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ORIGINAL PAPER

Formation of 4-Hydroxy-2-(*E*)-Nonenal in a Corn–Soy Oil Blend: a Controlled Heating Study Using a French Fried Potato Model

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Abstract The formation of 4-hydroxy-2-(*E*)-nonenal (HNE) in a corn–soy oil blend during frying was investigated. Frozen shoestring potatoes were fried once per hour at 180 °C for 8 h/day over a 4-day period. As a control, oil was also heated under identical conditions, except that no product was fried. HNE was quantified by GC–MS using a stable isotope dilution assay with pentaflurobenzyl hydroxylamine hydrochloride (PFBHA) and trimethylsilyl 2,2,2-trifluoro-*n*-(trimethylsilyl)acetimidate (BSTFA) derivatization. The HNE concentration in the potato fryers increased throughout the first day of frying. On subsequent days the daily maximum HNE concentration was reached after fresh oil was added and the fryer was brought to the frying temperature. The potato fryer oil reached a maximum concentration of 5.6 ppm during the second day of frying. Similarly, the HNE concentration of the oil in the control fryer increased throughout the first day of heating. On subsequent days the daily maximum HNE concentration varied throughout the experimental period. The control fryers reached a maximum concentration of 6.3 ppm at the end of the second day of heating. Throughout the experimental period there was a tendency for the oil in the control fryer to have a greater concentration of HNE than the oil in the potato fryer. Overall time of the experiment and heating with food versus heating without frying food and their interaction were significant in terms of HNE formation.

Keywords 4-hydroxy-2-(*E*)-nonenal · HNE · Unsaturated hydroxy aldehydes · Frying oil · Stable isotope dilution assay

Introduction

The compound 4-hydroxy-2-(*E*)-nonenal (HNE) is an oxygenated α,β-unsaturated aldehyde which is formed during the peroxidation of ω-6 polyunsaturated fatty acids. It has been known for some time that α,β-unsaturated carbonyl compounds are toxic, with the aldehydes being substantially more toxic than the other carbonyl compounds [1]. Interest in HNE came about in 1980 when Benedetti and colleagues discovered that HNE was the major cytotoxin formed during the peroxidation of liver microsomal lipids [2].

HNE has been linked to a number of health problems, including atherosclerosis, Alzheimer's and other neurodegenerative diseases, cirrhosis, diabetes and certain types of cancer [3]. With respect to diet and health, there is cause for concern because HNE has been discovered in a variety of food items [3], including mushrooms, meat, fish and fried foods.

It is now well known that HNE can be formed as a result of the autoxidation of culinary oils [4], and that the heating of these oils can accelerate this reaction [5]. Furthermore, after consumption HNE can be absorbed by the digestive tract [6], which may lead to birth defects [7].

The current methods for the quantification of HNE in frying oil are slow and often tedious. The use of one or more derivatization steps is necessary to increase the stability of the HNE molecule by converting one or more of the reactive functional groups into more stable forms. Stabilizing the HNE molecule arrests the concentration of

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HNE, which allows the HNE to be more accurately measured. The most widely used method for the quantification of HNE in oil requires chromatographic purification as well as 12+ hours of chemical reactions and sample workup procedures [8]. These steps add a considerable amount of time to the analysis of an oil sample, which vastly reduces sample throughput and increases the cost of the analysis. One alternative to the above cited method which does not fully stabilize the HNE molecule and also requires some chromatographic purification was developed by Surh and Kwon [9]. There has been some work done on a gas chromatography–mass spectrometry (GC–MS) based method for the detection of HNE in oil [10], but it has not been coupled with a quantification method.

Deep fat frying is an important cooking method; over 2 billion pounds of oil are used per year in the United States alone [11]. Despite the importance of deep fat frying, there has only been a limited amount of work done on HNE in frying oil. HNE has been quantified in frying oil samples from restaurants [9]; however, the researchers did not control the frying conditions or the material being fried. HNE has also been quantified during controlled frying of potatoes over an 8 h period [12]. In that study Seppanen and colleagues observed that oil before and after frying potatoes had a similar amount of HNE. Furthermore, the oil extracted from the potatoes had a similar amount of HNE as the oil in which they were fried.

The purpose of the present study was to quantify the formation of HNE over a multi-day controlled frying session designed to mimic an actual restaurant operation. We chose to use French fries as our model food because of the simple matrix and because potatoes are the most popular vegetable in the US with 125.6 lbs/capita consumed in 2007 [13]. A stable isotope dilution assay combined with a derivatization procedure allowed for relatively high sample throughput and adequate sensitivity for detection of HNE at low ppm levels.

Experimental

Chemicals and Materials

Fumaraldehyde dimethyl acetal, Amberlyst-15 catalyst, chlorotris(triphenyl-phosphine rhodium (I) (Wilkinson's catalyst), 5-bromo-1-pentene, 1-bromopentane, magnesium filings, hydrochloric acid, deuterium gas, butylated hydroxyl toluene (BHT), trimethylchlorosilane (TMCS), and 1-nonanol were obtained from Sigma–Aldrich (St. Louis, MO). Pentaflurobenzyl hydroxylamine hydrochloride (PFBHA) was obtained from Fluka (Buchs, Zurich, Switzerland) and trimethylsilyl 2,2,2-trifluoro-*n*-(trimethylsilyl)acetimidate (BSTFA) and dimethyldichlorosilane were obtained from Supelco (Bellefonte, PA). Diethyl ether, methanol, pentane, toluene, benzene and dichloromethane were obtained from Fisher Scientific (Fairlawn, NJ).

