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Effect of enhancement on the formation of heterocyclic amines in cooked pork loins: Preliminary studies

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ABSTRACT

Heterocyclic amines (HCAs) which are produced in meats cooked at high temperature a risk factor for certain human cancers. This study evaluated the effect of enhancement on HCA formation in cooked pork loins. Three samples of pork loin were prepared including non-injected loin, 12% water-injected loin, and 12% salt/phosphate injected loin. The HCAs were identified in all samples: PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine), MeIQx (2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline), and DiMeIQx (2-amino-3,4,8-trimethylimidazo [4,5-f]quinoxaline). Injection of salt/phosphate significantly reduced the level of PhIP by 42.7%, MeIQx by 79.0%, and DiMeIQx by 75.0%. Enhancement with water alone did not reduce HCA formation.

1. Introduction

Since the late 1970s when Japanese scientists associated potent mutagenic activity with *Salmonella typhimurium* T98 in the charred surface of broiled fish, the number of studies examining cooking-induced mutagens and carcinogens has dramatically increased. Cooking derived mutagens and carcinogens were later isolated and characterized as heterocyclic amines (HCAs). Further studies showed that HCAs are mainly formed in muscle foods, mainly meat and fish, via the Maillard reaction with creati(ni)ne, amino acids, and sugars as the precursors (Sugimura, 2002). The most common HCAs found in foods are the thermic HCAs, which include 2-amino-3-methyl-imidazo [4,5-*f*]quinoline (IQ), 2-amino-3-methylimidazo [4,5-*f*]quinoxaline (IQx), 2-amino-3,4-dimethylimidazo [4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline (MeIQx), DiMeIQx (2-amino-3,4,8-trimethyl-imidazo [4,5-*f*]quinoxaline), and 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP) (Knize, Dolbeare, Carroll, Moore, & Felton, 1994). These HCAs are listed in the U.S. Department of Health and Human Services' 12th Report of Carcinogens (2011) as compounds *reasonably anticipated to be a human carcinogen*. The International Agency for Research on Cancer (1993) categorized MeIQ, MeIQx, and PhIP as *reasonably anticipated to be a human carcinogen* and IQ as a *probable human carcinogen*. As with many chemical mutagens and carcinogens, HCAs have the capability to form DNA adducts (Sugimura, 2002). The epidemiological studies over the past 25 years have shown that the high intake of well-done meat and high exposure to meat carcinogens, particularly HCAs, increase the risk of human cancers, such as colorectal cancer in particular (Kampman, Slattery, Bigler, Leppert, & Samowitz, 1999). The total HCA-induced colorectal cancer risk depends on estimates of HCA levels in the diet and frequency of consumption (Sugimura, 2002). Cross et al. (2010) reported that intake of MeIQx (24.4 ng/1000 kcal) and DiMeIQx (1.74 ng/1000 kcal) were both associated with colorectal and colon cancer in the high meat consuming quintile group (66.5 g meal/1000 kcal). Nowell et al. (2002) reported, in a case-controlled study, that dietary HCA exposure was strongly associated with colorectal cancer risk. Estimates of HCA intake were: MeIQx, 94 ng/day; PhIP, 160 ng/day, and DiMeIQx, 7 ng/day. In a European cohort study Rohrmann, Hermann, and Linseisen (2009) found that dietary PhIP (45 ng/day) was associated with increased colorectal adenoma risk though the correlation for MeIQx (222 ng/day) and DiMeIQx (4 ng/day) was not significant. Wu et al. (2006) estimated from their US cohort study of 14,000 men that the daily mean HCA intake was; PhiP (103 ng/day), MeIQx (15 ng/day) and DiMeIQx (1.4 ng/day).

