In Vitro Binding of Benzo[*a*]pyrene by Extruded Potato Peels

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The effects of extrusion conditions on binding of the dietary carcinogen benzo[*a*]pyrene [B(a)P] were determined using an *in vitro* digestion model followed by HPLC with fluorescence detection. Extrusion at 110 °C and 30% feed moisture resulted in significantly less binding than did nonextruded peels (77% vs 84%), but other conditions did not affect binding. Peels bound more B(a)P than did wheat bran, cellulose, or arabinogalactan. B(a)P dosage was inversely related to the percentage bound. A model system containing cellulose with chlorogenic acid bound all B(a)P added, compared with only 66% by cellulose alone and 32% by a cellulose–quercetin mixture.

Keywords: *Benzo[a]pyrene; extrusion; dietary fiber; potato peels; phenolics*

INTRODUCTION

Potato peels, a byproduct of potato processing such as French fry and potato granule manufacture, may be used as a source of concentrated dietary fiber since they contain about 50% dietary fiber. *In vitro* binding of bile acids by peels has been studied to provide an estimate of their ability to reduce serum cholesterol when ingested (Camire et al., 1993).

Foods containing dietary fiber may also prevent cancer. Consumption of fiber-rich fruits, vegetables, and carbohydrates is associated with reduced incidence of colorectal adenomas (Sandler et al., 1993). Recent reviews (Klurfeld, 1992; Harris and Ferguson, 1993) have summarized possible mechanisms by which fiber-rich foods reduce the risk of cancer (Table 1). Animal feeding studies and *in vitro* assays have generally indicated a protective effect of dietary fiber against 1,2-dimethylhydrazine (Smith-Barbaro et al., 1981), heterocyclic amines (Kada et al., 1984; Sjodin et al., 1985; Mortomi and Mutai, 1986; Nishiyama et al., 1991) and polycyclic aromatic hydrocarbons (PAH) such as benzo[*a*]pyrene (Mirvish et al., 1981; Clinton and Visek, 1989). The predominant methods for evaluating the effectiveness of fiber *in vitro* have been mutagenicity as determined by the Ames assay of solutions after treatment with fiber or the detection of residual ^{14}C-labeled chemicals in the incubation solution.

Benzo[*a*]pyrene, also known as 3,4-benzopyrene or B(a)P, is a known mutagen and carcinogen which is widely distributed in the human environment (Adrian et al., 1984). Industrial combustion of organic matter and food processes such as smoking, frying, roasting, and broiling are major sources of B(a)P. B(a)P is readily oxidized by ozone, nitrogen oxides, sulfur oxides, and ultraviolet light.

The occurrence of B(a)P in drinking water and different foods such as fats, cereal products, roasted peanuts, heated oils, and smoke-cured products has been extensively reviewed and studied (Adrian et al., 1984; Kruijf et al., 1987). Levels of B(a)P in foods could range from a few to several hundred parts per billion. The daily total food and beverage intake of B(a)P by humans is estimated to vary from 0.25 to 2.5 μg (Dennis et al., 1983). Extrusion cooking is one form of thermal processing that does not appear to increase B(a)P and other PAHs in foods (Cripps and Dennis, 1984).

Table 1. Proposed Mechanisms for Inhibition of Mutagenesis and Carcinogenesis by Dietary Fiber[a]

1. binding of mutagens/carcinogens by dietary fiber constituents
2. increased water absorption → increased fecal bulk → reduced intestinal transit time
3. reduced pH due to bacterial fermentation of soluble fiber to short-chain fatty acids

[a] Adapted from Harris and Ferguson (1993).

Analysis of B(a)P has been troublesome due to the low concentrations (ppb) of B(a)P in foods and the light- and oxygen-sensitivity of B(a)P. Several researchers have used high-performance liquid chromatography (HPLC) with a fluorescence detector for high sensitivity and a reversed-phase column for good resolution (Tamakawa et al., 1992; Simko et al., 1993; Riha et al., 1992). Tamakawa et al. (1992) suggested extraction by *n*-hexane, cleanup with a Sep-Pak cartridge, and use of sodium sulfide (Na_2S) as an antioxidant, while other researchers used different extraction procedures and HPLC conditions.

The objectives of this study were to determine the *in vitro* binding of B(a)P by potato peels and the effects of extrusion cooking conditions on B(a)P binding using a modified HPLC procedure.

MATERIALS AND METHODS

Reagents. All solvents used throughout the experiment were of HPLC grade except cyclohexane (pesticide grade) (Fisher Scientific Co., Fair Lawn, NJ). Distilled water was used for incubation and extraction and HPLC-quality water for HPLC analysis. Hard red standard wheat bran was purchased from the American Association of Cereal Chemists (AACC) (St. Paul, MN). Cellulose, arabinogalactan, chlorogenic acid, quercetin, and benzo[*a*]pyrene (ca. 98% purity) were purchased from Sigma Chemical Co. (St. Louis, MO). Preparation of potato peels is shown in Figure 1.

