Sterol Oxidation Products in French Fries and in Stored Potato Chips

KEN LEE*, ANNE M. HERIAN1 and NANCY A. HIGLEY1

Department of Food Science, 1605 Linden Drive, University of Wisconsin-Madison, Madison, Wisconsin 53706

(Received for publication June 4, 1984)

ABSTRACT

Potato chips and french fries were analyzed by high performance liquid chromatography and thin layer chromatography for cholesterol and β-sitosterol oxidation products. Chips stored for 150 d at 23°C in unopened foil bags contained no detectable sitosterol oxidation products, but those held at 40°C contained 7α-hydroxysitosterol, 7β-hydroxysitosterol, and sitosterol β-epoxide only after an extended storage of 95 d. French fries as purchased contained sterol α- and β-epoxides, and 7α- and 7β-hydroxyesters. These sterol oxidation products were present in repeat samples from five different fast food restaurants. Ingestion of sterol oxides from potato chips is unlikely, whereas ingestion of sterol oxides from french fries is possible.

Few data are published on the types and amounts of sterol oxides in foods. Mixtures of oxidation products of cholesterol were found to be atherogenic (12,25,26). One hypothesis is that sterol oxides, rather than the native sterol, may be responsible for the atherogenic effects attributed to cholesterol (11).

It is possible that plant sterols oxidize to yield compounds analogous to cholesterol oxidation products (1,5). A major plant sterol, β-sitosterol, is structurally the same as cholesterol except for an ethyl side group as shown in Fig. 1.

About 30% of dietary cholesterol is absorbed compared to 5% of dietary β-sitosterol (18,23), but once it is absorbed, it is catabolized and esterified as is cholesterol (9,24). Placental transfer of plant sterols (23), and greater dietary absorption during infancy and childhood (17) has been reported. Infants fed vegetable oil formula accumulated plant sterols in their aortic tissue (15), and plant sterols were present in atheromatous lesions in adults (16). However, toxicity and presence of sitosterol oxidation products in foods has not been studied extensively.

It was noted in a recent review (8) that additional research is necessary to identify and quantify the sterol oxides which may exist in foods. We have developed procedures for extraction of oxides from foods with minimal interferences (11,15). We have also reported photo-induced 7α- and 7β-hydroxycholesterols in a dry egg nog mix (10). This study examines the sterol oxide content of french fries as purchased, as well as sitosterol oxide formation during high temperature storage of potato chips. These foods were studied as they contain lipids with a high potential for oxidative damage.

METHODS

Potato chips (Charles Chips, Mountville, PA) fried in cottonseed oil and packaged in foil were purchased locally in one lot. Chips were held in unopened bags at 40°C for 100 d or at room temperature (25±2°C) for 150 d. French fries were purchased from 5 different fast food restaurants at random times on 2 d each. Fries were analyzed on the day of purchase. β-Sitosterol was purchased from Aldrich Chemical. Cholesterol α-epoxide (m.p. 138-141°C) and sitosterol α-epoxide (m.p. 139-140°C) were synthesized according to Fieser and Fieser (7) and cholesterol triol (m.p. 228-230°C) was made by the procedure described by Knapp et al. (13). Cholesterol β-epoxide was synthesized by acetylation (6) and re-epoxidation (3) of cholesterol triol (13) as detailed previously (15). Cholesterol oxides including 25-hydroxycholesterol, 7α- and 7β-hydroxycholesteroles and 7-ketocholesterol were purchased from Steraloids (Wilton, NH) or Sigma. The purity of standards was checked.
by TLC (thin layer chromatography) and HPLC (high performance liquid chromatography).

Sterol extraction

About 50 g of potato chips were weighed and blended (Osterizer, Model 403) with 200 ml of chloroform:methanol (2:1) until homogeneous (about 30 s). The homogenate was filtered through glass wool into a 1000-ml separatory funnel with about 50 ml of distilled water and an additional 30 ml of chloroform. The chloroform layer containing the lipid was recovered, dried with sodium sulfate and chloroform removed under vacuum. About 5 g of lipid were refluxed for 90 min in 30 ml of 1.5 N KOH in 95% ethanol. After cooling, 100 ml of water were added and the sample was extracted three times with 100 ml of anhydrous ethyl ether (USP, J. T. Baker). The combined ether fractions were washed twice with 100 ml of 5% aq. NaHCO₃, washed twice with 100 ml of 0.2% aq. HCl, dried with sodium sulfate and vacuum distilled. The residue was purified through an arrestant column 3 x 16 cm to remove fatty acids and an argentated column 2 x 10 cm to remove pigments as described previously (11). The eluants were concentrated in vacuo and analyzed.

About 100 g of french fries were extracted as described above, except 250 ml of chloroform:methanol was used and only 3 g of lipid was saponified. An HPLC-analyzable sample was recovered after elution from the argentated column. All samples were stored in a freezer at -10°C under nitrogen and in the dark.

Analyses

TLC on pre-adsorbed Silica Gel 60 analytical plates (E. Merck, 200 µm) using anhydrous ethyl ether development and visualization by spraying with 50% aq. H₂SO₄ or 25% aq. p-toluene sulfonic acid (22) gave indications of composition. Identification of spots was by Rf values and color under visible and UV light before and after spraying. HPLC used a HIBAR LiChrosorb Si 60 (E. Merck) 5 µm column, 250 x 4 mm. Flow was maintained at 2.0 ml/min with a Waters 6000A pump and detection was with a Waters R401 differential refractometer. HPLC-grade solvents were from Burdick and Jackson and were degassed by sonication. Potato chip and french fry extracts were taken up in ether, 8 mg/ml, and 20 µl were injected. The sterol oxides were identified by retention times in comparison to standards and were quantified by peak area. Peroxide value was determined by titration with sodium thiosulfate (2).

