Effect of Cooking on Concentrations of *β*-Estradiol and Metabolites in Model Matrices and Beef

Eric Braekevelt,* Benjamin P. Y. Lau, Brett Tague, Svetlana Popovic, and Sheryl A. Tittlemier[†]

Food Research Division, Bureau of Chemical Safety, Health Products and Food Branch, Health Canada, 251 Banting Drive, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0K9. [†] Present affiliation: Grain Research Laboratory, Canadian Grain Commission, 303 Main Street, Winnipeg, Manitoba, Canada R3C 3G8.

Because beef food products are generally cooked prior to consumption, the behavior of chemicals in these cooked foods is important in estimating human exposure. The heat stability of the natural estrogen *β*-estradiol (*β*-E2) and its metabolites α-estradiol (α-E2), estrone (E1), and several catechol estrogens was examined in heated vegetable oil and aqueous solutions. The chemicals were also incorporated into regular and extra lean ground beef and subjected to cooking. E1 and E2 were stable in aqueous solutions at 100 °C, whereas the catechol estrogens exhibited first-order decay curves with half-lives of 2−10 min. Their stability improved to the same level as the other test chemicals when an antioxidant was added to the solution, suggesting that their disappearance was due to oxidation rather than thermal degradation. E1 and E2 were also stable in heated vegetable oil (160−180 °C), whereas catechol estrogen decreased 30−50% over the 2 h duration of the experiments. Chemical losses from cooked beef appear to be related to the fat content of the beef, with greater losses occurring in regular ground beef (25−30%), compared to extra lean ground beef (5−20%). This study shows that cooking reduces but does not eliminate the potential for dietary exposure to growth promoters in ground beef.

INTRODUCTION

The natural hormone *β*-estradiol (*β*-E2) regulates reproduction and growth in many animal species, including humans. Some important metabolites include α-estradiol (α-E2), estrone (E1), and the catechol estrogens 2-hydroxyestrone (2-OH E1), 4-hydroxyestrone (4-OH E1), 2-hydroxyestradiol (2-OH E2), and 4-hydroxyestradiol (4-OH E2). Although the metabolites of *β*-E2 exhibit less estrogenic activity than the parent compound (*1*), the catechol estrogens form adducts with DNA (*2*), and 4-OH E2 particularly has been shown to induce cancer (*3*). The chemical structures of these compounds are shown in **Figure 1**.

Estradiol has been administered to beef cattle to increase the rate of weight gain and improve feed efficiency (*4*). It is usually administered as a subcutaneous implant in the ear, either alone or in combination with other hormonal growth promoters. Although the use of hormones in food-producing animals is banned in the European Union, it is allowed in Canada and the United States. Naturally occurring hormones such as estradiol are regulated on the basis of 1% of the daily endogenous production by the most sensitive segment of the population (*5*).

As most animal-derived foods are cooked prior to consumption, the sensitivity of these chemicals to heat and their behavior in cooked matrices is important in determining consumers' exposure. The heat stability of these compounds was examined in several "model matrices", which were simplified systems that allowed rapid determination of heat stability without extensive extraction and cleanup procedures. The model matrices attempted to mimic conditions typically found during cooking and included vegetable oil and aqueous solutions. The test chemicals were then examined in more complex matrices by being incorporated into regular and extra lean ground beef, which was formed into patties and fried.

MATERIALS AND METHODS

Chemicals. Unlabeled α-estradiol (α-E2), *β*-estradiol (*β*-E2), estrone (E1), 2-hydroxyestrone (2-OH E1), 4-hydroxyestrone (4-OH E1), 2-hydroxyestradiol (2-OH E2), and 4-hydroxyestradiol (4-OH E2) were obtained from Sigma (Oakville, ON, Canada). Deuterated *β*-estradiol (*β*-E2-D$_4$), estrone (E1-D$_4$), 2-hydroxyestrone (2-OH E1-D$_4$), 4-hydroxyestradiol (4-OH E2-D$_5$), equilin (EQ-D$_4$), and dihydroequilin (DHEQ-D$_4$) were obtained from C/D/N Isotopes Inc. (Pointe-Claire, QC, Canada). The unlabeled chemicals were used as test chemicals, and the deuterated chemicals were used during sample preparation to monitor test chemical recovery and instrument performance. Acetonitrile (ACN), ethanol, ethyl acetate (EtOAc), hexane, methanol (MeOH), and water were OmniSolv grade (EMD Chemicals, Darmstadt, Germany) and used without further purification. Concentrated ammonium hydroxide was obtained from J. T. Baker Chemicals (Phillipsburg, NJ); sodium acetate and ascorbic acid were obtained from Sigma-Aldrich (Oakville, ON, Canada).

