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Inhibitory effects of pomegranate seed extract on the formation of heterocyclic aromatic amines in beef and chicken meatballs after cooking by four different methods

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ABSTRACT

Beef and chicken meatballs with a 0.5% (w/w) pomegranate seed extract were cooked using four different cooking methods (oven roasting, pan cooking, charcoal-barbecue, and deep-fat frying) and six heterocyclic aromatic amines; IQ, MeIQx, 4,8-DiMeIQx, PhIP, norharman, and harman were observed. In the beef meatballs, the highest inhibitory effects of pomegranate seed extract on heterocyclic aromatic amines formation were 68% for PhIP, 24% for norharman, 18% for harman, 45% for IQ, and 57% for MeIQx. Total heterocyclic aromatic amine formation was reduced by 39% and 46% in beef meatballs cooked by charcoal-barbecue and deep-fat frying, respectively. In the chicken meatballs, the highest inhibitory effects were 75% for PhIP, 57% for norharman, 28% for harman, 46% for IQ, and 49% for MeIQx. When the pomegranate seed extract was added to the chicken meatballs cooked by deep-fat frying, the total heterocyclic aromatic amine formation was inhibited by 49%, in contrast the total heterocyclic aromatic amine contents after oven roasting increased by 70%.

1. Introduction

People cook meat to increase its safety and palatability; however, heat-processing temperatures favor reactions between compounds inherent in meat and fish yielding genotoxic substances (Khan, Bertus, Busquets, & Puignou, 2009). Heterocyclic aromatic amines (HCAs) are mutagens produced at ppb levels when meat or fish are thermally processed (Kim & Lee, 2010). These compounds are formed in meats heated at 150 °C or higher and HCAs are produced as a result of Maillard reactions of creatine, sugars, and amino acids, which are all components present in mammalian muscular tissue (Gross & Gruter, 1992; Kim & Lee, 2010). More than 20 HCAs have been isolated and characterized from heated protein-rich foods and model systems (Ahn & Grün, 2005a). Most of them demonstrate potent mutagenicity in a bacteria mutagenicity test, and some of them have been classified by the International Agency for Research on Cancer as probable/possible human carcinogens. Their possible formation even during ordinary cooking processes implies frequent exposure by the general public.

The concentrations of HCAs can be dependent on meat type, quantity, cooking procedures, pH, water activity, carbohydrates, free amino acids, creatine, heat and mass transfer, lipid, lipid oxidation, and antioxidants (Oz, 2011; Pais, Salmon, Knize, & Felton, 1999). During cooking, the amounts of precursors at the meat surface may be enhanced by the transport of water and water-soluble precursors from the inner parts of the meat. This mass transport is essential for the formation of HCAs. A high cooking loss has been found to be related to the formation of large amounts of HCAs (Persson, Graziani, Ferracane, Fogliano, & Skog, 2003). In addition, cooking temperature, time of processing, as well as the cooking equipment and methods are known to influence the formation of HCAs in foods (Louis, Zheng, Jiang, Bogen, & Keating, 2007). Of the various parameters, cooking temperature is the most important in HCA generation (Persson et al., 2003).

Spices are a promising source of natural anti-oxidants, and some of them were tested to measure their effects on the formation of HCAs (Damašius, Venskutonis, Ferracane, & Fogliano, 2011). Several studies have shown that the concentrations of HCAs can be reduced by the addition of antioxidants such as vitamin E or antioxidant-containing spices or extracts such as rosemary, garlic, sage, thyme, green tea, and fruit or grape seed (Ahn & Grün, 2005b; Cheng et al., 2007; Damašius et al., 2011; Gibis & Weiss, 2010; Oz & Kaya, 2011; Quelhas et al., 2010; Smith, Ameri, & Gadgil, 2008; Tai, Lee, & Chen, 2001). For example, the scavenging effect of antioxidants on pyrazine cation radicals that participate in the formation of HCAs has been demonstrated by a decrease in electron spin resonance signals in the present of antioxidants (Kikugawa et al., 1999).

Pomegranate seed as a byproduct of pomegranate processing is about 20% (w/w) of the whole fruit (Jing et al., 2012). Recent studies have found that pomegranate seed may have the potential to be a good source of nutrients and antioxidants. It has been suggested that dietary supplementation with pomegranate seeds may prevent DNA damage (Guo, Deng, Xiao, Xie, & Sun, 2007), reduce the risk of cancer,

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and alleviate menopausal symptoms. The beneficial effects of pomegranate seeds may be related to the presence of a variety of biologically active compounds, particularly polyphenols which have been studied for their antioxidant effects (Jing et al., 2012). Although pomegranate seed extract (PSE) has been well investigated for their antimutagenic, anticarcinogenic, and antioxidant activity (Jing et al., 2012; Kanatt, Chander, & Sharma, 2010), there is no information with regard to the inhibitory effect of this extract on the formation of HCAs in cooked meat.

Meatballs are made by mixing ground meat, breadcrumbs, minced onion, salt, spices, possibly eggs, and some food filling materials. After mixing the mixture is divided into small amounts and rolled into small balls. Meatballs can be prepared using various cooking procedures (pan cooking, oven roasting, charcoal-barbecue, and deep-fat frying) thus HCAs are formed during cooking at various levels (Oz, 2011). Poultry meat is sometimes referred to as white meat, in contrast to red meat, for example, beef and pork. The main difference is the low fat content in poultry meat, but the amino acid composition and the content of glucose and creatine also differ. Therefore, the objective of this study was to investigate the effects of the pomegranate seed extract on the formation of HCAs in beef (red meat) and chicken (white meat) meatballs cooked by different methods.