RESULTS AND DISCUSSION

Potato chips heated at 40°C oxidized more rapidly than at room temperature, as indicated by higher peroxide values beginning at 49 d (Fig. 2), and a rancid taste and musty odor beginning at 24 d. However, oxidation products of β-sitosterol did not appear until 95 d of heating, corresponding to 230 meq peroxide per kg, and a paint-like odor (not tasted). Sitosterol oxidation products identified and quantified at 95 d by TLC and by HPLC were sitosterol β-epoxide, 6 µg/g lipid, 7α-hydroxysitosterol, 13 µg/g lipid, and 7β-hydroxysitosterol, 9 µg/g lipid. Total lipid in the chips was 35%. A chromatogram of the potato chip sterol extracted after 95 d at 40°C is shown in Fig. 3. The types of sitosterol oxidation products found in the 40°C chips were typical of products formed under autoxidizing conditions. After 100 d of heating at 40°C, generation of sterol oxides was no longer followed since samples could not be purified adequately for HPLC analysis. These oxidation products were a result of elevated temperature, as storage for 150 d at 23°C did not produce detectable oxidation products. The heated chips simulated warehouse conditions sometimes encountered in warmer parts of the country. Although the peroxide value of heated chips was much larger than of unheated chips, absence of light and of oxygen probably inhibited reaction of peroxides with β-sitosterol.

Sterol oxidation products were detected in french fries as shown in Table 1. Since the friers used in fast-food restaurants sometimes include beef tallow and hydrogenated vegetable oils, our values likely include both cholesterol and plant sterol oxides. The HPLC conditions used here were not able to differentiate cholesterol from β-sitosterol.

Lipid in french fries ranges from 17 to 19% wet-basis (19), thus the concentration of sterol oxides in this food as eaten is lower than the levels in Table 1. Duplicates for each restaurant were similar in sterol oxide composition and level, in spite of sampling at various times on different days. Thus the frying oil may be in a steady state with the 7β-hydroxysterols in larger amounts than the 7α-hydroxysterols. Smith (20) suggested that the 7β-epimer is more stable, causing it to predominate. In the
French fry, cholestan-5,6β-epoxy-3β-ol was predominant, suggesting the β-epoxy epimer was more stable. Chicoye et al. (4) reported preferential formation of the β-isomer of cholestan-5,6-epoxy-3β-ol in irradiated yolk solids. The absence of 5-cholestan-3β-ol-7-one was likely due to destruction by saponification (11).

It has been hypothesized that oxygen species are present in food which attack cholesterol to produce cholesterol epoxide, or cholestan-5,6-epoxy-3β-ol (14). The epoxide can be hydrated to the angiotoxic cholestan-3β,5α,6β-triol (21). Four of the five fries tested contained α- or β-epoxides.

Abusive storage conditions of foil-bagged potato chips caused sitosterol oxidation products to appear, at a point far beyond consumer acceptability. Thus ingestion of sterol oxides from potato chips stored under usual conditions is not likely. French fries as purchased from five fast-food restaurants all contained sterol oxidation products. This may be due to the higher temperature and presence of light and oxygen in the frying operation. Thus ingestion of sterol oxides from French fries and other fried foods is possible. The amounts and significance of these oxidation products in the diet remains to be studied.

**ACKNOWLEDGMENT**

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and by grant No. IN35V19 from the American Cancer Society.

**REFERENCES**


**TABLE 1. Levels of sterol oxidation products in french fries.**

<table>
<thead>
<tr>
<th>Common name:</th>
<th>α-epoxide</th>
<th>β-epoxide</th>
<th>7β-hydroxy</th>
<th>7α-hydroxy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholestan-5, 6α-epoxy-3β-ol</td>
<td>Cholestan-5, 6β-epoxy-3β-ol</td>
<td>5-Cholestan-3β,7β-diol</td>
<td>5-Cholestan-3β,7α-diol</td>
<td></td>
</tr>
<tr>
<td>Restaurant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>19</td>
<td>25</td>
<td>39</td>
<td>tr</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>27</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>nd</td>
<td>3</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>23</td>
<td>44</td>
<td>21</td>
</tr>
<tr>
<td>E</td>
<td>17</td>
<td>18</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td>nd</td>
<td>18</td>
<td>30</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>tr</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>nd</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>nd</td>
<td>2</td>
<td>62</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*nd = Not detected due to co-eluting substances on HPLC.

*tr = Traces on TLC but not quantifiable by HPLC.
5. daly, g. g., e. t. finocchiaro, and t. richardson. 1983. characterization of some oxidation products of β-sitosterol. j. agric. food chem. 31:46-50.
6. fieser, l. f., and b. k. bhattacharya. 1953. cholestanetriol and ketone 104 from a variety of sources. j. am. oil chem. soc. 75:4418-4423.
7. fieser, m., and l. f. fieser. 1967. reagents for organic syntheses. vol. 1. john wiley and sons, inc., new york, ny.
10. herian, a. m., and k. lee. 1984. 7α- and 7β-hydroxycholesterols formed in a dry egg nog mix exposed to fluorescent light. j. food sci. (accepted).
11. higley, n. a., a. m. herian, k. lee, and s. taylor. 1984. cholesterol oxides in meats. meat sci. (submitted).
15. lee, k., a. m. herian, and t. richardson. 1984. detection of sterol epoxides in foods by colorimetric reaction with picric acid. j. food prot. 47:340-342.