All glassware was deactivated by treating with a 5% solution of dimethyldichlorosilane in toluene (Sigma–Aldrich product #33065-U), following the manufacturers directions.

Oil Quality Measurement

The fatty acid composition of fresh oil (Table 1) was determined using AOCS methods Ce 1-62 and Ce 2-66 [14]. Official methods of analysis were used to measure percent free fatty acids (%FFA, AOCS method Ca 5a-40), photometric color index (PCI, AOCS method Cc 13-c 50), percent polymerized triacylglycerols (%PTAG, AOCS method Cd 22-91) and percent total polar materials (%TPC, based [15] on AOCS method Cd 22-91).

Synthesis of 4-Hydroxy-(*E*)-2-Nonenal and [$^2H_2$-8,9]-4-Hydroxy-(*E*)-2-Nonenal

4-Hydroxy-(*E*)-2-nonenal (HNE) for calibration was synthesized according to the method of Chandra and Srivastava [16]. [*8,9*-$^2H_2$]-4-hydroxy-(*E*)-2-nonenal ($^2H_2$-HNE) was synthesized using the same method, except that [*4,5*-$^2H_2$]-1-bromopentane was used in place of the 1-bromopentane (See Fig. 1). [*4,5*-$^2H_2$]-1-Bromopentane was synthesized from 5-bromo-1-pentene using a modification of the procedure described by Lin and colleagues [17] for the synthesis of [*5,6*-$^2H_2$]-hexan-1-ol from 5-hexen-1-ol as follows: Two grams of 5-bromo-1-pentene was placed in a glass reaction vessel containing 10 mL of benzene and 15 wt% (0.3 g) of Wilkinson's catalyst; the vessel was sealed with a rubber septum and then filled with deuterium gas by alternating evacuation of the system and flushing with deuterium gas. Pressure was maintained at approximately 10 psi. The deuteration process was periodically monitored by GC–MS. After completion of the reaction the catalyst was removed by centrifugation. The benzene was removed under a gentle vacuum using a rotary evaporator and the

Table 1 Fatty acid composition of corn–soy oil blend

Fatty acid	Percent
Palmitic (16:0)	10.9
Stearic (18:0)	3.0
Oleic (18:1)	25.4
Linoleic (18:2)	54.1
Linolenic (18:3)	3.5
Arachidic (20:0)	0.4
Other	2.7

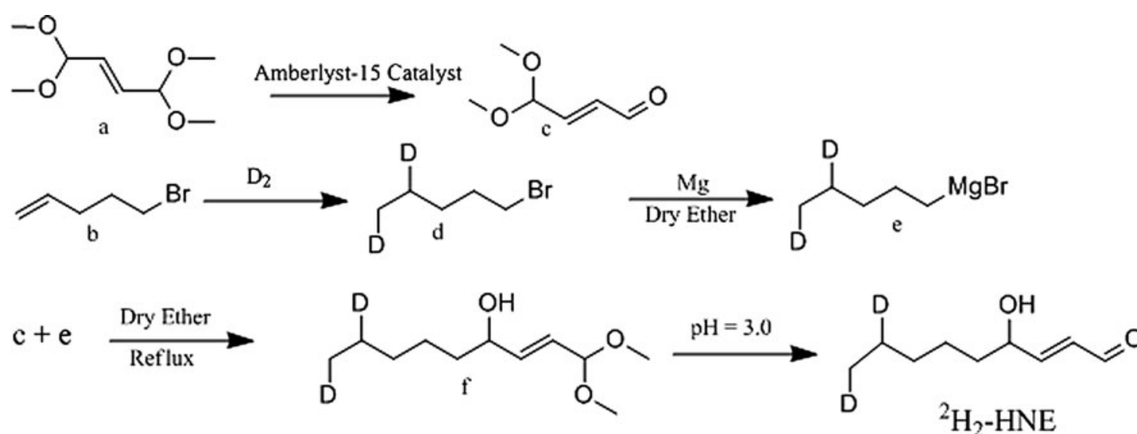

Fig. 1 Schematic route for the synthesis of $^2H_2$-HNE

[*4,5-$^2H_2$*]-5-bromopentane was recovered by vacuum distillation at room temperature (<100 mTorr). Compound purity was >99% by GC. MS–EI spectrum was as follows: 39(39), 40(24), 41(44), 42(45), 43(60), 44(95), 45(70), 55(6), 57(21), 73(100), 74(12), 93(5), 106(2), 107(6), 109(5), 152(5), 154(5). See Fig. 2 for a mass spectral comparison of the synthesized HNE and $^2H_2$-HNE.