*Author to whom correspondence should be addressed. E-mail eric. braekevelt@hc-sc.gc.ca.
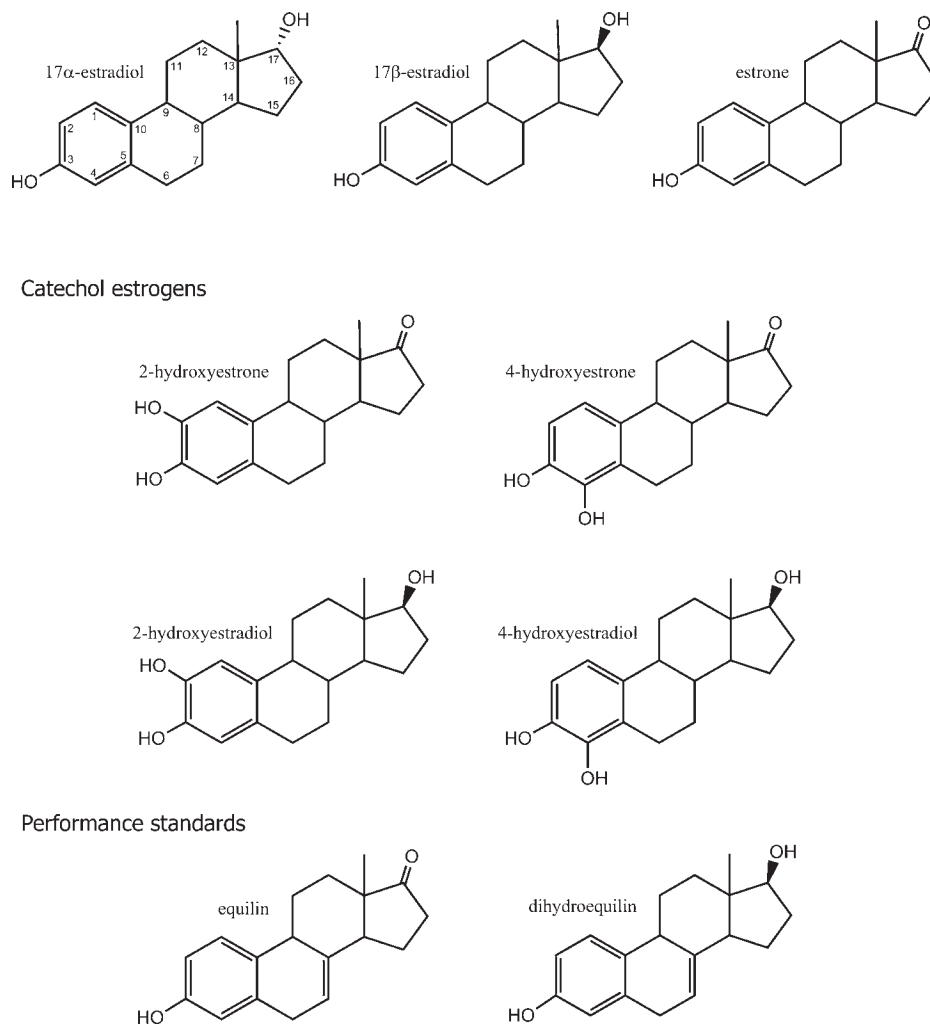
Figure 1. Structures of the test chemicals and internal standards.

Model Matrices. The model matrices included 0.1 M acetate buffer (pH 5.0), 5% ethanol in water, and vegetable oil. The aqueous solutions (20 mL/vial) were dispensed into a number of 20 mL glass scintillation vials and spiked with a mixture of the test chemicals at a concentration of 100 ng/g. The vials were sealed with foil-lined screw-top caps and immersed in a beaker of boiling water (100 °C). Triplicate vials were removed at measured time intervals of up to 120 min, cooled rapidly by immersion in an ice bath, then immediately extracted and analyzed according to methods described below. Controls of unheated solution were kept in the ice bath throughout the experiment and extracted and analyzed at the end of the experiment. Vegetable (canola) oil was dispensed into screw-top glass test tubes (10 mL/tube) and spiked with the test chemicals at a concentration of 100 ng/g. Test tubes were sealed with Teflon-lined screw caps and placed in a beaker of heated vegetable oil (160−180 °C). Triplicate test tubes were removed at measured time intervals, cooled rapidly, extracted, and analyzed together with controls of unheated solution. The temperatures of the oil and water heating baths were monitored with a mercury-in-glass thermometer throughout the experiments.

Beef Tissue. Samples of regular (approximately 25% fat) and extra lean (<10% fat) ground beef were obtained at retail stores in Ottawa, Canada. The test chemicals were added to each tissue at a concentration of 100 ng/g of meat as it was homogenized in a stand mixer. Patties of 100 g and approximately 1.5 cm thickness were formed with a glass Petri dish cover and then wrapped in foil and frozen at −20 °C until use.

Two experiments for each type of ground beef were conducted approximately 40 days apart. The meat was fortified with the test chemicals at day 0. The first cooking experiment took place the day after fortification (day 1), and the second experiment was conducted on day 40. Frozen patties were allowed to warm to room temperature before being cooked. Four beef patties were used for each cooking experiment: two patties were cooked, and the other two were not cooked. Each patty to be cooked was placed in a tared aluminum weighing dish and cooked uncovered for 7 min per side in an electric frying pan at a setting of 177 °C. Patties were removed from heat and the internal temperature was immediately recorded with a digital probe (0.1 °C resolution): the average internal temperature was 71.8 ± 0.9 °C. The patties were then allowed to cool and were reweighed. Cooked patties were removed from their weighing dishes, and the dishes were reweighed to determine the weight of the collected juices. Both the cooked and uncooked patties were broken by hand into small pieces, and 1.0 g samples were taken in triplicate from each patty for analysis.

