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Inhibitory effects of apple peel polyphenol extract on the formation of heterocyclic amines in pan fried beef patties

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ABSTRACT

The efficacy of polyphenol-rich dried apple peel extract (DAPP) to inhibit the formation of heterocyclic aromatic amines (HCAs) during frying of beef patties was assessed after DAPP was applied at 0.1, 0.15 and 0.3% (w/w) either on the surface of the patties or mixed inside the patty prior to frying. 2-Amino-3,8-dimethylimidazo[4,5f]quinoxaline (MeIQx), 2-amino-1-ethyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,4,8-dimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx) were quantified after frying. HCA concentrations decreased ($p < 0.05$) upon both surface and mixed applications of DAPP at all of the tested doses. Surface application of 0.3% DAPP showed greater ($p < 0.05$) inhibitory effects on HCA formation by 68% for MeIQx, 56% for 4,8-DiMeIQx and 83% for PhIP as opposed to 41%, 21% and 60% respectively, for the mixed DAPP application of 0.3%. The present study results indicate that surface application of DAPP in meat preparation prior to pan-frying can be a useful approach to minimize the formation of genotoxic HCAs in fried beef patties.

1. Introduction

Epidemiological data has indicated that food-derived carcinogens from cooked meat and meat products predispose individuals to gastrointestinal cancer, particularly of the colon, which is the second leading cause of cancer mortality in North America and most industrialized countries (Rohrmann, Hermann, & Linseisen, 2009; Goldman & Shields, 2003). Heterocyclic amines (HCAs) are carcinogenic and/or mutagenic compounds mainly formed in muscle foods, especially meat and fish, via the Maillard reaction with creat-i(ni)ne, amino acids and sugars as the precursors (Kizil, Oz, & Besler, 2011). The major classes of HCAs include amino-imidazo-quinolines, or amino-imidazo-quinoxalines (called IQ-type compounds). 2-Amino-3,8-dimethylimidazo[4,5f]quinoxaline (MeIQx), 2-amino-1-ethyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,4,8-dimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx) are the most abundant of the HCAs formed in grilled beef, bacon, fish and poultry (Knize, Dolbeare, Carroll, Moore, & Felton, 1994; Skog, Johansson, & Jägerstad, 1998; Sander, Linseisen, & Rohrmann, 2010). Although 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (IQx) is less abundantly formed following frying or grilling, it is considered among the most carcinogenic of the HCAs (Moller et al., 2002). It has been reported that there exists a high risk for colorectal cancer for individuals who frequently eat well-done grilled meat that potentially contains elevated levels of HCAs (Sinha et al., 1998). The levels of HCAs formed in meats prepared by common household cooking methods are normally small (0.1–50 ng/g) (Santos et al., 2004; Puangsombat, Gadgil, Houser, Hunt, & Smith, 2012); however, the amounts in meats or poultry that are cooked well-done, or the grilled pan scrapings often used for gravy, can be as high as 500 ppb (Skog et al., 1998).

In view of the risks associated with consuming HCAs, there is a need to reduce exposure by blocking HCA formation such as adding an ingredient during the cooking of meats to prevent their production (Balogh, Gray, Gomaa, & Booren, 2000). Fortification of foods with food-derived antioxidants such as polyphenols has been under active research since free radicals are thought to be involved in HCA formation through the Maillard reaction (Schwab et al., 2000). A large population study has suggested protection against the development of HCA-induced colon cancer by high dietary intake of flavonoids (Rohrmann et al., 2009), which are found in fruits and vegetables along with teas, chocolate and red wine. Both individual phytochemicals and plant extracts have been reported to inhibit the generation of HCAs from meats, which includes flavonoids from black, white and green tea (Dhawan et al., 2002; Quelhas et al., 2010), the flavonoid chrysin (Turesky, Taylor, Schnackenberg, Freeman, & Holland, 2005), citrus flavonoids (Bear & Teel, 2000) as well as a variety of phenolic-rich fruit and vegetable extracts (Edenharder, Sager, Glatt, Muckel, & Platt, 2002; Cheng, Wu, Zheng, & Peng, 2007). Apple is a rich source of flavonoids and the direct mixing of a single dose of a crude apple extract (0.1%) into ground beef patties was shown to inhibit HCA formation caused by frying, which

Abbreviations: DAPP, dried apple peel powder; HCAs, heterocyclic amines.