B(a)P stock standard solution (approximately 1 mg/mL in acetonitrile) was covered with aluminum foil and stored at 4 °C. Working standard solution was prepared daily by diluting with acetonitrile. The stock solution was stable for at least 1 month. **Caution**: Benzo[*a*]pyrene is a known carcinogen and mutagen. Care should be taken to prevent inhalation and skin contact. B(a)P may be effectively destroyed in waste solutions and glassware with a solution of 0.4 M potassium permanganate in 3 M sulfuric acid (Castegnaro and Sansone, 1986).

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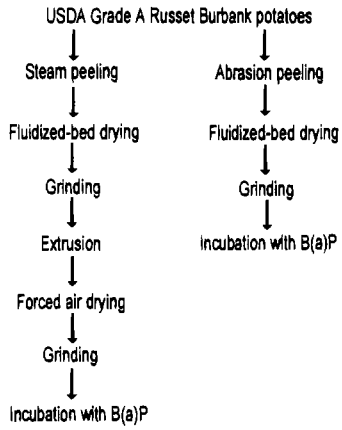

Figure 1. Processing of potato peels prior to incubation with benzo[*a*]pyrene.

Extrusion. Dried, ground potato peels were extruded in a Werner-Pfleiderer (Ramsey, NJ) ZSK-30 twin-screw extruder with heating gradually increased in seven individually controlled zones from the feed end to the die end of the barrel. Barrel temperature profiles were approximately 27–38–54–93–104–110–110 or 27–49–88–110–130–150–150 °C. The experimental design was a 2 × 2 factorial with two final zone barrel temperatures (110 or 150 °C) and two feed moisture levels (30% or 35%). A K-Tron volumetric feeder (K-Tron Corp., Pitman, NJ) was used to maintain feed rate at 11.4 kg/h. Screw speed was 300 rpm. The screw configuration was recommended by the manufacturer for cornmeal; screw specifications have been previously reported (Arora et al., 1993). A single 4 mm die hole was used. Melt temperature (temperature of material within the extruder), torque, and die pressure were read directly from the extruder control panel. Duplicate samples were collected for each set of extrusion conditions. Peels were dried postextrusion at 93 °C to ca. 5% moisture, ground as before but to pass 0.131 mm (no. 80) mesh without raising sample temperature, and stored in Ziploc bags at room temperature.

***In Vitro* Binding.** The *in vitro* binding of B(a)P to extruded steam potato peels, unextruded steam peels, unextruded abrasion peels, AACC wheat bran, cellulose, and arabinogalactan was measured in triplicate. Blanks without added binding substances were carried through the same procedure as samples. All glassware was rinsed with acetone before use and wrapped with aluminum foil to avoid photodegradation.

To each KIMAX 35 mL glass screw-capped centrifuge tube were added 0.100 g of sample and 2 mL of 0.01 N HCl. Each sample then received 20 μL of 1 μg/mL B(a)P standard solution under nitrogen to avoid oxidation. The tubes were capped and incubated in a water bath (Fisher Scientific, Versa-Bath Model 236, Springfield, NJ) at 37 °C for 1 h with shaking at 50 rpm to simulate gastric conditions. Samples were then neutralized with 0.1 N NaOH to pH 7.0. Thirteen milliliters of 10 mg/mL porcine pancreatin (5X USP, ICN Biochemicals, Cleveland, OH) in 0.01 M (pH 7.0) phosphate buffer was added under nitrogen to each tube. Tubes were incubated in the water bath for another hour under the same conditions as before. After incubation, the samples were centrifuged at 2000*g* for 10 min in a Beckman tabletop centrifuge (Model TJ-6R, Palo Alto, CA). The supernatant was removed by pipet for extraction.

Extraction. To a 125 mL glass separatory funnel were added 10 mL of supernatant and 20 mL of cyclohexane and shaken vigorously for 2 min. To break the emulsion, 5 mL of ethanol was added and swirled gently. After the separation of layers, the lower aqueous layer was transferred to another 125 mL separating funnel and extracted with 15 mL of cyclohexane. Both cyclohexane layers were combined and cleaned with 15 mL of water. After the aqueous layer was discarded, the cyclohexane extract was filtered into a 500 mL round-bottom flask over anhydrous sodium sulfate in a glass funnel. The separatory funnel was rinsed twice with 10 mL of cyclohexane, and rinses were also filtered over the sodium sulfate. The combined cyclohexane extract was rotary evaporated (Buchler Instruments, Fort Lee, NJ) to 4 mL and transferred to a 7 mL glass vial. The flask was rinsed twice with 1 mL of cyclohexane, and rinses were combined in the glass vial. The combined concentrate was evaporated to almost dryness under nitrogen and stored at 4 °C.