HNE and $^2H_2$-HNE stock solutions were prepared in methanol containing 0.1% butylated hydroxytoluene (BHT) to stabilize the compounds. HNE was synthesized in enough quantity (>400 mg) to allow for the accurate preparation of standard solutions; however, only a few milligrams of $^2H_2$-HNE were synthesized which made it difficult to accurately weigh $^2H_2$-HNE on an analytical balance. Instead the actual concentration of $^2H_2$-HNE was determined by gas chromatography (GC) using a calibration procedure of HNE against a stable internal standard (1-nonanol). Using a GC equipped with a flame ionization detector (FID) and a cool on-column injector a response factor (2.20) was calculated for HNE against 1-nonanol. The GC conditions used are described later. This approach is similar to the method described by Schuh and Schieberle [18]. After the calibration was complete a known amount of 1-nonanol was added to the $^2H_2$-HNE solution to determine the exact concentration of the $^2H_2$-HNE and to monitor the degradation of the standard compound over time. The $^2H_2$-HNE solution was stored at −70 °C when not being used. Throughout the course of 9 months of experiments the $^2H_2$-HNE standard solution degraded to a level equivalent to 81.7% of its original concentration.

Quantification of HNE

In order to quantify by GC–MS the amount of HNE in the oil, it was necessary to first extract HNE from the oil followed by a derivatization procedure. Derivatization of HNE is crucial for the analysis of this compound by GC–MS since it increases the volatility and thermal stability of the molecule. The method used for the extraction of HNE was based on the method of Lang and colleagues [19]. One gram of oil was weighed on an analytical balance into a centrifuge tube, 5.0 μL of a 0.606 μg/μL solution of $^2H_2$-HNE in methanol (∼3 ppm in the oil) was added to the oil and the tube vortex mixed for 1 min to completely integrate the $^2H_2$-HNE into the oil. The HNE was extracted two times with water as follows: 10 mL of distilled water was added, the solution was shaken for 20 min on a multi-mixer [∼70% power, Mistral multi-mixer (Lab-line Instruments, Melrose Park, IL)] and then centrifuged for 15 min at 3,000*g* [IEC HNSII centrifuge (Thermo Fisher Scientific, Waltham, MA)]. The aqueous layers were pooled and subjected to the derivatization step described below.

The method used for the derivatization of HNE was based on the procedure of Kawai and colleagues [10]. This method was chosen because it stabilizes HNE [20] so that GC–MS can be used, which is a requirement for the use of the stable isotopologue of HNE as the internal standard. The combined aqueous layer was mixed with 1 mL of a methanolic solution containing 25 mg/mL PFBHA and 0.1% (w/v) BHT and then the solution was sonicated [Branson 2200 sonicator (Branson ultrasonics, Danbury, CT)] for 1 h. The HNE-oxime that formed was then extracted into pentane (2 × 10 mL). The pentane extract was evaporated to dryness under a stream of dry N_2 at 40 °C. A few drops of oil remained in the tube, to which 200 μL of 10% TMCS in BSTFA was added. In a sealed test tube this mixture was heated at 90 °C for 1 h then allowed to cool. Finally, 1.0 mL of dichloromethane was added to the reaction mixture and the solution transferred to a 2 mL vial for storage at −20 °C until analysis by GC–MS.

