Incorporation of the Toxic Aldehyde 4-Hydroxy-2-*trans*-nonenal into Food Fried in Thermally Oxidized Soybean Oil

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ABSTRACT: The toxic aldehyde 4-hydroxy-2-*trans*-nonenal (HNE) is an oxidation product of linoleic acid and is formed during the thermal oxidation of soybean oil at frying temperature. This investigation was conducted to determine whether HNE would be incorporated into food fried in thermally oxidized soybean oil. Commercially available liquid soybean oil was heated at 185°C for 5 h prior to frying uniform pieces of potato (1 × 0.5 × 7 cm). The oil was sampled prior to and after frying and was analyzed for the presence of HNE and other polar lipophilic aldehydes and related carbonyl compounds by HPLC. The oil was also extracted from the fried potato pieces and was analyzed identically to the frying oil. HNE was found to be a major polar lipophilic compound in the thermally oxidized frying oil, as previously published by this laboratory, and in the oil extracted from the fried potato. Similar concentrations of HNE were found in the oil prior to and after frying and in the oil extracted from the fried potato (57.53 ± 16.31, 52.40 ± 6.10, and 59.64 ± 11.91 mg HNE per 100 g oil, respectively). These results indicate that toxic HNE was readily incorporated into food fried in thermally oxidized oil; extensive consumption of such fried foods could be a health concern.

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KEY WORDS: Fried potato, hydroxyaldehydes, 4-hydroxynonenal, soybean oil, thermal oxidation.

The process of lipid peroxidation is a free radical-mediated deterioration of FA in the presence of air. During lipid peroxidation, hydroperoxides are formed, which then undergo decomposition and yield FA oxy free radicals and hydroxy radicals *via* β-cleavage. Subsequently, this chain cleavage leads to the formation of a wide variety of secondary lipid peroxidation products, including aldehydes, ketones, and other carbonyl-containing compounds. Of particular interest are the group of aldehydes known as 4-hydroxyalkenals, some of which have been shown to be cytotoxic and mutagenic (1). The toxicity of these compounds arises from the high reactivity of their three main functional groups: the aldehyde group, the α,β C=C double bond, and the hydroxyl group. Reaction of the β-carbon with thiol (SH) and amino (NH_2) groups results in protein and DNA modifications. It has been reported (2,3) that 4-hydroxyalkenals at cellular concentrations of more than 100 μM result in acute effects including inhibition of catabolic functions such as mitochondrial respiration and anabolic functions including protein, DNA, and RNA synthesis leading to cell death. Cellular concentrations of 4-hydroxyalkenals in the range of 1–20 μM reportedly cause partial inhibition of protein and DNA synthesis (3). 4-Hydroxyalkenal concentrations in this lower range are speculated be also produced *in vivo* in response to oxidative stress (3).

One particular 4-hydroxyalkenal, 4-hydroxy-2-*trans*-nonenal (HNE), is formed from the oxidation of n-6 FA, including linoleic acid, which is found in soybean oil and other polyunsaturated vegetable oils. The toxicity of HNE was noted when a fraction of oxidized methyl linoleate that contained HNE was administered orally to mice; severe lymphocyte necrosis in the thymus was observed 24 h after ingestion (4). The LD_{50} for HNE in mice has been reported as 68.6 mg (0.44 mmol)/kg body weight, given intraperitoneally (3). Recent experiments have related HNE toxicity to the incidence of atherosclerosis (5,6); LDL oxidation; stroke; Parkinson's, Alzheimer's, and Huntington's diseases (7–12); and liver disease (13), among others. HNE forms conjugates with proteins containing cysteine, histidine, and lysine residues and can damage DNA by inducing gene mutations and can alter the structure and function of cancer-related proteins (14).

It has been suggested that the toxicity of thermally oxidized oils derives from low M.W. products of oxidation such as hydroxy- and hydroperoxyalkenals rather than hydroperoxides (15) and that the nonvolatile secondary oxidation products are more likely to be detrimental to health than other oxidation products of thermally oxidized oil (16). In previous investigations we observed the detrimental effect on rats of consumption of thermally oxidized oil. When rats were fed diets containing 15% thermally oxidized soybean oil (heated 6 h at 185°C) for 4 wk, liver size was significantly increased as compared to rats fed unheated oil, indicating toxicity (Seppanen, C.M., A.S. Csallany, and D.D. Gallaher, unpublished data).