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was related to the presence of proanthocyanidins, phloridzin and chlorogenic acid (Cheng et al., 2007). It is possible that the inhibition of HCA formation could be further enhanced using dried apple peel powder (DAPP) as peels have markedly higher levels of total antioxidant capacity than either the flesh plus peel or flesh of all the apple varieties examined (Wolfe, Wu, & Liu, 2003).

In the present work, we investigated the use of DAPP in a dose response manner to minimize the production of the three most abundant HCAs, MeIQx, 4,8-MeIQx and PhIP in fried beef patties at a high frying temperature (223 °C). As ingredients have generally been applied upon the surface of meats to prevent HCA production, studies are lacking regarding the direct comparison of the blending of additives within the meat product versus their application on the meat surface in terms of the formation of HCAs after frying. An additional aim was therefore to study the influence of surface versus mixed application of DAPP on HCA formation.

2. Materials and methods

2.1. Materials

The standards IQx, MeIQx, 4,8-DiMeIQx and PhIP and deuterated internal standards of 2-amino-3-trideuteromethylimidazo[4,5-*f*]quinoline (D_3-IQx), 2-amino-8-methyl-3-trideuteromethylimidazo[4,5-*f*]quinoxaline (D_3-MeIQx) and 2-amino-1-trideuteromethyl-6-phenylimidazo[4,5-*b*]pyridine-D3 (D_3-PhIP) were obtained from Toronto Research Chemicals (North York, ON). Ammonium acetate and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO). Deionized water was produced using a Sybron/Branstead PCS unit (Barnstead/Thermolyne, Dubuque, IA). Solid-phase extraction OASIS mixed cation exchange columns (MCX) were purchased from Waters (Bristol, CT). Ethyl acetate, acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Fisher Scientific (Fairlawn, NJ). The DAPP samples were provided by Leahy Orchards Inc. (Franklin, QC). DAPP contains phloridzin quercetin 3-O-glucoside, quercetin 3-O-galactoside, quercetin 3-O-arabinoside, quercetin 3-O-xyloside and quercetin 3-O-rhamnoside as the major polyphenols (Denis et al., 2013). The total phenolic content of DAPP used in the present study was 2.76 g gallic acid equivalent/100 g dry weight.

2.2. Sample preparation and thermal treatment of ground beef patties

Freshly ground beef (advertised as 85% lean beef) was purchased from a local supermarket (Marché Richelieu, Sainte Anne de Bellevue, QC) and used in the frying tests within 1 h of purchase. Samples of 100 g ground beef were used to prepare beef patties with thickness about 1.5 cm and diameter about 10.2 cm using a burger patty maker (Ares, Pointe-Claire, QC). The treatments consisted of 0, 0.1, 0.15 and 0.3% of DAPP (w/w) composed of 100% dried apple peel with a high flavonoid content (Denis et al., 2013). DAPP was either applied to the surface of the ground beef patties or thoroughly mixed and kept at ambient conditions for 30 min prior to frying. Ground beef patties without added DAPP were treated as controls for each experiment. The ground beef patties were pan fried on each side for 10 min at 223 °C using a Cleveland Tilting Skillet Model SET-10 (Burkett Restaurant Equipment and Supplies, OH), preheated to 223 °C (Balogh et al., 2000; Knize et al., 1994). Following frying, the samples were immediately ground using a Robot Coupe Blixer (Robert Coupe, MS).