2. Materials and methods

2.1. Chemicals

All chemicals and solvents used were of HPLC or analytical-reagent grade, and water was purified using the Milli-Q gradient A10 system (Millipore, Billerica, MA, USA). All the solutions were filtered through a 0.45 μm filter before being injected into the HPLC system.

The HCA compounds studied are as follows: 1) 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2) 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 3) 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 4) 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), and 5) 1-methyl-9*H*-pyrido[3,4-*b*]indole (harman) purchased from Toronto Research Chemicals (Toronto, Canada). The 9*H*-pyrido[3,4-*b*]indole (norharman) was purchased from Sigma (Steinheim, Germany). For the solid phase extraction, an Oasis MCX cartridge (3 cm^3/60 mg, Waters, Milford, Massachusetts, USA) and an Accubond C_{18} cartridge (3 cm^3/200 mg, Agilent Technologies, Santa Clara, CA, USA) were used.

Zinc sulfate heptahydrate was purchased from Riedel-de Haen (Seeize, Germany). HPLC-grade methanol and acetonitrile, acetic acid, potassium ferrocyanide trihydrate, ammonia (25%), sodium carbonate, and hydrochloric acid (37%) were all purchased from Merck (Darmstadt, Germany). DPPH (1,1-Diphenyl-2-picryl-hydrazyl radical), Folin–Ciocalteu, and gallic acid (Sigma, Steinheim, Germany) were also used.

Stock standard HCA solutions of 100 mg/l in methanol were prepared and used for further dilutions. Standard solutions of 0.1 mg/l, 0.2 mg/l, 0.5 mg/l, 1 mg/l, 5 mg/l, and 10 mg/l in methanol were prepared for both calibration and standard addition purposes. Standards were filtered through a 0.45 μm filter before being injected into the HPLC system.

2.2. Determination of cooking loss

Cooking loss was measured as the weight difference between the meatballs before and after cooking.

2.3. Determination of total phenolics

The total amount of phenolic compounds (TFC) was determined using Folin–Ciocalteu reagent (Singh, Chidambara Murthy, & Jayaprakasha, 2002) and expressed in gallic acid equivalents (GAE). The dry extract and meatballs were diluted in methanol (2000 times for extract and 10 times for meatballs) and 0.5 ml of this solution was mixed with 2.5 ml of Folin–Ciocalteu reagent and 2 ml of 7.5% Na_2CO_3 solution. After 30 min of incubation the absorbance was measured at 760 nm, using a UV/Vis spectrophotometer and compared with a gallic acid calibration curve. All measurements were made in duplicate.

2.4. Determination of radical scavenging activity using DPPH method

The radical scavenging activities (RSA) of the extract and meatballs were measured using the DPPH radical-scavenging assay method (Singh et al., 2002). The dry extract and meatballs were dissolved in methanol. Five milliliters of a 0.1 mM methanolic solution of DPPH was added to the tubes containing 0.5 milliliters of diluted extract (2000 times) and meatballs (10 times) and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min. The control was prepared as above without any extract or meat samples and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\text{Radical scavenging activity } \% = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right] \times 100$$

2.5. Meatball preparation and cooking conditions

The chicken and beef used to make the meatballs were purchased from a local market in Izmir, Turkey. First the chicken and beef were ground and then the fat content of the ground chicken and beef was adjusted to 25%. To give a good structure to the meatballs, unsalted breadcrumbs were added at 20% (w/w). The size of the meatballs was 1.3 cm thick and 5 cm in diameter and the weight of the meatball was about 30 g. Pomegranate seed extract purchased from Balen, Turkey was added to the ground meat at 0.5% (w/w).

The cooking methods most commonly used in Turkey were used: pan cooking, oven roasting, charcoal barbecue, and deep-fat frying. For deep-fat frying, fresh sunflower oil was used. When the temperature of oil increased to 150 °C, the meatballs were fried for 5 min in a commercial stainless steel deep-fat fryer. The pan cooking process was carried out with a commercial pan (metal), which was preheated until the surface temperature was 180 °C and then, the meatballs were fried for 8 min per side without fat or oil. For the charcoal-barbecue, approximately 1 kg of charcoal was placed in the bottom of an oven, and 100 ml of gasoline was poured onto charcoal to start the fire. When all the flames had subsided, the charcoal was leveled by raking. The meatballs were then barbecued over the charcoal for 10 min per side, total cooking time 20 min, the distance between the samples and the charcoal was about 8 cm. The surface temperature of the samples was measured as about 280 °C. For oven roasting, the meatballs were placed in an oven for 27 min at 180 °C. Temperatures were measured using a laser infrared thermometer (Hongtai HT866, Guangdong, China). All experiments were repeated twice. For every replicate, three meatballs were used for each process. After completing all the cooking processes, the cooked meatballs were cooled at room temperature. Then, the cooked meatballs were homogenized using a kitchen blender to produce a uniform sample. The meatball samples were stored at −18 °C. Prior to analysis, samples were thawed in a refrigerator at 4 °C for 12–24 h.

2.6. Extraction and analysis of heterocyclic aromatic amines

Extraction and purification of HCAs were performed according to the method developed by Özdestan, Kaçar, Keşkekoğlu, and Üren (in press).