We reported that HNE is the major polar lipophilic aldehyde produced during the thermal oxidation of soybean oil, which contains about 51% linoleic acid, as well as 4-hydroxyhexenal, 4-hydroxyoctenal, and 4-hydroxydecenal (17). Furthermore, measurable amounts of HNE were detected in thermally oxidized soybean oil after 2 h of heating at 185°C (18). We subsequently determined that HNE is present after only 1 h of heating at frying temperature (Seppanen, C.M., and A.S.

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Csallany, unpublished data). Deep-frying of food results in the uptake of oil into it. Oil absorbed into food during frying has been shown to reflect the composition of the frying oil (19–21). Thus, it is expected that food fried in oil that contains HNE would also contain this toxic aldehyde. Therefore, the current investigation was undertaken to determine the extent of incorporation of HNE into food fried in thermally oxidized soybean oil.

EXPERIMENTAL PROCEDURES

Chemicals and materials. 2,4-Dinitrophenylhydrazine (DNPH) and hexanal were obtained from Sigma (St. Louis, MO); HPLC-grade acetone, dichloromethane, and methanol, from Mallinckrodt (Paris, KY); HPLC-grade hexane and hydrochloric acid from Fisher Scientific (Fair Lawn, NJ); and HPLC-grade water from EM Science (Gibbstown, NJ). Silica gel TLC plates (Al Sil G, aluminum-backed, 20 × 20 cm, 250 μm layer) were obtained from Whatman Ltd. (Maidstone, Kent, England). All solvents used were HPLC grade.

Preparation of thermally oxidized soybean oil. Portions (300 g) of commercial liquid soybean oil purchased at a local grocery store were heated at 185 ± 3°C in a 1000-mL beaker on a hotplate for 5 h. The thermally oxidized oil was sampled prior to and immediately after frying the potato pieces, and the analysis for polar lipophilic aldehydes and carbonyl compounds was conducted immediately. Three separate portions of thermally oxidized soybean oil were prepared.

Preparation of fried potato. Russet potatoes were peeled and cut into uniform strips (1 × 0.5 cm × 7 cm). Batches of freshly cut potato (10 g wet basis) were fried for 3 min each in the thermally oxidized soybean oil. After frying, the potato pieces were placed on absorbent paper to cool and to remove excess surface oil. In the first heating session, two batches of potato samples were fried (3 min/batch) for a total frying time of 6 min. Four batches of potato samples were fried (3 min/batch) in the second heating session: total frying time was 12 min. During the third heating session, six batches of potato samples were fried (3 min/batch) for a total of 18 min of frying time. Sufficient time was allotted between batches to allow the oil temperature to return to 185°C.

Extraction of oil from fried potato. Portions of fried potato samples (10 g wet basis) were prepared by combining two batches of fried potato samples from the same heating session. The first frying resulted in one portion of fried potato sample; the second frying yielded two portions of fried potato samples; and the third frying resulted in three portions of fried potato samples. The six portions of fried potato samples were each homogenized with 20 g anhydrous Na_2SO_4 and 50 mL hexane. For each sample, the hexane supernatant was removed, and the potato/Na_2SO_4 slurry was extracted two subsequent times with 50 mL of hexane. The hexane portions were combined and evaporated under vacuum. The oil extracted from the fried potato was immediately analyzed for HNE and other polar lipophilic carbonyl compounds.