Sample Extraction and Cleanup. Cooled samples from the model matrix experiments were spiked with 100 ng of each of a mixture of deuterated recovery standards (β-E2-D$_4$, E1-D$_4$, 2-OH E1-D$_4$, and 4-OH E2-D$_5$). Each scintillation vial from the aqueous experiments was emptied into a 6 mL LC-18 solid phase extraction (SPE) cartridge (Supelco Canada Ltd., Oakville, ON, Canada), which had been preconditioned with 5 mL of MeOH followed by 5 mL of 20% MeOH in 0.1% ascorbic acid solution. The cartridge was rinsed with 5 mL of 20% MeOH in 0.1% ascorbic acid solution, allowed to dry on the vacuum manifold for approximately 5 min, and then eluted with 5 mL of MeOH. Vegetable oil samples were extracted with 5 mL of MeOH followed by 5 mL of ACN. The combined extracts were defatted with hexane, evaporated to dryness under N$_2$ in a water bath held at 60 °C, reconstituted in 5 mL of 20% MeOH in 0.1% ascorbic acid solution, and cleaned up on C18 cartridges as described above.

Ground beef samples (1.0 g) were spiked with 100 ng of each of the deuterated recovery standards and ground with 2.0 g of silica. The homogenized mixture was transferred to a 50 mL centrifuge tube, and then 10 mL of 0.1 M acetate buffer with 0.1% ascorbic acid (pH 5.0), 10 mL of

Article

J. Agric. Food Chem., Vol. 59, No. 3, 2011 **917**

Table 1. MRM Parameters of the Test Chemicals and Standards

| compound | type | MRM transitions | | cone voltage (V) | collision energy (eV) |
		precursor ion	product ions		
E1	test chemical	269	143, 145	60	50, 35
E1-D$_4$	recovery std	273	145, 147	60	50, 35
α-E2	test chemical	271	145, 183, 239	50	45, 45, 40
β-E2	test chemical	271	145, 183, 239	50	45, 45, 40
β-E2-D$_4$	recovery std	275	147, 187, 243	50	45, 45, 40
2-OH E1	test chemical	285	159, 161, 175	60	40, 40, 40
2-OH E1-D$_4$	recovery std	289	163, 177	60	40, 40
4-OH E1	test chemical	285	159, 161, 175	60	40, 40, 40
2-OH E2	test chemical	287	147, 161, 199, 255, 269	60	45, 45, 40, 40, 35
4-OH E2	test chemical	287	147, 161, 199, 255, 269	60	45, 45, 40, 40, 35
4-OH E2-D$_5$	recovery std	292	163, 260	60	40, 45
EQ-D$_4$	performance std	271	117, 145	50	47, 35
			227, 241	55	30, 30
DHEQ-D$_4$	performance std	273	145, 183, 213	55	42, 42, 35

ACN, and 5 mL of hexane were added. The tube was vigorously shaken (~150 rpm) on a mechanical shaker for 10 min and centrifuged at 6000 rpm for 10 min, and the hexane layer (containing neutral lipids) was removed and discarded. The mixture was defatted again with 5 mL of hexane, then extracted twice with 5 mL of EtOAc after the pH of the aqueous phase had been increased with 200 μL of concentrated ammonium hydroxide. EtOAc extracts were transferred to a new centrifuge tube and evaporated to dryness. Extracts were reconstituted in 5 mL of 20% MeOH in 0.1% ascorbic acid solution and applied to a C18 cartridge (preconditioned with 5 mL of MeOH followed by 5 mL of 20% MeOH in 0.1% ascorbic acid solution). The cartridge was rinsed with 5 mL of 20% MeOH in 0.1% ascorbic acid solution, allowed to dry on the vacuum manifold for approximately 5 min, and then eluted with 5 mL of MeOH. The eluate was evaporated to dryness, reconstituted in 2 mL of 4:1 EtOAc/MeOH (v/v), and applied to a 3 mL LC-NH2 cartridge (Supelco Canada Ltd.) that had been preconditioned with 3 mL of EtOAc and 3 mL of 4:1 EtOAc/MeOH. The eluate and an additional cartridge rinse of 3 mL of 4:1 EtOAc/MeOH were collected.

All samples were evaporated to dryness and reconstituted in 350 μL of 20% MeOH in 0.1% ascorbic acid solution. The performance standards EQ-D$_4$ and DHEQ-D$_4$ (100 ng/mL) were added to all samples at reconstitution to monitor matrix effects (e.g., suppression or enhancement of ionization in the MS source) Samples were then sonicated for 10 min and transferred to vials for LC-MS/MS analysis.

LC-MS/MS Analysis. Samples were analyzed on an Agilent 1100 LC system (Agilent Technologies, Canada), coupled to a Quattro Ultima triple-quadrupole mass spectrometer (Waters Corp., Manchester, U.K.). Injections of 20 μL were chromatographed on a Waters XBridge C18 column (2.1 mm × 150 mm; 3.5 μm). The column temperature was held at 10 °C. Mobile phase A was 100% H$_2$O, and mobile phase B was 2:1 (v/v) ACN/MeOH. The mobile phase gradient was from 35% B at 0.150 mL/min for 1 min, to 55% B over 2 min, held for 8 min, to 80% B over 4 min, held for 3 min at 0.175 mL/min, then to 35% B over 0.5 min, and held for 4 min. Flow was then reduced to 0.150 mL/min with 35% B and held for 5.5 min to re-equilibrate the column.