Table 2. Potato Peel Extrusion Conditions[a]

barrel temp,[b] °C	feed moisture, %	melt temp, °C	torque,[c] %	die pressure, kPa
110	30	126	30	898.3
110	35	122	23	689.5
150	30	150	23	482.6
150	35	146	17	482.6

[a] Average of duplicates. [b] Final barrel temperature. [c] Reading during extrusion minus torque (14%) when barrel was empty at screw speed of 300 rpm.

Table 3. *In Vitro* Binding of Benzo[*a*]pyrene[a]

sample	barrel temp, °C	feed moisture, % (db)	B(a)P bound, % of blank
steam peels	110	30	77.43 ± 4.19 ab
steam peels	110	35	81.10 ± 2.63 abc
steam peels	150	30	82.10 ± 3.71 ac
steam peels	150	35	79.08 ± 2.63 abc
steam peels			83.95 ± 2.45 c
abrasion peels			75.03 ± 0.61 b
wheat bran			67.57 ± 1.46 d
cellulose			61.20 ± 0.62 d
arabinogalactan			13.23 ± 0.85 e

[a] Values shown are mean ± standard deviations ($n = 6$); values followed by the same letter in a column are not significantly different ($p < 0.05$), using Tukey's HSD test.

HPLC Quantification of B(a)P. The residue was dissolved in 1 mL of acetonitrile and sonicated well. The HPLC system consisted of a Waters Millipore Model 510 pump, a VICI injector (Valco Instruments Co. Inc., Houston, TX) with a 10 μL injection loop, a Waters 470 scanning fluorescence detector, and a Hewlett-Packard 3396A integrator. The analysis was performed using a Zorbax C_{18}, 250 × 4.6 mm i.d. column at room temperature. The mobile phase used was 85% acetonitrile in water with a flow rate of 1.5 mL/min. The detector was operated with an excitation wavelength of 381 nm and an emission wavelength of 407 nm. The B(a)P standard solution was 18 ng/mL in acetonitrile and was injected after every three samples. Peak height was used for quantification. Percent of B(a)P binding by samples was calculated according to

$$\frac{\text{B(a)P}_{\text{blank}} - \text{B(a)P}_{\text{sample}}}{\text{B(a)P}_{\text{blank}}} \times 100\% \qquad (1)$$

B(a)P Concentration Curve. The amount of B(a)P added in the first experiment was based on the daily per capita intake reported by Dennis et al. (1983). Various amounts of B(a)P were added to 0.100 g samples of peels extruded at 110 °C and 30% moisture to estimate dose response. To one set of triplicate samples was added 20 μL of acetonitrile instead of B(a)P solution to serve as a zero level and to ascertain whether peels contained any endogenous B(a)P. For each level (0, 250, 2500, 5000, 10000, and 20000 ng/20 μL), triplicate blanks were prepared simultaneously as peel samples. Incubation, extraction, and HPLC analyses were performed as before, but the higher concentrations required dilution prior to HPLC analysis.

Effect of Phenolics. To test whether phenolic compounds as well as fiber participate in B(a)P binding, three sets of samples and blanks in triplicate were assayed: 0.100 g of α-cellulose, 0.099 g of cellulose with 0.0011 g of chlorogenic acid, and 0.099 g of cellulose with 0.0011 g of quercetin.

Peel Color. Color of ground peels was determined with a HunterLab Labscan II spectrocolorimeter (Reston, VA).

Statistical Analysis. The MGLH ANOVA program (SYSTAT, Evanston, IL) was used to evaluate the effects of barrel temperature and feed moisture on the percent of B(a)P binding

Table 4. Benzo[a]pyrene Bound by Potato Peels Treated with Various Amounts[a]

B(a)P added, ng	B(a)P recovered from peels, ng	B(A)P recovered from blanks, ng	% bound	B(A)P bound, ng/g
20000	349.46 ± 179.52	723.34 ± 91.25	51.69 ± 24.82 a	3507.80 ± 1813.94 b
10000	133.30 ± 23.07	226.37 ± 120.61	42.12 ± 10.19 a	1359.67 ± 233.94 a
5000	70.82 ± 28.64	156.52 ± 73.89	54.75 ± 18.30 a	706.46 ± 283.45 a
2500	14.98 ± 2.05	48.43 ± 5.16	69.06 ± 4.23 a	152.73 ± 20.72 a
250	3.63 ± 1.14	9.01 ± 4.76	79.33 ± 6.48 a	37.05 ± 11.58 a
0	ND[b]	ND		0

[a] Means ± standard deviations for triplicate samples of 0.100 g of potato peels extruded at 110 °C and 35% feed moisture and corresponding reagent blanks. Values within columns followed by different letters are significantly different ($p \leq 0.05$, least significant difference test). [b] None detected.