Measurement of polar lipophilic aldehydes and carbonyl compounds in oil. The method described by Seppanen and Csallany (17) was used to analyze the thermally oxidized soybean oil and oil extracted from the fried potato. Aliquots of the oxidized oil and the oil extracted from the fried potato (2 and 1 g, respectively) were reacted in duplicate with 5 mL DNPH reagent (prepared by combining 10 mg recrystallized DNPH with 20 mL of 1 N HCl) overnight at room temperature to form hydrazone derivatives with the aldehydic secondary oxidation products. The DNPH derivatives were extracted from the oil first with methanol/water (75:25 vol/vol) and then dichloromethane. The lipophilic DNPH derivatives were then separated into three groups by TLC using dichloromethane as the solvent: the polar carbonyl compound derivatives, the nonpolar carbonyl compound derivatives, and the osazones. The polar carbonyl compounds were eluted from the TLC plates with methanol; then the solvent was evaporated by N_2 gas to 1 mL. Aliquots (100 μL) of the concentrated DNPH derivatives were separated and quantified by HPLC on a reversed-phase C18 column [Ultrasphere ODS, 25 cm × 4.6 mm i.d., 5 μm particle size, (Altex, Berkeley, CA)] with a guard column (2 cm × 2 mm i.d.; ChromTech, Apple Valley, MN). The group of lipophilic polar carbonyl hydrazones, which includes HNE, was eluted with isocratic elution for 10 min with methanol/water (50:50 vol/vol) followed by a linear gradient to 100% methanol for a total elution time of 40 min at a flow rate of 0.8 mL/min. Absorbance was monitored at 378 nm. Disposable syringes used for sample injection were equipped with a 0.2 μm polyvinylidene difluoride filter (ChromTech). A mixture of hexanal-, 2-heptenal-, and decanal-DNPH standards were used daily to measure the reproducibility of the HPLC system before the application of samples.

Quantification of DNPH derivatives. HPLC peak areas were converted to mass by using a peak area of 13,000 mV equivalent to 1 ng pure HNE-DNPH standard. This value was determined by repeated injections of various concentrations of pure HNE-DNPH standard and comparing the peak area response to the concentration.

Statistical analysis. Differences between the treatments, the oil sampled prior to frying, the oil sampled after frying, and the oil extracted from the fried potato were compared using the ANOVA test. A P-value < 0.01 was considered to indicate a significant difference between treatments.

RESULTS AND DISCUSSION

In the present experiment, the soybean oil used for frying the potato pieces was heated for 5 h prior to frying in order to allow for formation of measurable amounts of HNE. Our previous investigations have shown that HNE is detectable in thermally oxidized soybean oil after 2 h of heating at 185°C (18). Recent investigations in this laboratory revealed that a measurable amount of HNE exists in soybean oil after only 1 h of heating (unpublished data). The amount of HNE increases during continued heating, reaching a maximal concentration after about 6 h at 185°C, after which the concen-

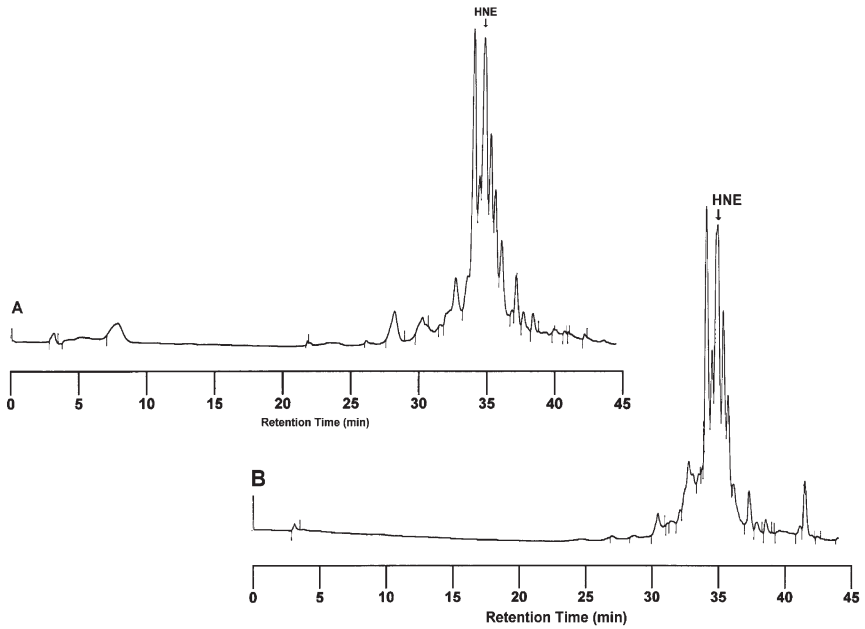

FIG. 1. HPLC separation of 2,4-dinitrophenylhydrazine (DNPH) derivatives of polar aldehydes and carbonyl compounds from thermally oxidized soybean oil (heated 5 h at 185°C). (A) Oil sampled prior to frying potato pieces. (B) Oil sampled immediately after frying for 10 min. The identified compound is 4-hydroxy-2-*trans*-nonenal (HNE). Other peaks are unidentified. For experimental conditions see Experimental Procedures section.

tration decreases. We reported previously that unheated soybean oil does not contain HNE (18).