The MS was operated with electrospray ionization in negative ion detection mode, with a capillary voltage of 3.0 kV and an ion source temperature of 130 °C. The desolvation gas (N$_2$) temperature was 350 °C; flow was 500 L/h. The collision gas was argon at a pressure of 2.26×10^{-3} mbar. The resolution of both MS1 and MS2 was 0.5 amu at half-height (< 1.4 amu at base). Detection was performed in multiple reaction monitoring (MRM) mode; MRM parameters are presented in **Table 1**.

Data Analysis and Method Performance. Data analysis was performed using the Masslynx 4.1 Datasystem (Waters, Manchester, U.K.) on the LC-MS/MS system. At least two MRM transitions were monitored for each compound. The most abundant MRM transition was used for compound quantitation. Detection was confirmed when (a) the chemical retention time was within 2.5% of its retention time from the calibration standard; (b) the peak area ratios of the secondary MRM transition relative to the primary transition were within 20% of the average value obtained from the standards; and (c) the instrument detection limit (LOD)

Table 2. Recoveries of Test Chemicals (Relative to Deuterated Recovery Standards) from Fortified Matrices (Mean ±1 SD)

| test chemical | recovery standard | aqueous (*n* = 9) | vegetable oil (*n* = 6) | ground beef (*n* = 6) | |
				uncooked	cooked
E1	E1-D$_4$	80 ± 22	79 ± 44	94 ± 9	103 ± 4
α-E2	β-E2-D$_4$	110 ± 7	45 ± 22	93 ± 18	86 ± 8
β-E2	β-E2-D$_4$	93 ± 7	59 ± 9	105 ± 9	115 ± 7
2-OH E1	2-OH E1-D$_4$	96 ± 5	87 ± 2	73 ± 16	98 ± 5
4-OH E1	2-OH E1-D$_4$	105 ± 5	75 ± 1	65 ± 12	89 ± 16
2-OH E2	4-OH E2-D$_5$	94 ± 5	134 ± 4	76 ± 9	102 ± 11
4-OH E2	4-OH E2-D$_5$	92 ± 3	97 ± 5	78 ± 12	98 ± 4
overall		96 ± 15	75 ± 30	84 ± 18	99 ± 12

was exceeded. The LOD is the lowest detectable concentration at which the signal-to-noise ratio (S/N, based on peak-to-peak noise) of the analyte peak in the MRM chromatogram of the quantitation transition was better than 3:1 and was estimated by extrapolating the response of the analyte at the lowest calibration concentration to the level equivalent to S/N = 3. The quantitation limit (LOQ) was defined as 3 × LOD. Test chemicals were quantified using a 7-point calibration curve (1/3X weighted linear regression; concentration range from 1.0 to 200 ng/mL).

A number of deuterated recovery standards were added before the extraction step to monitor extraction efficiency. Test chemical concentrations were corrected for recovery using the closest structurally similar deuterated analogue (**Table 2**). Overall recovery of the test chemicals from the aqueous model matrices relative to the recovery standards was 96 ± 15% (**Table 2**). The extraction and cleanup procedure was not optimized for lipid-rich matrices, which was reflected in lower and more variable analyte recovery from vegetable oil (75 ± 30%). Overall relative recovery of the test chemicals in cooked ground beef was 99 ± 12%. In uncooked beef, recoveries of the catechol estrogens (75 ± 13%) were significantly lower than recoveries of E1, α-E2, and β-E2 (97 ± 14%).

RESULTS

Model Matrices. E1, α-E2, and β-E2 were stable in aqueous solutions at 100 °C, but the catechol estrogens were not stable in solutions without antioxidant (**Figure 2A**). The latter compounds exhibited first-order decay curves, from which half-lives were calculated. The 4-OH catechol estrogens exhibited higher stability than the 2-OH catechol estrogens (half-lives of < 2 min for the 2-OH catechol estrogens vs > 10 min for the 4-OH catechol estrogens). The stability of all the catechol estrogens improved to the same level as the other test compounds when an antioxidant (0.1% ascorbic acid) was added to the solution (**Figure 2B**).

