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Effect of different types of sugars in a marinating formulation on the formation of heterocyclic amines in grilled chicken

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ABSTRACT

The aim of the study was to determine the effect of different types of sugar on the formation of heterocyclic amines (HCA) in marinated grilled chicken. Chicken breast samples were marinated with table sugar, brown sugar, and honey for 24 h at 4 °C. The internal temperature, weight loss, free amino acids, sugars, and HCA were determined. The concentrations of all types of HCA (except IQx) in samples that were marinated with table sugar were significantly higher ($p < 0.006$) than brown sugar; whereas those were marinated with honey had the lowest HCA concentrations. A substantial reduction in the concentration of MeIQ, PhIP, DiMeIQx, IQ, IQx, and norharman was achieved in chicken marinated with honey. A correlation study indicated that adding honey into the recipe retarded the formation of most HCA (MeIQ, DiMeIQx, IQ, IQx, norharman, and harman), whereas table sugars enhanced the formation of all HCA except norharman, harman, and AαC.

1. Introduction

Heterocyclic amines (HCA) are an important class of toxic compounds that are usually formed in a complex reaction involving free amino acids, reducing sugars and creatine through the Maillard reaction (Liao, Wang, Xu, & Zhou, 2010). More than 25 types of HCA have been isolated and identified in cooked foods (Oz, Kaban, & Kaya, 2010). Depending on the formation process, HCA can be classified into two groups: thermic HCA and pyrolytic HCA. Pyrolytic HCA are formed when proteinaceous foods are heated above 300 °C, whereas thermic HCA are formed below 300 °C (Skog & Solyakov, 2002). The International Agency for Research on Cancer classified IQ (2-amino-3-methylimidazo[4,5-f]-quinoline), MeIQ (2-amino-3,4-dimethyl-3H-imidazo[4,5-f]quinoline), MeIQx (2-amino-3,8-dimethlimidazo[4,5-f]-quinoxaline), DiMeiQx (2-amino-3,4,8-trimethyl-3H-imidazo[4,5-f]quinoxaline), and PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) as probable human carcinogens (class 2A) (IARC, 1993). Epidemiological studies that were conducted to assess the cancer risk according to the dietary HCA intake in human subjects suggested that excess consumption of HCA in meat products can cause colon cancer (Skog & Solyakov, 2002), breast and prostate cancer (Hwang & Ngadi, 2002), and lung cancer (Sinha et al., 1995).

Formation of HCA has been reported in cooked food, especially in broiled and grilled meat and fish dishes (Felton, Fultz, Dolbeare, & Knize, 1994). Sinha et al. (1995) found the concentration of PHiP in grilled chicken breast to be as high as 480 ng/g. Salmon et al. (1997) found that grilled chicken breast contained as much as 15 ng/g of MeIQx and less than 6 ng/g of DiMeIQx. Oz et al. (2010) found the total of three HCA (IQx, IQ, and MeIQ) in barbecued (grilled) chicken was approximately 10 ng/g.

In the United States, Sweden, New Zealand, Japan, and Singapore the average intake of meat and fish is estimated to range from 80–160 g/day; likewise, the average daily consumption of meat and fish in Malaysia is estimated at 104 g/day (Jahurul et al., 2010). Grilled meat is a popular dish all over the world, and the meats are often marinated before being grilled. Marinating is performed for a variety of reasons, including the improvement of flavour, tenderness, and moistness of the cooked product (Yusop, O'Sullivan, Kerry, & Kerry, 2010). In addition, marinades can act as a barrier to avoid a direct contact between the meat and the flame (Liao et al., 2010), and therefore, marinating is expected to be able to reduce the concentration of HCA in grilled meats.