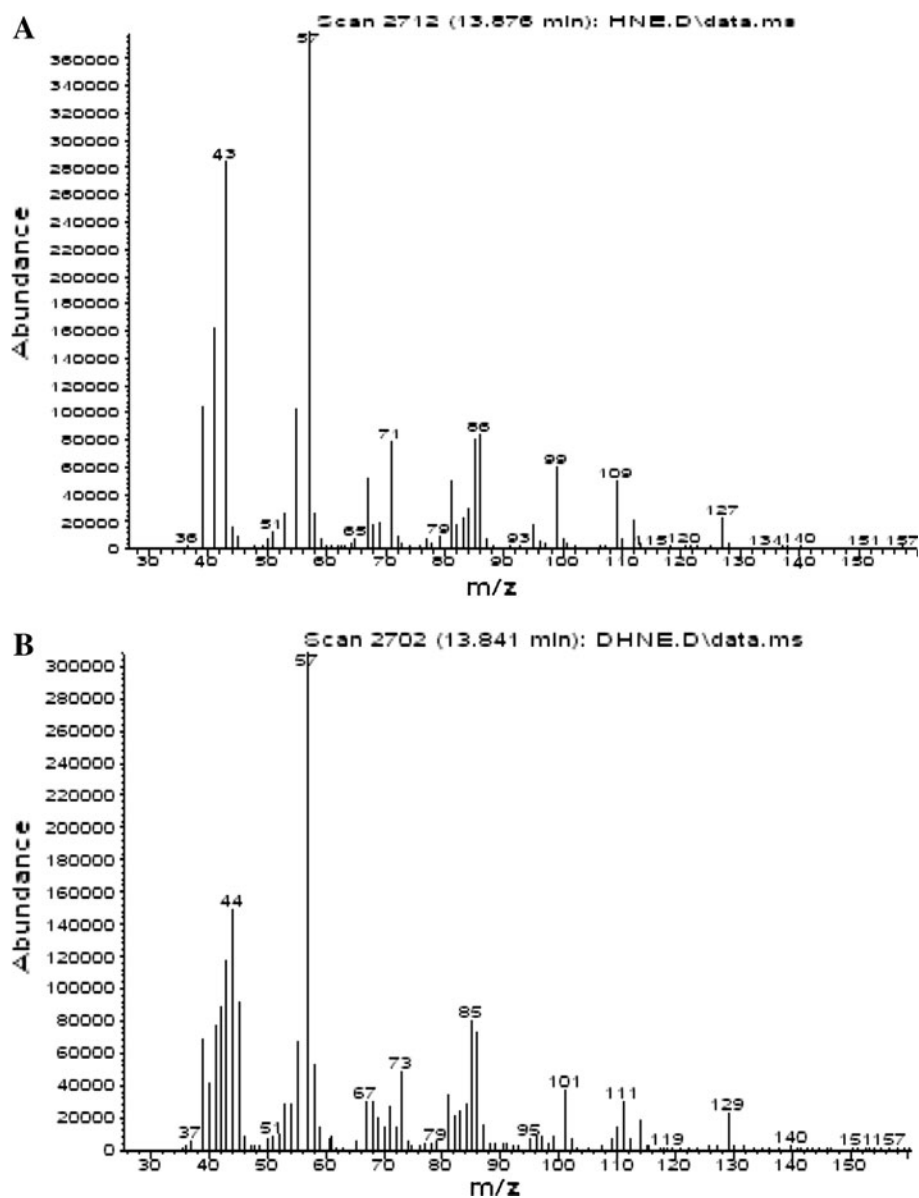

Fig. 2 Electron-impact (EI) mass spectra for **a** HNE and **b** $^2H_2$-HNE

Gas Chromatography-Mass Spectrometry

An HP5890 Series II Gas Chromatograph (GC)/HP5790B Mass Selective Detector (MSD) (Agilent Technologies, Inc., Santa Clara, CA) system was used for GC–MS analysis.

GC Settings for Analysis of Underivatized HNE

In order to prevent the thermal degradation of HNE, solutions containing HNE or $^2H_2$-HNE plus 1-nonanol (1 μL) were injected by the cool on-column method (+3 °C temperature tracking mode) into the GC–MSD system. Separations were performed using a Sac-5 capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Supelco, Bellefonte, PA). Helium was used as the carrier gas at an initial flow rate of 1.0 mL/min at 40 °C. The GC oven temperature was programmed from 40 to 230 °C at a rate of 10 °C/min with an initial and final hold times of 5 and 20 min, respectively. The MSD conditions were as follows: capillary direct interface temperature, 250 °C; ionization energy, 70 eV; mass range, 35–300 amu; electron multiplier voltage (Autotune + 200 V); scan rate, 4.39 scans/s.

GC Settings for Analysis of Derivatized HNE

The sample (2 μL) was injected in the hot splitless mode (280 °C; 1 min valve-delay, 50 mL/min split vent flow) into the GC–MSD system. Separations were performed using a Sac-5 capillary column (30 m× 0.25 mm i.d. × 0.25 μm film thickness; Supelco, Bellefonte, PA).

Helium was used as the carrier gas at an initial flow rate of 1.0 mL/min at 40 °C. The GC oven temperature was programmed from 40 to 300 °C at a rate of 10 °C/min with an initial and final hold times of 5 and 20 min, respectively. The MSD conditions were as follows: capillary direct interface temperature, 250 °C; ionization energy, 70 eV; selected ions monitored, *m/z* 226, *m/z* 228, *m/z* 242, *m/z* 244 and *m/z* 352; electron multiplier voltage (Autotune + 200 V); scan rate, 3.03 cycles/second.

Calculations

Based on previously published research [10], ion *m/z* 352 is characteristic of derivatized 4-hydroxy-2-alkenals, while ions *m/z* 226 and *m/z* 242 are characteristic of PFBHA-BSTFA derivatized HNE, if they occur along with *m/z* 352 and at the correct GC retention time (Fig. 3). Since one end of the $^{2}H_2$-HNE molecule is two mass units heavier than the non-deuterated HNE, ions *m/z* 228 and *m/z* 244 are characteristic of $^{2}H_2$-HNE when occurring with ion *m/z* 352. Both HNE and $^{2}H_2$-HNE eluted from the GC column as two peaks due to the stereochemistry of the imine formed (two isomers for each compound were formed) during derivatization. Both peaks were used for quantification. At the expected GC retention times peaks that contained ions *m/z* 226, *m/z* 242, and *m/z* 352 was considered to be positive identification for HNE and, likewise, peaks that contained the ions *m/z* 228, *m/z* 244, and *m/z* 352 was considered to be positive identification for $^{2}H_2$-HNE (Fig. 4).

To quantify HNE in a sample, a known amount of HNE and a known amount of $^{2}H_2$-HNE were added to oil samples of a known mass. These samples were then subjected to the analysis to build a standard curve. The best standard curve to calculate HNE concentration was based on the sum of the *m/z* 226 and *m/z* 242 ion peak areas in all HNE peaks divided by the sum of the *m/z* 228 and *m/z* 244 ion peak areas in all $^{2}H_2$-HNE peaks. The data used to calculate the standard curve also allowed for the calculation of a response factor (1.16) for derivatized HNE against derivatized $^{2}H_2$-HNE (See Fig. 5). To quantify HNE in an unknown sample the sum of *m/z* 226 and *m/z* 242 ion peak areas for both HNE-derivatives were compared against the sum of the *m/z* 228 and *m/z* 244 ion peak areas for both $^{2}H_2$-HNE-derivatives. The concentration of HNE present in any given sample was calculated using the following equation:

$$C_{\mathrm{HNE}} = \frac{R_{\mathrm{f}} * M_{\mathrm{IS}} * \mathrm{Area} \sum (m/z : 226, m/z : 242)}{M_{\mathrm{sample}} * \mathrm{Area} \sum (m/z : 228, m/z : 244)}$$

where C_{HNE} is the concentration of HNE (ppm, μg/g) in the sample, M_{IS} is the mass (μg) of $^{2}H_2$-HNE added to the sample, R_{f} is the response factor of HNE against $^{2}H_2$-HNE, and M_{sample} is the mass (g) of the oil sample.