In heated vegetable oil, E1, α-E2, and β-E2 were stable over the 2 h duration of the experiments (**Figure 3A**). Catechol estrogen
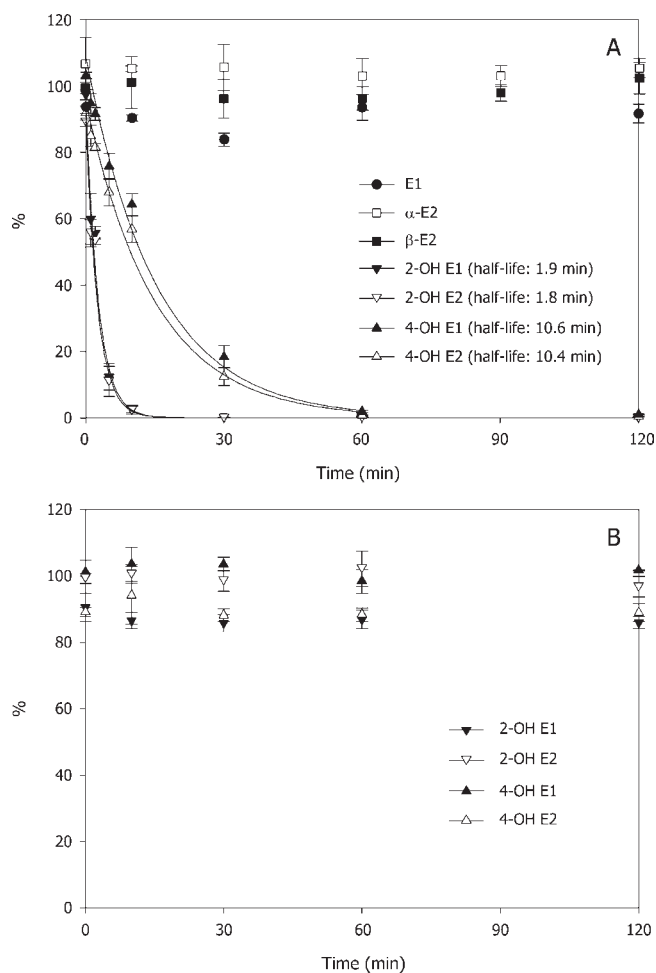
Figure 2. Stability of test chemicals in heated aqueous solutions: (**A**) 0.1 M acetate buffer (pH 5.0, no antioxidant); (**B**) 0.1 M acetate buffer (pH 5.0), 0.1% ascorbic acid added as antioxidant. Each data point represents the average of three replicates; error bars are ±1 SD.

concentrations at the beginning of the experiments were significantly lower than those at the end (Student's *t* test, $p < 0.01$), suggesting that some degradation occurred (**Figure 3B**). The 4-OH catechol estrogens were significantly less stable than the 2-OH catechol estrogens ($p < 0.001$), decreasing by approximately 50% over the duration of the experiments compared to 25−35% for the 2-OH catechol estrogens. Half-lives were not calculated because the chemicals did not exhibit first-order decay. The 4-OH-substituted compounds were the most stable of the catechol estrogens in the aqueous model matrices, whereas the 2-OH compounds were the more stable chemicals in vegetable oil.

Beef Tissue. Triplicate analyses of regular and lean ground beef yielded an average test chemical relative standard deviation (RSD) within patties of $7.0 \pm 3.6\%$ ($n = 32$), indicating that the mixing process homogenously distributed the test chemicals within the ground beef. Although experiments from day 40 had similar within-patty variability (RSD = $5.3 \pm 3.5\%$), there were sometimes statistically significant differences in test chemical concentrations between day 1 and day 40 experiments. This was not consistently observed for any particular chemical, nor were day 40 experiments consistently lower than day 1 experiments. Results were normalized to the average uncooked patty concentration for a particular experiment to account for test chemical losses during patty storage and to allow direct comparison of day 1 and day 40 experiments.
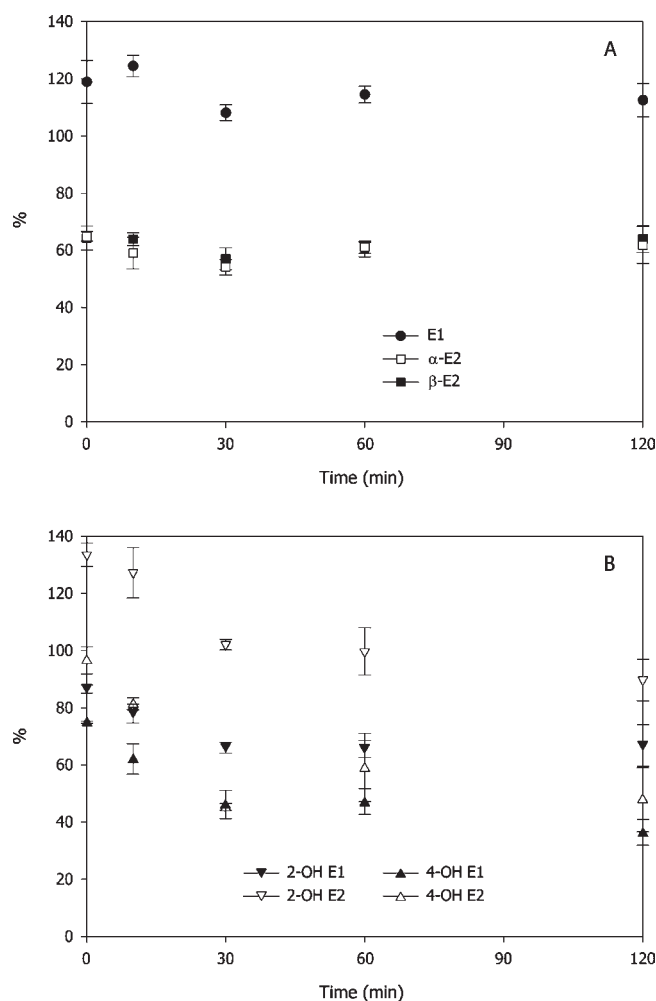

Figure 3. Stability of test chemicals in heated canola oil (160 °C): (**A**) E1, α-E2, and β-E2; (**B**) catechol estrogens. Each data point represents the average of three replicates; error bars are ±1 SD.