The HPLC separation of the DNPH derivatives of polar lipophilic carbonyl compounds in thermally oxidized soybean oil is demonstrated in Figures 1A and 1B. The oil was analyzed after 5 h of heating at 185°C, prior to and immediately after frying the potato pieces. The similarity of these two chromatograms indicates that frying of the food for 10 min had very little effect on the composition of the polar lipophilic aldehydes in the oil. As shown in the figures, HNE was a major compound in the thermally oxidized oil, as we have reported earlier (18).

The chromatogram shown in Figure 2 represents the DNPH derivatives of the polar lipophilic carbonyl compounds in the oil extracted from the potato fried in the thermally oxidized oil. The absorbed oil had the same distribution profile of polar lipophilic aldehydes as the thermally oxidized frying oil. Several additional peaks also were detected that were not in the frying oil. These additional compounds were derived from the potato during frying. HNE remained a major polar lipophilic aldehyde in the oil after frying and HNE was also a major polar lipophilic compound in the oil extracted from the fried potato.

The total amount of polar lipophilic aldehydes and related carbonyl compounds in the thermally oxidized oil sampled before and after frying and the oil extracted from the potato were determined by summing the individual peak areas measured by HPLC (Fig. 3). There were no statistically significant differences in the total polar lipophilic aldehyde content among the three oils. The oil extracted from the potato had a somewhat higher amount of total polar lipophilic compounds owing to the presence of additional compounds arising from the potato.

The concentrations of HNE in the oil analyzed before and after frying and in the oil extracted from the fried potato are presented in Figure 4. These values were 57.55 ± 10.96 mg HNE per 100 g oil for the oil prior to frying, 43.87 ± 4.12 mg HNE per 100 g oil after frying, and 51.23 ± 5.48 μg HNE per 100 g oil for the oil extracted from the fried potato. There were no statistically significant differences between the HNE concentrations in these three oils. This demonstrates that HNE, a highly toxic aldehyde, was incorporated with the oil into the fried potato at the same concentration as it existed in the thermally oxidized frying oil. The average concentration of oil in the potato was 9.65%; therefore, the HNE concentration in the fried potato was calculated to be 4.90 ± 0.47 mg HNE per 100 g fried potato.

Since linoleic acid is a precursor for HNE and since soybean oil contains about 51% linoleic acid (22), other oils containing high concentrations of linoleic acid may be equally susceptible to the formation of considerable amounts of HNE with heat treatment. Thermally oxidized corn oil, which contains about 58% linoleic acid, has a similarly high concentration of HNE compared to soybean oil under similar heat treatment (23).

The results of this study indicate that when HNE is present in the frying oil, it will be present in similar concentration in the oil absorbed by the fried food. This is the first known report of the measurement of HNE in fried food under controlled conditions. The conditions used in this experiment are somewhat more extreme than what may be typically found in