Numerous strategies have been used to limit formation of HCAs, including lowering cooking times, lowering temperatures (Knize et al., 1994; Skog, Steineck, Augustsson, & Jägerstad, 1995), and precooking food in a microwave (Felton, Fultz, Dolbeare, & Knize, 1994). A high cooking loss has been found to be related to the formation of large amounts of HCAs (Knize et al., 1994; Skog et al., 1995); thus, reducing the cooking loss is likely to reduce HCA formation in meat

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products. Persson, Sjöholm, and Skog (2003) showed that addition of water-binding ingredients (1.5% sodium chloride and 0.3% sodium tripolyphosphate) to beef burgers improved the cooking loss and also decreased the formation of PhIP, MeIQx, and DiMeIQx when beefburgers were fried at 180 °C and 220 °C.

Case-ready fresh meat products are defined as products that come in a packaged state from the supplier and are not repackaged at the store (Belcher, 2006). The prevalence of case ready products sold in the U.S.A. has grown at a tremendous rate, increasing from 50% in 2002 to 64% of total fresh meat packaged in 2007 (Baczwaski & Mandigo, 2003; Belcher, 2006), and the number is expected to rise above 70% by 2010 (Young, 2009). Two technologies that are commonly used in case-ready meat products are enhancement and marination. Enhancement is the process of injecting a solution of water, salt, and sodium phosphates that typically adds 7 to 15% addition to the beginning weight of fresh meat to improve the eating quality (juiciness, tenderness, and flavor) of the final product (Baczwaski & Mandigo, 2003; Knock et al., 2006; Sheard & Tali, 2004). Marination expands the solution by using ingredients with additional flavor and texture profiles. A marinade typically contains the same ingredients as an enhancement solution plus color and flavor components such as caramel coloring and dressing with spices (Baczwaski & Mandigo, 2003). In the Sealed Air Corporation, the National Cattlemen's Beef Association, and the National Pork Board assessed case-ready meat products in the U.S.A. and it was found that, within enhanced meat products, pork had the greatest number of products (35%), followed by chicken (19%), beef (13%), and turkey (6%); within marinated meat products, pork also had the greatest number of products (42%), followed by beef (30%), chicken (16%), and turkey (12%) ("Today's Retail Meat Case," 2007).

Non-meat ingredients used in enhanced meat products play various roles. Water is used mainly to dissolve other non-meat ingredients and increase yield; it also contributes to juiciness and tenderness (Miller, 1998). Salt and phosphate are sometimes used alone but often are used in combination to provide a synergistic action (Sheard & Tali, 2004). Salt is added at low levels (0.1 to 3.0%) to increase water-holding capacity, improve texture by solubilizing of meat myofibrillar proteins, and improve flavor (Baczwaski & Mandigo, 2003; Miller, 1998). Salt concentration is not a regulated ingredient but is self-limiting because high concentrations will negatively affect palatability of the products (Alvarado & Mckee, 2007). Sodium phosphates increase water-holding capacity by increasing meat pH and also by interacting with meat myofibrillar proteins to increase their ability to hold water inside the meat during cooking, resulting in increased juiciness and tenderness of cooked meat products (Baczwaski & Mandigo, 2003; Dušek, Kvasnička, Lukášková, & Krátká, 2003; Miller, 1998). There are different forms of sodium phosphates available, such as sodium hexametaphosphate, sodium tripolyphosphate, and tetrasodium pyrophosphate. Sodium tripolyphosphate was reported to have the best effect in retaining the additional water associated with solution enhancement in meat products (Baublits, Pohlman, Brown, & Johnson, 2006). When phosphates are used to increase the water-holding capacity properties of meat, USDA requires that phosphate concentrations are no higher than 0.5% of the finished product weight (Alvarado & Mckee, 2007).

Although some research has been conducted regarding methods to minimize HCA formation in cooked meat products, details of the effects of increasing water-holding capacity of fresh meats by means of enhancement and on HCA formation are still lacking. The aim of this study was to study the effect of enhancement on HCA formation in cooked pork loins.