Table 3. Average Weights (± Standard Deviations) of Beef Patties and Juices in the Cooking Experiments (Grams, Mean ±1 SD, *n* = 2)

	uncooked patty	cooked patty	juices
regular ground beef			
experiment 1 (day 1)	100.0 ± 1.0	69.1 ± 0.7	7.1 ± 1.6
experiment 2 (day 40)	99.8 ± 0.0	67.7 ± 0.1	7.2 ± 2.2
extra lean ground beef			
experiment 1 (day 1)	98.7 ± 0.0	68.9 ± 1.1	2.8 ± 0.2
experiment 2 (day 40)	97.8 ± 0.7	62.1 ± 1.8	6.3 ± 0.3

Mass loss after cooking (approximately 30−35%) was similar for both regular and extra lean ground beef patties (**Table 3**). To compensate for ground beef mass losses during cooking, concentrations (ng/g) in both cooked and uncooked patties were converted to chemical totals (ng) by multiplying by patty weight. The results from the day 1 and day 40 experiments were very similar, and the results presented in **Figure 4** are an average of the two days.

In regular ground beef, total losses from the patty after cooking were 25−30% for most test chemicals (**Figure 4**). Differences in chemical amounts between cooked and uncooked patties were statistically significant for all test chemicals ($p < 0.001$). The 4-OH catechol estrogens exhibited statistically greater ($p < 0.001$) losses than the other chemicals, with average losses of 35−40%. The apparent difference in chemical stability between the 2-OH and 4-OH catechol estrogens is the same as that
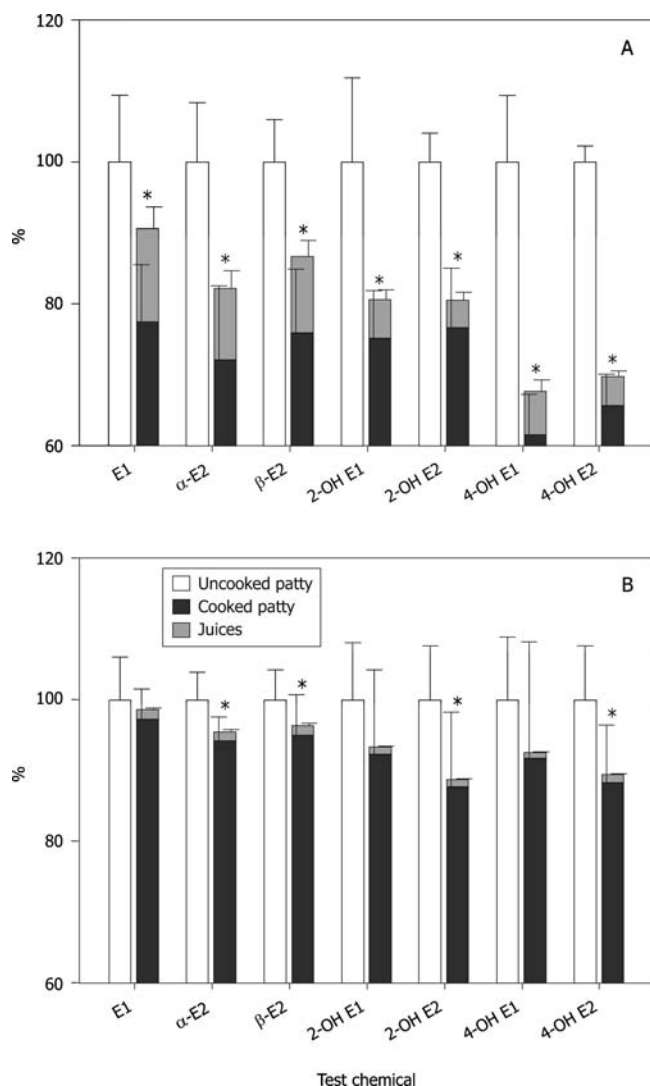
Article

J. Agric. Food Chem., Vol. 59, No. 3, 2011 **919**

Figure 4. Stability of test chemicals in ground beef (uncooked patty normalized to 100%): (**A**) regular ground beef; (**B**) extra lean ground beef. Each bar represents the average of duplicate patties for two experiments performed 40 days apart; error bars are ±1 SD, and asterisks indicate a statistically significant difference between cooked and uncooked patties ($p < 0.05$).

observed in heated vegetable oil, but in the aqueous model matrices the 4-OH catechol estrogens were more stable than the 2-OH catechol estrogens.

In the extra lean ground beef, chemical losses were much lower than in regular ground beef (5–20 vs 25–30%). Differences in chemical amounts between cooked and uncooked patties were statistically significant ($p < 0.05$) for α-E2, β-E2, 2-OH E2, and 4-OH E2. The catechol estrogens exhibited similar losses as the other chemicals, suggesting similar stability. There was no statistically significant difference in stability observed between 2-OH and 4-OH catechol estrogens.