Statistical Analysis

Data were analyzed using SAS 9.2 (SAS, Cary, NC). An analysis of variance was performed on the data set with the overall time of the experiment and heating with frying versus heating without frying as the independent factors. To further analyze the data independent *t*-tests were performed comparing heating with frying and heating without frying while holding the time of the experiment constant.

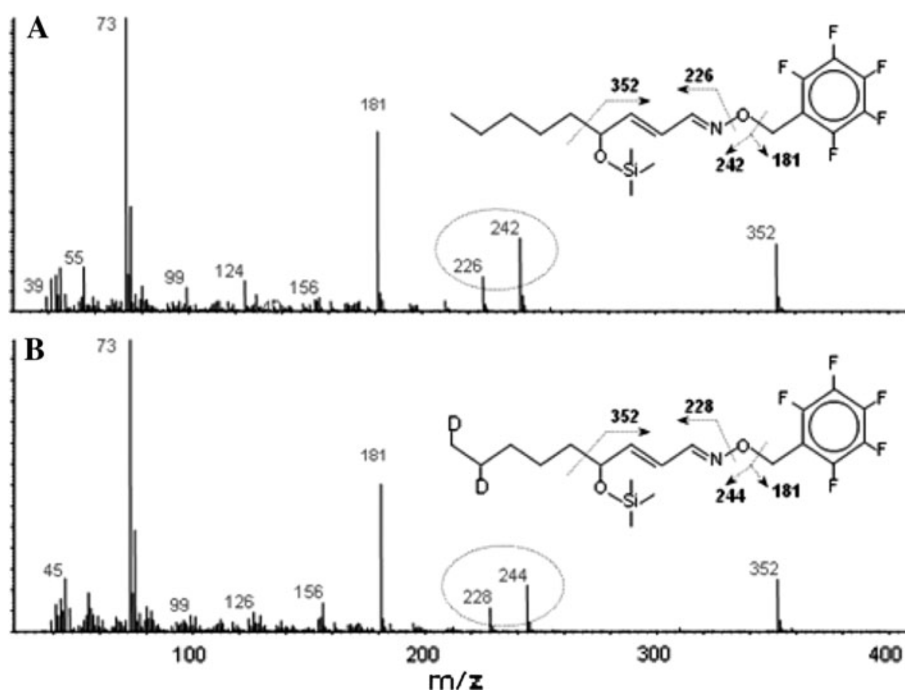

Fig. 3 Electron-impact mass spectra for PFBHA–BSTFA derivatized **a** HNE and **b** $^{2}H_2$-HNE