2. Materials and methods

2.1. Chemicals

The HCA standards IQ (2-amino-3-methyl-imidazo [4,5-*f*]quinoline), IQx (2-amino-3-methyl-imidazo [4,5-*f*]quinoxaline), MeIQ (2-amino-3,4-dimethyl-imidazo [4,5-*f*]quinoline), MeIQx (2-amino-3,8-dimthylimidazo [4,5-*f*]quinoxaline), 4,8-DiMeIQx (2-amino-3,4,8-trimethyl-imidazo [4,5-*f*]quinoxaline), TriMeIQx (2-amino-3,4,7,8-tetramethyl-imidazo [4,5-*f*]quinoxaline), and PhIP (2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine were obtained from Toronto Research Chemicals (Toronto, Canada). Ammonium acetate and triethylamine were purchased from Aldrich Chemicals (Milwaukee, WI, USA). Phosphoric acid and trichloroacetic acid were obtained from Sigma Chemicals (St. Louis, MO, USA). Deionized water was processed by a Sybron/Branstead PCS unit (Barnstead/Thermolyne, Dubuque, IA, USA). The solid-phase extraction Extrelut NT 20 columns and diatomaceous earth refill material were purchased from VWR International (Bristol, CT, USA). Bond Elut propyl-sulfonic acid (PRS) cartridges, C-18 cartridges, and the coupling adaptors were purchased from Varian Sample Preparation (Harbor City, CA, USA). Solvents and chemicals such as acetonitrile (high-performance liquid chromatography [HPLC] grade), methanol (HPLC grade), and sodium hydroxide (ACS-grade) were purchased from Fisher Scientific (Fairlawn, NJ, USA).

2.2. Preparation of enhanced pork samples

Enhanced pork loin samples were prepared at the Kansas State University meat laboratory by using a multi-needle brine injector (Model N30 Wolftec, Inc., Werther, Germany). Two individual loins were selected, and each was divided into three sections. Each of sections was randomly assigned to one of three treatments: (1) no injection, (2) injection with 12% water, or (3) injection with 12% enhancement brine (0.5% sodium chloride and 0.35% sodium tripolyphosphate). After pumping, loins were vacuum packed and held for 72 h at 4 °C to allow the injected solution to equilibrate throughout. The experiment was replicated three times.

2.3. Sample preparation

After the equilibration, loins were sliced at a thickness of 2 cm with a meat slicer (Cabela's commercial grade slicer, 1/3 hp, Sidney, NE, USA) and then stored at 4 °C before cooking. The samples used for chemical analyses were further chopped and ground with a food processor (KitchenAid, model KFP 750) and refrigerated at 4 °C before analysis.

2.4. Chemical analyses

2.4.1. pH measurement

The sample pH was measured according to the method of Jang et al. (2008) with slight modifications. Five grams of finely ground sample was added to 45 mL of distilled water and blended for 30 s at a medium speed in a Waring blender (Waring Laboratory, Torrington, CT, USA). The pH of each sample was measured with an Accumet AP115 portable pH meter (Fisher, Pittsburgh, PA, USA).

2.4.2. Creatine determination

Creatine content was determined according to the method described by Polak, Došler, Žlender, and Gašperlin (2009) with slight modifications. A 0.25-g finely ground sample was homogenized for 5 min at 9500 rpm (IKA, Ultra-Turrax T18) (Wilmington, NC, USA) in 100 mL trichoroacetic acid (30 g/L in distilled water), and then the samples were filtered through Whatman #4 filter paper. Twenty milliliters of the filtrate was defatted with 10 mL diethylether, and then samples were shaken vigorously and allowed to stand for 10 min to separate the phases. After the phases were separated, 4 mL of defatted extract (bottom layer) was mixed with 2 mL of diacetyl (0.2 g/L in distilled water) and 2 mL of 1-napthol (25 g/L in 20 g/L of sodium hydroxide solution). The mixture was heated for 5 min at 40 °C. Each sample's absorbance was measured at 520 nm against a reagent blank. The creatine content was expressed as milligram per gram of meat sample.