2.3. Solid phase extraction of heterocyclic amines

The extraction of HCAs (Santos et al., 2004) was performed on 1.5 g beef patty samples placed into 50 mL glass extraction tubes into which were added 50 μL of the trideuterated IQ, MeIQx and PhIP internal standard (IS) mixture (D_3-PhIP, 0.2 μg/mL; D_3-IQ, 0.5 μg/mL; D_3-MeIQx, 0.5 μg/mL methanol). There was no commercially available internal standard for 4,8-MeIQx-D3; therefore, we used the most structurally similar compound available, MeIQx-D3, as the internal standard for 4,8-MeIQx. For the calibration standards, 1.5 mg of "blank" beef (cooked at 223 °C for 5 min on each side) was extracted similarly but with the addition of a 100 μL of a mixture of unlabeled standards of 4,8-DiMeIQx, MeIQx (0.00, 0.005, 0.01, 0.02, 0.05, 0.1 and 0.2 μg/mL methanol) and PhIP (0.0, 0.02, 0.05, 0.1 and 0.2 μg/mL) for the external calibration curve. A 6 mL aliquot of 1 M sodium hydroxide was then added to all beef samples and vortexed for 2 min, which was followed by the addition of 8 mL ethyl acetate. After vortexing for another 2 min, the samples were centrifuged at 748 × *g* for 5 min. After removal of 6 mL of supernatant, an additional 6 mL ethyl acetate was added and the extraction procedure was repeated as described above. The ethyl acetate supernatants containing the extracted HCAs were combined and evaporated to dryness under nitrogen. The dried samples were re-solubilized in 2 mL methanol prior to cleaning and concentration by solid phase extraction (SPE) using mixed mode 1 cc, 30 mg, OASIS MCX cartridges (Waters, MA). The cartridges were preconditioned with 1 mL of methanol and equilibrated with 1 mL of water and the samples were loaded. The cartridges were first rinsed with 1 mL of water containing 2% formic acid, followed by 1 mL of methanol and then the HCAs were eluted twice with 0.75 mL methanol containing 5% ammonium hydroxide into Eppendorf tubes. Eluted samples were dried and stored at −30 °C prior to liquid chromatography-tandem mass spectrometry (LC–MS/MS). Prior to LC–MS/MS analysis, dried samples were reconstituted in 100 μL methanol and filtered using 0.45 μm polypropylene spin filters into LC vials prior to injection of 20 μL into the LC–MS/MS instrument.

2.4. LC–MS/MS analysis

LC–MS/MS analysis was performed on a Shimadzu Nexera UHPLC system (Tokyo, Japan) coupled with a QTRAP 5500 hybrid triple quadrupole-linear ion trap (AB Sciex, Concord, ON, Canada) mass analyzer in positive ion mode. Chromatographic separation was achieved using a Phenomenex Gemini-NX column (100 × 4.6 mm, 5 μm particles) with mobile phases A: 20 mM ammonium acetate, pH 6 and B: acetonitrile. The elution gradient consisted of starting conditions at 5% B held for 1 min, followed by a linear increase to 30% B at 15 min, to 60% B at 18 min and 90% B at 18.5 min and held for an additional 1.5 min at a flow rate of 1 mL/min. Ion source conditions were as follows: ion spray source voltage, 5000 V; curtain gas, 40 psi; temperature, 550 °C, nebulizer/drying gases, 60 psi; declustering potential, 80 V. Each multiple reaction monitoring transition was optimized using standard HCA solutions and the following transitions and optimized parameters were used: IQx *m/z* 200 → 185 with collision energy (CE) of 36 V and *m/z* 200 → 158 (CE 46 V); IQ-D_3 *m/z* 202 → 184 (CE 41 V) and *m/z* 202 → 157 (CE 47 V); MeIQx *m/z* 214 → 199 (CE 34 V) and *m/z* 214 → 131 (CE 51 V); MeIQx-D_3 *m/z* 217 → 199 (CE 34 V) and *m/z* 217 → 131 (CE 51 V); 4,8-DiMeIQx *m/z* 228 → 213 (CE 32 V) and *m/z* 228 → 212 (CE 42 V); PhIP *m/z* 225 → 210 (CE 39 V) and *m/z* 225 → 140 (CE 61 V); PhIP-D_3 *m/z* 228 → 210 (CE 39 V) and *m/z* 228 → 140 (CE 61 V). The first transition was used for quantification and the second was used for confirmation.

Data were acquired using Analyst 1.5.1 software and processed (peak integration and quantitation) using MultiQuant 2.1 for quantitative HCA analysis. For each analyte, an external calibration curve was produced and the resulting μg/mL values were calculated for each beef extract sample. Three independent experiments were conducted for each study and triplicate samples were used. These values were then transformed into ng HCA/g beef for comparison between control (no treatment) and different samples treated with DAPP. Final results are shown after converting HCA quantities as a function of the amount contained in the controls (percent of control). The precision of the method for the analysis of HCAs is seen in Fig. 1 from the standard curves along with the percent accuracies and r^2 values. The precision