tubes were closed and subjected to 180 °C ± 2 °C in hot oven.

III. Result and Discussion

A. Trans Fat Formation and Lipid Oxidation during Extended Oil Life Conditions

In the first 5 days of treatment, *trans* fat level in *extended oil life* conditions increased from 0.22% to 0.36% (Fig. 1). These results contrasted to the previous reports done in shorter period of heat treatment time. Tsuzuki [7] heated six vegetable oils, including cooking oil, canola oil, corn oil, rice bran oil, safflower oil, and sesame oil. Total amounts of *trans* fat in these vegetable oils did not change significantly during 4-hour treatment. Liu, Stephen Inbaraj, and Chen [8] also found that no *trans* fat was formed after heating hydrogenated and un-

hydrogenated soybean oil for 24 hours. The previous treatment durations properly were not sufficient to observe the increment in *trans* fat level.

The *trans* fat increment was caused by singlet oxygen and free radicals. Under daylight, at a specific wavelength, available sensitizers in ground singlet state (^{1}Sen) are converted to be in excited singlet state (^{1}Sen*). The ^{1}Sen* intersystem crosses to produce excited triplet sensitizers (^{3}Sen*). The ^{3}Sen* transfers energy to a triplet oxygen to yield ground state sensitizers and singlet oxygen. Singlet oxygen reacts with double bonds in unsaturated fatty acids via the Ene reaction [9]. This reaction produces allyl hydroperoxides, in which parts of the double bonds are in *trans* configuration and detected as *trans* fat. At the same time, as free radicals generated during lipid oxidation, a free radical can react reversibly to a double bond to form a radical adduct. When a double bond is reconstructed, *trans* configuration is favored because a *trans* double bond is more thermodynamically stable [10].

After 5 days of thermal treatment, *trans* fat was not accumulated in the oil sample. From the sixth day, *trans* fat level fluctuated around a value of 0.33%. The dilution by new palm olein was supposed to have an effect on *trans* fat formation.

During *extended oil life* treatment, the changes in lipid oxidation, monitored by the peroxide value (PV) and anisidine value (AV), were in agreement with previous study. Various types of oil were reported by Guillén and Cabo to have different oxidation stages [18]. Initially, hydroperoxides are formed, and this stage ends when the hydroperoxides level begins to decrease. Next, the secondary products such as aldehydes are mainly generated. PV of palm olein in this study increased and reached a maximum value (4.98 mequiv O_2/kg) on the first day of heat treatment (Fig. 2). Therefore, the initial stage of lipid oxidation was within the first day of heat treatment. The next stage of lipid oxidation was from the second day to the sixteenth day. During this stage, the PV decreased and fluctuated around a value of 1.78 mequiv O_2/kg (Fig. 2) and the AV increased gradually from 68.18 to 117.02 (Fig. 3).

B. Trans Fat formation and Lipid Oxidation during Limited Oxidation Condition

In the *limited oxidation* conditions, where the palm olein was kept from air and daylight, the *trans* fat value still increased during the first 4 days of treatment from 0.21% up to 0.29%, and fluctuated around 0.25% from the fifth day to the sixteenth day (Fig. 1).

Although the *trans* fat patterns are similar, oxidation levels in *extended* and *limited exposure* conditions are in a discrepancy. Palm olein was gradually oxidized as it was exposed to air and daylight up to 16 days. However, in contrast to the condition discussed above, PV in the *limited oxidation* conditions decreased immediately after the first hour of treatment (Fig. 2), and the AV mostly remained stable at a value of 2.82 (Fig. 3) from the first day until the sixteenth day. There was much less extent of oxidation in *limited oxidation* conditions.