For regular ground beef, 8.0% (±1.6%) of the lost mass was recovered in the collected juices, whereas 4.5% (±2.0%) was recovered for lean ground beef. The difference in the mass of recovered juices between the two types of meat was statistically significant ($p < 0.01$). Approximately 10–20% of the total amount of E1, α-E2, and β-E2 was found in the regular ground beef patty juices, whereas only about 5% of the catechol estrogens were found in the juices. A similar pattern was also observed in the extra lean beef, although the total chemical amounts in the

juices were lower (E1, α-E2, and β-E2, 1.6%; catechol estrogens, 1.1%). The differences in concentration between the catechol estrogens and the other test chemicals in the juices were statistically significant ($p < 0.05$) in all experiments.

DISCUSSION

Model Matrices. E1, α-E2, and β-E2 were stable in both the aqueous and vegetable oil model matrices throughout the duration of the experiments. In contrast, the catechol estrogens were not stable: these compounds underwent decreases of 50% within a few minutes in antioxidant-free aqueous solutions and in several hours in vegetable oil (**Figures 2** and **3**). The catechol estrogens are known to rapidly oxidize to quinones, and antioxidants are often added to laboratory solutions to minimize losses of these compounds during sample preparation and analysis (6). The addition of 0.1% ascorbic acid to the aqueous test systems resulted in an increase in catechol estrogen stability to the same level as the other test chemicals, suggesting that degradation of the catechol estrogens was due to oxidation rather than thermal degradation. Indeed, these compounds were more stable in heated vegetable oil than the antioxidant-free aqueous solutions despite the higher temperature of the oil. Degradation rates are usually related to maximum temperature and duration of exposure at that temperature (7), but the nature of the sample matrix also appears to affect chemical stability. The effect of adding an antioxidant to the vegetable oil was not examined because catechol estrogen degradation rates were quite slow over the short time period generally required to cook foods in hot oil. Interestingly, t he 4-OH-substituted compounds were the most stable of the catechol estrogens in the aqueous model matrices, whereas the 2-OH compounds were the more stable chemicals in vegetable oil.

Beef Tissue. Cooking reduces the amounts of E1, E2, and catechol estrogens in ground beef. The reduction appears to be related to the fat content of the beef, with greater losses occurring in fattier samples. Uncooked regular ground beef contained about 25% fat (measured gravimetrically after cyclohexane–isopropanol extraction (8)), and the juices collected from cooked regular ground beef were predominantly (∼75%) fat. In contrast, the extra lean beef was about 4% fat, and the juices collected from cooking were only 2% fat. The greater test chemical losses observed in the regular ground beef patties may have been due to their association with the fats, which liquefied during cooking and were easily lost from the patties. The 30–35% difference in mass between the uncooked patties and the cooked patties (including juices) was presumably evaporated water.

The catechol estrogens behaved similarly in both regular and extra lean ground beef. There was no significant difference in losses between the 2-OH catechol estrogens and E1, α-E2, and β-E2 in both types of ground beef. The 4-OH catechol estrogens exhibited statistically greater ($p < 0.001$) losses than the other chemicals in regular ground beef. This was was similar to their behavior in vegetable oil and may be a result of the high fat content of the regular ground beef. However, all of the test chemicals including the catechol estrogens exhibited similar stability in the extra lean ground beef. Although some differences were statistically significant, maximum losses were <20%. The beef matrix appears to impart some stability to the more labile compounds, either by shielding them from high cooking temperatures or by the presence of matrix components with antioxidant properties.

A consistently greater proportion of E1 and E2 was found in the juices compared to the catechol estrogens. This difference may

be due to differences in polarity between the two compound groups. Losses of persistent organic pollutants (POPs) from salmon have been strongly correlated with lipid loss from the cooked tissue (*9*). This was attributed to the affinity of POPs for lipid phases, which are subsequently lost from tissue during cooking. The more hydrophobic compounds such as E1 and E2 may be similarly associated with the lipid phase. In contrast, the more polar catechol estrogens may instead associate with proteins and other cellular components and remain within the cooked tissue. The relative decrease in the catechol estrogens in the juices may also be a result of chemical degradation from the more intense heating that the juices were subjected to compared to the bulk of the beef patty, although as demonstrated in the vegetable oil experiments, these chemicals appear to be relatively stable in fatty matrices for short time periods, even at elevated temperatures.

There is some evidence of degradation of the catechol estrogens before cooking, as their recoveries in raw meat were significantly lower than those of the other test chemicals (**Table 2**). This may be due to storage conditions or the presence of oxidizing agents in the uncooked meat. It appears that either freezing the patties slows the oxidation process or this reaction was complete before the patties were frozen, as catechol estrogen concentrations in uncooked patties were similar between day 1 and day 40 experiments. Catechol estrogen recoveries were greater in cooked ground beef (**Table 2**), suggesting that oxidizing agents that may have been present in uncooked ground beef were removed by cooking.

The results presented here are generally consistent with research done on other veterinary drugs. Chemicals that were stable in heated aqueous solutions were generally also stable in cooked food matrices (*10−15*). Prolonged cooking at high temperature (such as deep frying) may be more likely to inactivate chemicals, but grilling or roasting meat even to "well done" generally does not achieve temperatures above 80 °C (*16*), consistent with the average internal temperature of 72 °C observed in this study.