Table 5. Benzo[a]pyrene Binding by Cellulose with or without Added Phenolics[a]

sample	B(a)P recovered, ng	% bound	B(a)P bound, ng/g
cellulose	0.53 ± 0.18 b	66.50 ± 11.20 b	10.50 ± 1.79 b
cellulose + chlorogenic acid	ND[b]	100 c	15.66 c
cellulose + quercetin	1.08 ± 0.08 c	32.00 ± 5.20 a	5.01 ± 0.81 a

[a] Means ± standard deviations for triplicate samples of 0.100 g of cellulose or 0.099 g of cellulose + 0.0011 g of chlorogenic acid or quercetin. Values within columns followed by different letters are significantly different ($p \leq 0.05$, least significant difference test). [b] None detected.

by extruded steam potato peels. Tukey's honest significant difference test was used to compare samples at the 5% level of probability. Relationships between B(a)P binding and color were determined by Pearson's correlation test.

RESULTS AND DISCUSSION

During extrusion at 110 °C, torque and die pressure were higher than at 150 °C (Table 2). Binding of B(a)P by extruded steam potato peels was not significantly affected by barrel temperature, feed moisture, or their interaction. Unextruded steam peels bound more B(a)P than did peels extruded at barrel temperature of 110 °C and feed moisture of 30%, while no significant difference of binding was found with steam potato peels extruded at other conditions (Table 3). Binding of B(a)P by abrasion potato peels was lower than that by steam potato peels. Harris et al. (1991) observed that more 1,8-dinitropyrene was bound by potato skin than by potato flesh. Abrasion peels contain approximately 25% dietary fiber and 50% starch, which may have maintained B(a)P in suspension. Wheat bran and cellulose were not different in binding of B(a)P but were lower than peels. Binding by arabinogalactan, a soluble fiber, was significantly lower than binding by all other materials tested.

Gulliver et al. (1983) showed that about 85% of the polycyclic aromatic hydrocarbon carcinogen 7,12-dimethylbenzo[a]anthracene was bound by both cellulose and lignin during *in vitro* studies, which was similar to our results. Our results also agreed with the *in vitro* studies conducted by Harris et al. (1993), which showed the individual insoluble fibers such as cellulose could bind a hydrophobic carcinogen, whereas soluble fibers such as pectin, gum arabic, and β-glucan inhibited binding. Clinton and Visek (1989) studied the anticarcinogenic effect of wheat bran on B(a)P when tested in rats. Ten percent wheat bran reduced exposure of the intestine to dietary B(a)P as indicated by the inhibition of intestinal B(a)P hydroxylase, and 10% wheat bran was effective in limiting the B(a)P hydroxylase activity when rats were fed charcoal-broiled beef, a natural source of B(a)P in the diet of humans. Cellulose and lignin had no effect on B(a)P *in vivo*. Animal testing is therefore expected to confirm that potato peels reduce colon cancer risk. Potato peels bound lower percentages of high doses of B(a)P (Table 4). Above 5000 ng, about 50% of B(a)P was bound, but 79% binding was found for the 250 ng dose. Only 2–3.5% of added B(a)P was recovered from reagent blanks, and we assume that similar losses occurred in tubes containing peels. Such losses, presumably due to B(a)P oxidation and adsorption to glassware, verify the need for reagent blanks throughout this procedure.

The mechanism for B(a)P binding and/or destruction is not clear. Kitts et al. (1993) studied the interaction between dietary B(a)P and glucose–lysine Maillard reaction products in rats and showed the anticarcinogenic and antimutagenic effects of Maillard reaction products in the gastrointestinal tract. Extruded potato peels had darker colors (lower *L* value) than unextruded peels due to the Maillard reaction products formed during extrusion cooking (Arora et al., 1993). However, in this experiment (data not shown) lightness (*L* value) was not correlated with the binding of B(a)P by potato peels.

Potato peels also contain free phenolics and flavonols which may interact directly with B(a)P or its diol epoxide to minimize mutagenicity (Newmark, 1987). No B(a)P was recovered from the supernatant of a cellulose–chlorogenic acid mixture (Table 5). Chlorogenic acid, the predominant phenolic acid in potatoes, may react with B(a)P to form an insoluble complex. Cellulose and quercetin bound less B(a)P than did cellulose alone. Quercetin may not prevent B(a)P mutagenicity by the same mechanism(s) as chlorogenic acid. Quercetin inhibits cytochrome P-450 oxidases required for B(a)P activation and also may prevent the binding of B(a)P to DNA (Leighton et al., 1992). The interaction of dietary components with phenolic compounds requires further study to minimize exposure of intestinal tissue to B(a)P and other dietary carcinogens. In particular, this study did not account for emulsification by bile salts, which may keep carcinogens in solution (Ferguson et al., 1990).

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