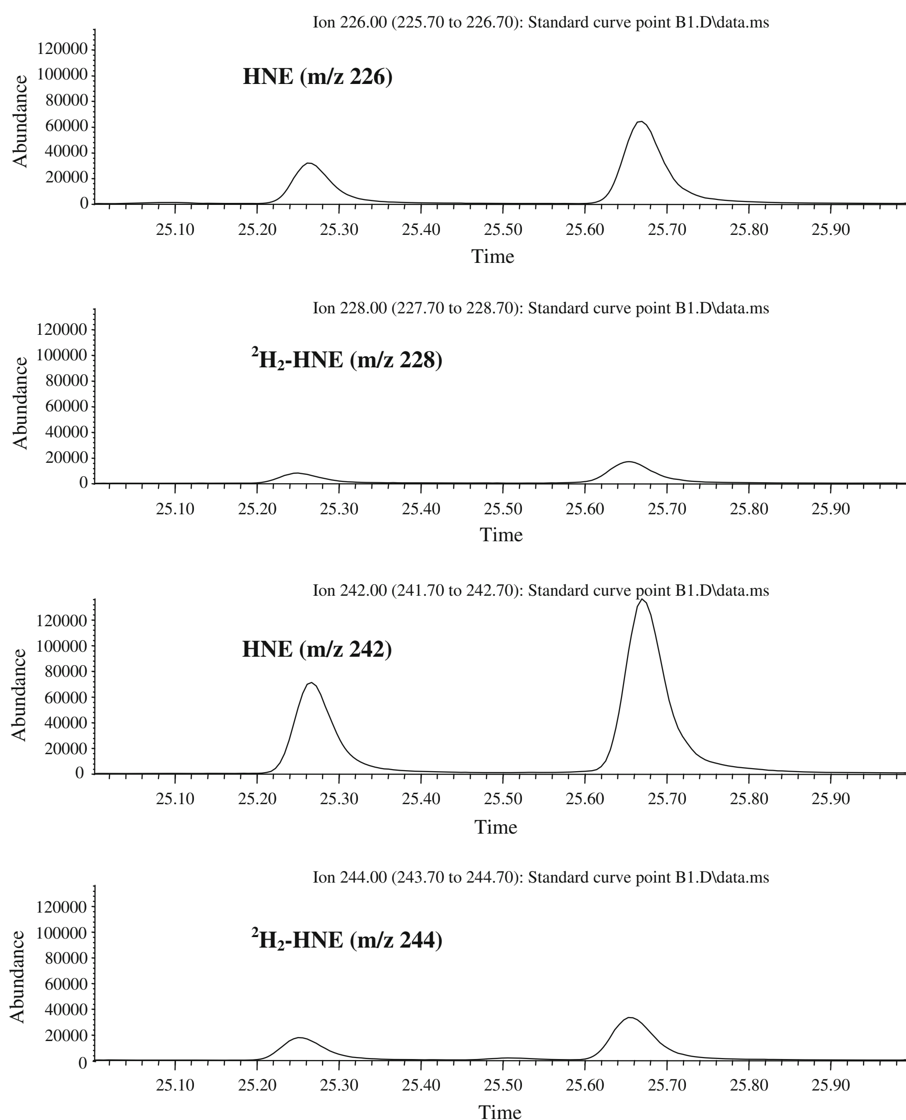

Fig. 4 GC–MS selected ions used to measure HNE and $^2H_2$-HNE

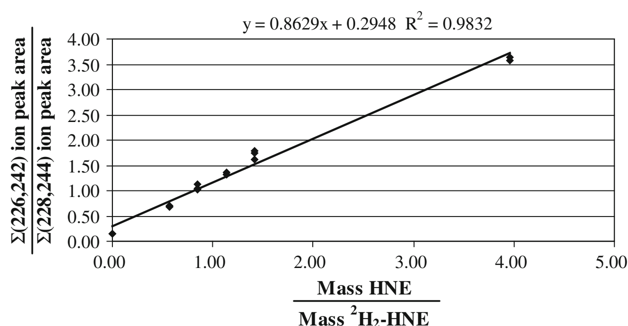

Fig. 5 Calibration curve for PFBHA–BSTFA derivatized HNE and $^2H_2$-HNE

Heating of Oil with Frying

Frozen extra long shoestring 1/4 inch frozen potatoes (#1249861, Sysco, Houston, TX) (French fries, 9% of the oil weight) were fried [515D 15 lb deep fat fryer (Star, St. Louis, MO)] in a corn-soy oil blend [Advantage clear frying oil #65412 (Cargill, Minnetonka, MN)] for 4.5 min at 180 °C, once per hour, for a total of eight fryings per day. The air/oil interface had a surface area of 850 cm^2. The fryers were operational for 8 h per day, for a minimum of 4 days. Frying conditions were chosen based on previous studies (Tompkins et al., 1999) and to optimize taste. At the beginning of each day, the oil was weighed, then sufficient fresh oil was added to bring the total oil weight to 6.8 kg.

Four oil samples for HNE analysis were taken each day; one immediately before the fryer was turned on, one after the oil had come to the frying temperature (15 min), one 4 h into heating, and one 8 h into heating. Oil samples for HNE analysis were pipetted into Pyrex test tubes, immediately immersed in liquid nitrogen to cool, then stored at 70 °C until analysis.

At the end of each day the oil was filtered (Magnesol PR2002 filtering machine; Dallas Group of America, Whitehouse, NJ) as per manufacturer's directions without use of any filtering substrate. After filtration oil samples were taken to measure %FFA, PCI, %PTAG and %TPC.

Heating of Oil Without Frying

Oil was prepared and heated in an identical manner to the frying experiment, except that no food product was fried in the oil. The oil was heated in the fryers for 8 h per day. At the beginning of each day oil was removed until the oil level was identical to the oil level of fryer that fried French fries, and then was topped off to 6.8 kg. At the end of the day the oil was filtered. Samples were taken at the same rate for both heating and frying.

All unused oil was stored at 5 °C in the dark, and a fresh container was opened at the beginning of each frying experiment. The French fries were received frozen and stored in their original packaging at −25 °C in the dark.

Results and Discussion

As mentioned previously, HNE is highly unstable and is prone to degradation during sample storage, extraction, derivatization reactions and chromatographic analysis. In addition, HNE is also similar with respect to its chemical properties to numerous other organic constituents (e.g. carbonyls, alcohols and fatty acids) found in culinary oils. These other compounds, if extracted, could potentially interfere with HNE during extraction, derivatization and chromatography. Use of water as an extracting solvent to isolate HNE is quite common [19], and has a main advantage that extraction of other non-polar oil compounds is minimal. The actual percentage yield of HNE during this extraction step is low and might vary depending upon the condition of the oil (i.e., fresh versus oxidized or dirty oil). Also, derivatization reactions might not always progress to the same extent for each sample extract. An excellent way to minimize the influence of the above variables is the use of stable isotope dilution analysis (SIDA), a technique pioneered in the 1970s [21, 22] which is now used as a standard method for the analysis of some compounds [23].

A high linoleic acid (54%) corn-soy oil blend was used in this study. This oil was specifically chosen because it is high in linoleic acid, a known precursor of HNE [24].