Because the beef cooking experiments presented here were conducted with fortified rather than incurred tissues, some aspects of estrogen metabolism, such as the formation of glucuronides and other conjugates, were not addressed. Cooking may disrupt the equilibrium between a parent compound and its metabolites, which may free a pool of bound chemical or result in its redistribution in different compartments (*17*). However, relatively nonpolar compounds such as those examined in this study (log K_{OW} > 3) partition primarily into neutral lipids (*9*), and this physical process appears to be independent of the mode of administration. For instance, the nonpolar veterinary drug levamisole (log K_{OW} = 2.9) behaved similarly in fortified and incurred tissues (*14*). Experiments using fortified tissues can nonetheless provide information on chemical behavior that can then be used to guide the design of a more resource-intensive incurred study.

In summary, cooking reduces but does not eliminate the potential for dietary exposure to estradiol and its metabolites in ground beef. With the exception of the 4-OH catechol estrogens in regular ground beef, total losses were < 20%. A greater proportion of the test chemicals was found in the collected juices of the fattier beef samples. Exposure to these chemicals could be reduced slightly if the juices are not consumed with the meat,

but in all cases, at least 60% of the introduced chemical remained in the tissue after cooking.

LITERATURE CITED

(1) Hoogenboom, L. A. P.; de Haan, L.; Hooijerink, D.; Bor, G.; Murk, A. J.; Brouwer, A. Estrogenic activity of estradiol and its metabolites in the ER-CALUX assay with human T47D breast cells. *APMIS* **2001**, *109*, 101−107.

(2) Cavalieri, E. L.; Stack, D. E.; Devanesan, P. D.; Todorovic, R.; Dwivedy, I.; Higginbotham, S.; Johansson, S. L.; Patil, K. D.; Gross, M. L.; Gooden, J. K.; Ramanathan, R.; Cerny, R. L.; Rogan, E. G. Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10937−10942.

(3) Liehr, J. G.; Fang, W.-F.; Sirbasku, D. A.; Ari-Ulubelen, A. Carcinogenicity of catechol estrogens in Syrian hamsters. *J. Steroid Biochem.* **1986**, *24*, 353−356.

(4) Maume, D.; Deceuninck, Y.; Pouponneau, K.; Paris, A.; le Bizec, B.; André, F. Assessment of estradiol and its metabolites in meat. *APMIS* **2001**, *109*, 32−38.

(5) U.S. Food and Drug Administration. Guidance for industry. *General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals*; U.S. Food and Drug Administration: Washington, DC, 2006.

(6) Devanesan, P.; Todorovic, R.; Zhao, J.; Gross, M. L.; Rogan, E. G.; Cavalieri, E. L. Catechol estrogen conjugates and DNA adducts in the kidney of male Syrian golden hamsters treated with 4-hydroxyestradiol: potential biomarkers for estrogen-initiated cancer. *Carcinogenesis* **2001**, *22*, 489−497.

(7) Ibrahim, A.; Moats, W. A. Effect of cooking procedures on oxytetracycline residues in lamb muscle. *J. Agric. Food Chem.* **1994**, *42*, 2561−2563.

(8) Smedes, F. Determination of total lipid using non-chlorinated solvents. *Analyst* **1999**, *124*, 1711−1718.

(9) Bayen, S.; Barlow, P.; Lee, H. K.; Obbard, J. P. Effect of cooking on the loss of persistent organic pollutants from salmon. *J. Toxicol. Environ. Health A* **2005**, *68*, 253−265.

(10) McCracken, R. J.; Kennedy, D. G. The bioavailability of residues of the furazolidone metabolite 3-amino-2-oxazolidinone in porcine tissues and the effect of cooking upon residue concentrations. *Food Addit. Contam.* **1997**, *14*, 507−513.

(11) Rose, M. D.; Farrington, W. H. H.; Shearer, G. The effect of cooking on veterinary drug residues in food: 3. sulphamethazine (sulphadimidine). *Food Addit. Contam.* **1995**, *12*, 739−750.

(12) Rose, M. D.; Bygrave, J.; Sharman, M. The effect of cooking on veterinary drug residues in food. Part 9. Nitroimidazoles. *Analyst* **1999**, *124*, 289−294.

(13) Rose, M. D.; Shearer, G.; Farrington, W. H. H. The effect of cooking on veterinary drug residues in food: 1. Clenbuterol. *Food Addit. Contam.* **1995**, *12*, 67−76.

(14) Rose, M. D.; Argent, L. C.; Shearer, G.; Farrington, W. H. H. The effect of cooking on veterinary drug residues in food: 2. Levamisole. *Food Addit. Contam.* **1995**, *12*, 185−194.

(15) Rose, M. D.; Farrington, W. H. H.; Shearer, G. The effect of cooking on veterinary drug residues in food: 7. Ivermectin. *Food Addit. Contam.* **1998**, *15*, 157−161.

(16) Moats, W. A. Inactivation of antibiotics by heating in foods and other substrates − a review. *J. Food Prot.* **1988**, *51*, 491−497.

(17) Rose, M. D.; Shearer, G.; Farrington, W. H. H. The effect of cooking on veterinary drug residues in food: 5. Oxfendazole. *Food Addit. Contam.* **1997**, *14*, 15−26.