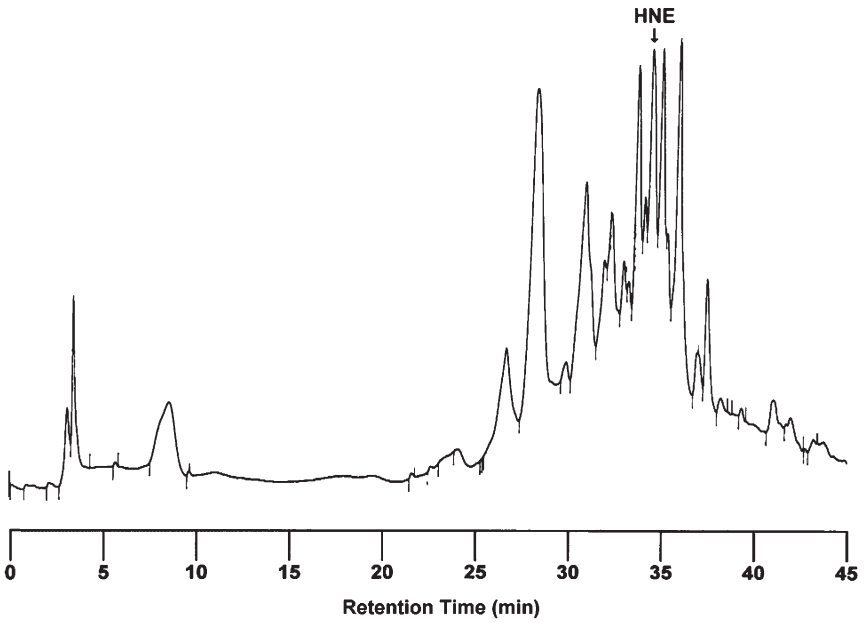

FIG. 2. HPLC separation of DNPH derivatives of polar aldehydes and carbonyl compounds from oil extracted from potato pieces fried in thermally oxidized soybean oil (shown in Fig. 1). The identified compound is HNE. Other peaks are unidentified. See Figure 1 for abbreviations, and Experimental Procedures section for separation conditions.

various frying operations. However, if liquid soybean oil is used for frying in home or commercial operations and heated for 2 h or more, toxic HNE will form in the frying oil and HNE will be incorporated into the fried food: The amount will depend on the amount of oil absorbed during frying. Repeated exposure to small amounts of toxic HNE may result in biological changes and could be detrimental to health. The fact that highly toxic HNE is one of the major polar aldehydes formed in thermally treated soybean oil and corn oil, and possibly in other high linoleic acid-containing oils, and the fact that HNE incorporates into fried food, together with the frying oil, are a concern for public health.

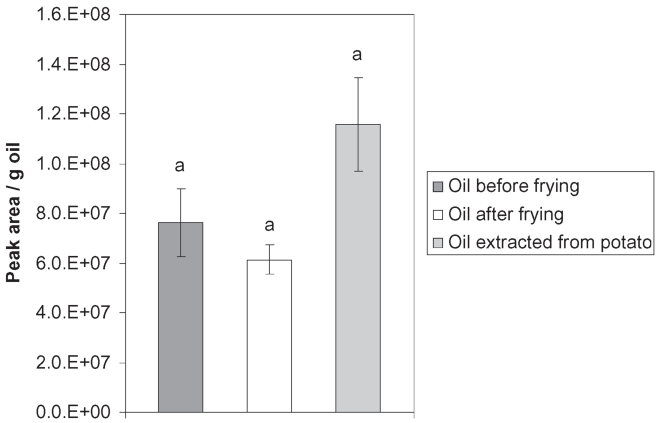

FIG. 3. Total polar lipophilic aldehydes and related carbonyl compounds, determined as the sum of the individual HPLC-separated DNPH derivatives of polar compounds, in thermally oxidized soybean oil heated at 185°C for 5 h, in thermally oxidized soybean oil after 10 min of frying, and in oil extracted from potato pieces fried in thermally oxidized soybean oil. Abbreviations are given in Figure 1. Bars represent mean ± SEM; oil before frying, $n = 8$; oil after frying and oil extracted from potato, $n = 13$. The presence of the same letter indicates no statistically significant differences between samples.

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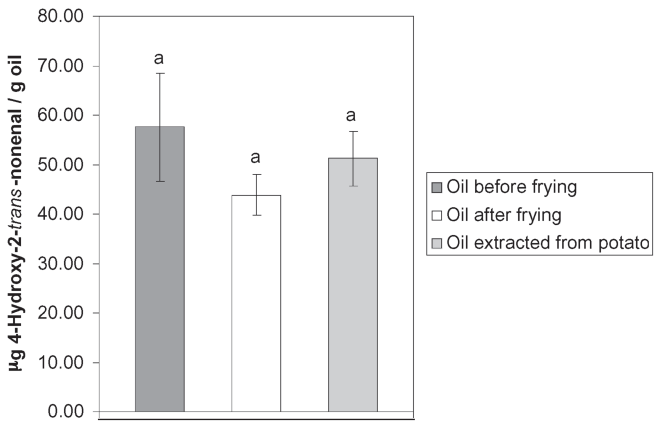

FIG. 4. HNE in thermally oxidized soybean oil heated at 185°C for 5 h, in thermally oxidized soybean oil after 10 min of frying, and in oil extracted from potato pieces fried in thermally oxidized soybean oil. Abbreviations are given in Figure 1. Bars represent mean ± SEM; oil before frying, $n = 8$; oil after frying and oil extracted from potato, $n = 13$. The presence of the same letters indicate no statistically significant differences between samples.

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