Changes in the %FFA and PCI were as expected based on previous studies (Figs. 6, 7, respectively). The steady increase of percent free fatty acids in the oil over the course of frying followed an expected trend [25]. The %FFA in the heated without frying oil did not increase nearly as rapidly throughout the experiments; this trend

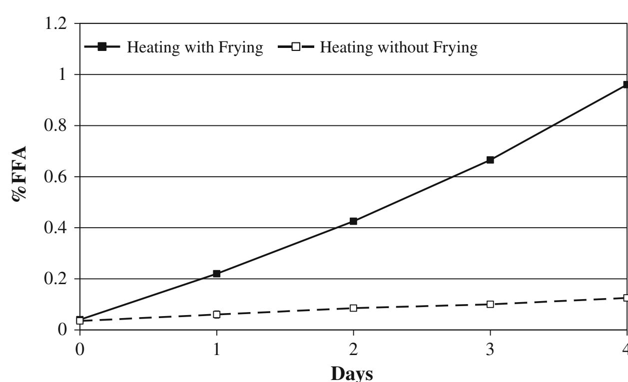

Fig. 6 Percent free fatty acids (%FFA) in corn-soy oil during heating with and without frying of frozen shoestring potatoes

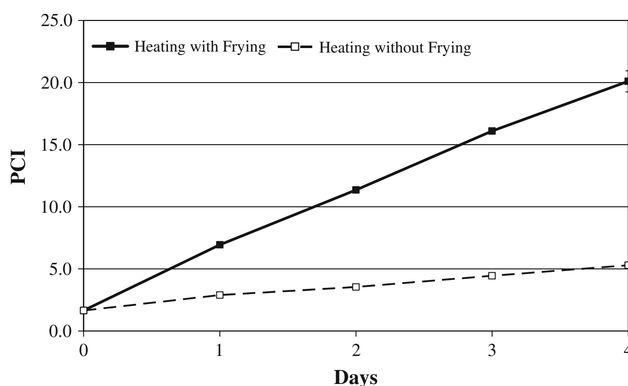

Fig. 7 Photometric color index (PCI) in corn-soy oil during heating with and without frying of frozen shoestring potatoes

has been previously reported [26]. It has been observed that the color of oil as measured by the PCI gets darker as the amount of frying increases [26]. The PCI increased at a much slower rate in oils heated without any food frying than in the oils heated with intermittent potato frying [27].

Changes in the %PTAG and %TPC were as expected based on previous studies (Figs. 8, 9, respectively). The %TPC tends to increase in frying oil throughout the frying life of the oil [28], but the %TPC also tends to increase similarly in heated oils [27]. The %PTAG during frying tends to increase throughout frying [27], and the %PTAG in the heated oil sample increased at a similar rate [29]. The results in the present study are not exactly the same as other researchers, but the other investigators did not study the same corn-soy blend oil. However, the trends in the present study are similar to the trends others have reported, which indicates that the degradation of the corn-soy oil during frying was not atypical.

Each trial was conducted from 4 to 7 days. Only the first 4 days of trial 1 are included, because the final 3 days showed the same trend as the fourth day.

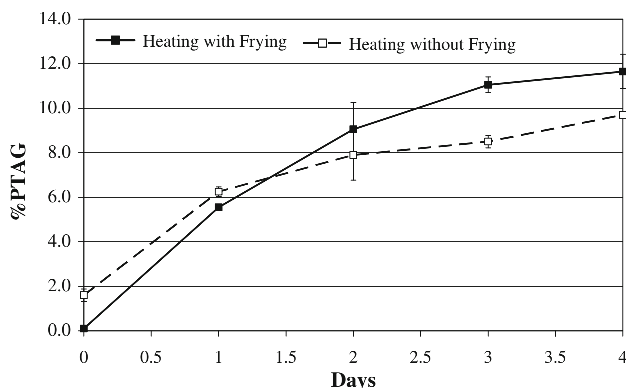

Fig. 8 Percentage of polymerized triacylglycerols (%PTAG) in corn-soy oil during heating with and without frying of frozen shoestring potatoes

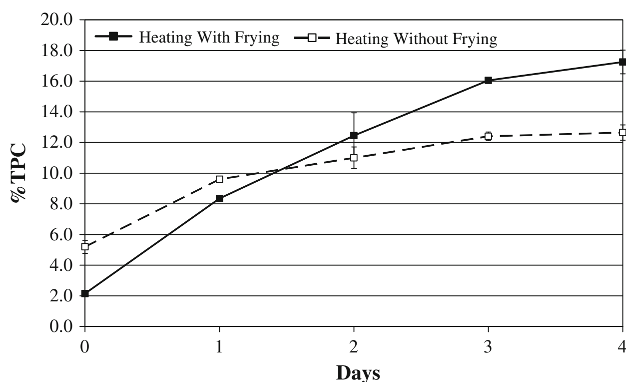

Fig. 9 Percentage total polar content (%TPC) in corn-soy oil during heating with and without frying of frozen shoestring potatoes

The analysis of variance indicated that the independent factors (overall time of the experiment and heating with frying versus heating without frying) and their interaction were significant at $P < 0.05$. Due to noise in the data, many data points were not statistically different from one another. Early on in the experiment, the treatment did not have a significant effect, by the fourth day of the experiment, the treatment was found to be statistically significant at $P < 0.05$. If the effect of frying French fries was small and if it took numerous fryings for an effect to become apparent, it would explain why it took several days to see a statistical difference between the treatments.

Previous studies have shown that HNE concentrations in frying oils range from 0.2 [30] to 60 ppm [12]. Culinary oils that have been heated but not used to fry food had reported HNE concentrations ranging from 2.47 [8] to 42 ppm [5]. It should be noted that HNE formation is temperature dependent for many of the oils that have been tested [31, 32].