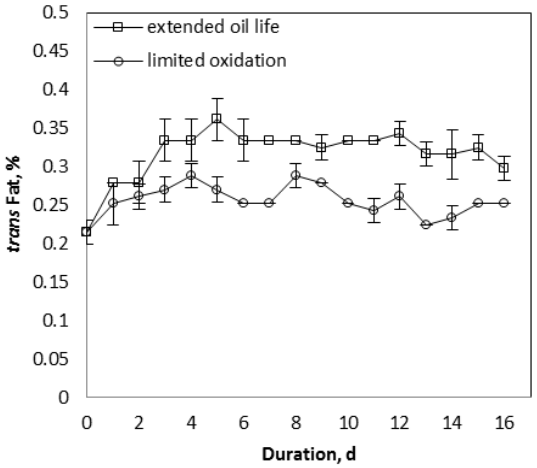

Figure 1. *Trans* Fat value during *extended oil life* and *limited oxidation* conditions

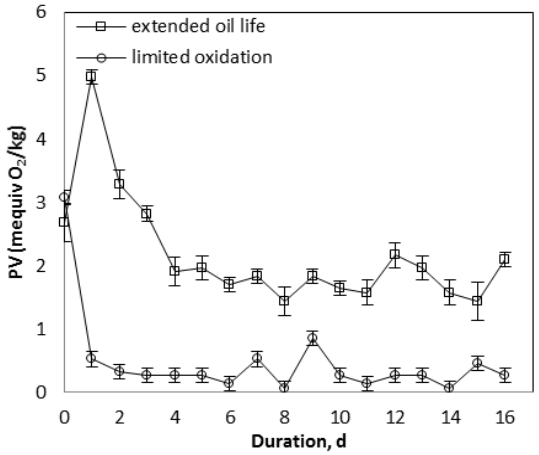

Figure 2. Peroxide value (PV) during *extended oil life* and *limited oxidation* conditions

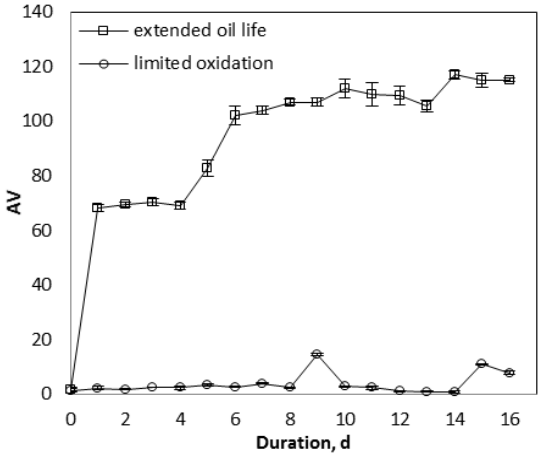

Figure 3. Anisidine value (AV) during *extended oil life* and *limited oxidation* conditions

Peroxide composition, represented by the PV during initial period of treatment was probably decomposed under *limited oxidation* conditions. The hemolytic cleavage in the oxygen-oxygen bond was favored, compared with oxygen-hydrogen cleavage, due to the lower bond energy [19]. The oxygen-oxygen cleavage resulted in alkoxy and hydroxy radicals.

Hydroxy radical (HO·) was reported to be a strong enough radical to abstract hydrogen from other molecules as well as to add to a double bond [20], [21]. This oxygen reactive species can abstract hydrogen from a nearby lipid to generate a lipid radical, which then reacts with triplet oxygen to form a hydroperoxide (expressed by PV). Hydroperoxide is able to be further decomposed into aldehyde (expressed by AV). However, due to the lack of triplet oxygen, under the *limited oxidation* conditions, the lipid autoxidation occurred to a much lesser extent. Therefore, the PV and AV were at a minimal level.

At the same time, the *cis* double bonds were available to react with the hydroxy radicals. The resulting hydroxylated fatty acid radicals were limited to the *cis*/*trans* isomerization, but not the lipid oxidation. Due to the lack of triplet oxygen, newly formed lipid radicals, caused by hydroxylated fatty acid radicals, cannot produce hydroperoxides. Instead, the hydroxylated fatty acid radicals can eliminate hydroxy radicals to reform unsaturated fat. The *trans* configuration was favored due to higher thermodynamic stability. From the fifth day, *trans* fat increment was not observed any further. The amount of *cis* double bonds was supposed to reduce significantly, so *cis* double bonds were involved in *cis/trans* isomerization with a less extent. Importantly, the initial peroxide should be a concern in the study of *trans* fat formation during thermal treatment in the conditions which are lack of oxygen.

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- *trans* Fat formation and the ability of β-carotene in anti-trans fat formation
- The relationship between *trans* fat and lipid oxidation products of palm olein under thermal treatments.
- Monitoring lipid oxidation and *trans* fat formation in several edible oils with distinguished fatty acid profiles.