During heating with frying, the concentration of HNE rose throughout the first day (Fig. 10). As the day progressed, the rate of the increase in HNE concentration slowed. In subsequent days there was a spike in the HNE concentration when the fryers were brought up to temperature, which was the daily maximum concentration attained.

During heating without frying the HNE concentration also rose throughout the first day (Fig. 10). Much like the heating with frying described above, the greatest increase of HNE concentration was when the fryer came up to the frying temperature. Unlike the frying trials, the highest HNE concentrations were not seen immediately after the

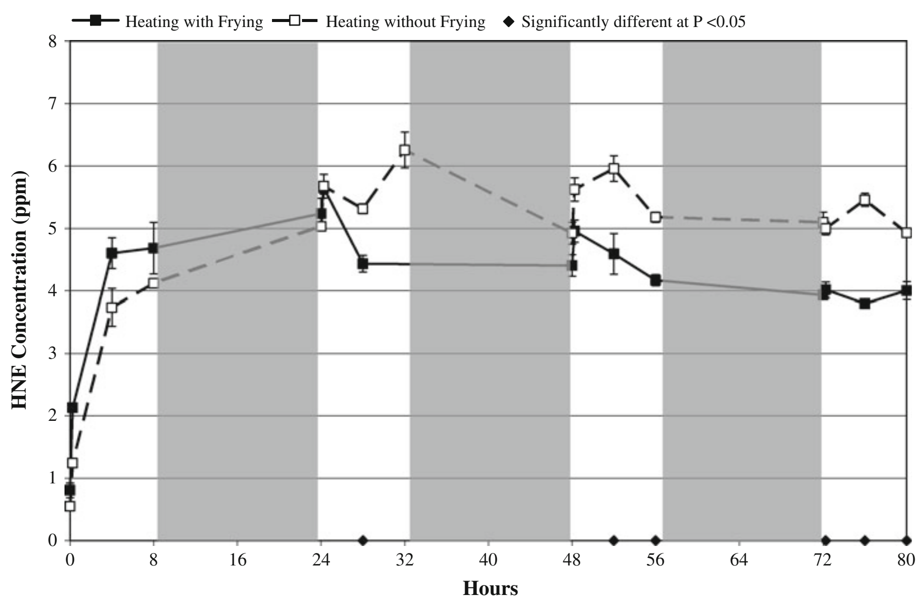

Fig. 10 Concentration of HNE (in parts per million, ppm) over a 4-day period (*grey shaded* areas indicate times when the fryers were off; diamonds on the *x*-axis at specific time intervals indicate that those concentrations are significantly different at $P < 0.05$)

fryers came up to the frying temperature, but in the middle or at the end of the day.

Since the oil chosen was high in linoleic acid, it was hypothesized that a relatively high concentration of HNE would be observed. Other controlled frying studies using French fries have reported up to 60 ppm of HNE [12]. HNE concentrations below 10 ppm were observed in the present study. The lower levels of HNE observed in this study are possibly due to differences between a small scale bench top experiment and a pilot plant scale experiment. Although the HNE concentrations are nearly an order of magnitude lower then previous studies, the results are probably very accurate due to the use of SIDA.

There are some general trends of HNE formation, regardless of the food being fried. Throughout the first day of heating or frying, the HNE concentration steadily increases, and throughout the rest of the oil's lifetime the HNE concentration remains relatively stable. When fresh oil is added to the fryer and the fryer brought up to the frying temperature, there is a spike in the HNE concentration. This is probably due to the fresh linoleic acid which is added, which subsequently oxidizes as it is heated.

It was initially hypothesized that frying in heated oil would form as much or even more HNE then heating alone. Based on the concentrations of HNE in previous studies, oils used to fry potatoes contained the same amount or greater amounts of HNE than heated oil [5, 12]. The experimental data show that if the fries did have an effect, it was the opposite of what was previously reported. It is likely that the addition of water (via the potatoes) into the system increased the rate of HNE decomposition. It was previously reported that the addition of water increases the decomposition rate of the frying oil [26]. HNE is an unstable compound, an increase in the degradation of the oil as a whole should increase the degradation of HNE. Another explanation for the decrease of HNE concentration in frying oil is the absorption of HNE by the fries; however, previous research has indicated that this is not a major factor [12].

While there was a significant difference between heated oil and frying oil, it was not a very large difference. Previous research [12] has shown that frying potatoes had no effect on the concentration of HNE in the oil. A possible reason no differences were observed is that the quantitation methods used by other investigators were less sensitive to small differences in HNE concentration than the SIDA used in this report. The duration of their experiment was also shorter in that they fried only a maximum of six batches of fries per experiment. A significant difference between the oil sample from the oil used for frying versus the oil sample heated without frying was not observed until the 28th hour of the experiment (12th batch of fries). It is possible that the addition of potatoes has only a slight impact on the HNE concentration in the oil, so it takes many batches of fries in order to effect a large enough difference to be significant.

Conclusions

Throughout this study relatively low concentrations of HNE were observed compared to what had been previously published in the literature. Despite these low values, the data indicate that the addition of French fries to the oil during heating does not substantially increase the amount of HNE in frying oil when compared to heating without any food frying. Since most previously published studies on HNE formation over time were limited to heated oils (without the addition of food), the present results provide important new information that helps explain the extent to which HNE forms in oil used for frying food.

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