HCA are generated through the Maillard reaction, which involves free amino acids, creatinine, and reducing sugars (glucose and fructose, directly or *via* hydrolysis of sucrose) as precursors (Hwang & Ngadi, 2002). Table sugar provides >95% of sucrose (Dawling, 1990), and acts as a sweetening agent, bulking agent, preservative; sugar also provides colouring in marinades (Abdulmumeen, Risikat, & Sururah, 2012). Brown sugars may have 90% sucrose and a high mineral content with a distinctive brown

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colour because of the presence of molasses. Honey is composed primarily of simple sugars, such as glucose (30%), fructose (38%) and sucrose (1.3%) (Shin & Ustunol, 2004). Honey is known as an alternative to table sugar, and honey has been used as a source of digestible sugars since ancient times (Shin, Rodgers, Gomaa, Strasburg, & Gray, 2002).

Various authors have shown that the concentration of HCA can be reduced by adding ingredients to marinades before cooking, such as tomatoes (Persson, Graziani, Ferracane, Fogliano, & Skog, 2003), tart cherry tissue (Britt, Gomaa, Gray, & Booren, 1998), and spices (such as onion and garlic) (Shin, et al., 2002). Studies by Shin and Utsunol (2004) reported the total HCA as 24 ng/g (i.e., MeIQx, DiMeIQx, and PhIP) in fried chicken that was marinated with mixed ingredients, such as lemon juice, soy sauce, minced garlic, clover, and (buckwheat) honey. Salmon et al. (1997) reported a 92–99% decrease in PhIP in whole chicken breast that was marinated with a mixture of brown sugar, olive oil, vinegar, garlic, mustard, lemon juice, and salt. Although honey and brown sugar were used in marinades, they did not compare the effect of different types of sugar in their study.

The objective of this study was to determine the effects of different types of sugar in marinades on the formation of HCA in grilled chicken. Three different types of sugars, namely table sugar, brown sugar, and honey, were used. The internal temperature of the chicken throughout the grilling process was monitored and measured. The weight loss of chicken before and after grilling was also measured. The reducing sugar and free amino acid concentrations of unmarinated (control) and marinated chicken, and the HCA concentrations of grilled chicken (unmarinated and marinated) were determined. To the best of our knowledge, our work is the first study that focused on the effect of different types of sugars (table sugar, brown sugar, and honey) in marinades on HCA formation in grilled meat.

2. Materials and methods

2.1. Preparation of chicken marinades

Raw chicken breasts were purchased from the local wholesale market at Selangor, Malaysia and stored at −20 °C until use. Onion (fresh), table sugar, brown sugar, lemongrass (fresh), salt, turmeric powder, and cooking oil were purchased from a wholesale market (Sri Kembangan, Selangor), whereas pure honey *(Koompassia Excels)* was purchased from an established honey farm in Bukit Katil, Melaka, Malaysia. The purchased table sugar, brown sugar, and honey were used to reflect the common cooking practice in marinating grilled chicken.

The marinades containing table sugar, brown sugar and honey were prepared the next day. The other ingredients, onion (fresh), lemongrass (fresh), salt, turmeric powder, and cooking oil, were the same for all three marinades. The marinade recipe was obtained from a qualified chef from the Department of Food Service and Management, Faculty of Food Science and Technology, UPM, Malaysia. The relative sweetness of the three sugars was first determined with the sugar Brix calculation ratio, using the method described by Lacey, Hancock, and Ramsey (2009), before they were used for marinating. The formulation of each sugar is described in Table 1.

The frozen raw chicken breasts were thawed at 4 °C for six hours, and then cut into small cubes (1 × 1 cm). For every 100 g of chicken breast cubes, the following ingredients were used: fresh onion (15 g), fresh lemongrass (10 g), turmeric powder (5 g), salt (1 g), and cooking oil (1 ml). The weight of three sugars, i.e., 10.0 g for table sugar, 10.3 g for brown sugar, and 14.7 g for honey, were added individually into the standard recipe. All of the ingredients were mixed for 3 min at speed No. 2 using a Waring Blender (model MX337, Panasonic Corp, Osaka, Japan) and then the marinade was thoroughly mixed by hand with the chicken breast cubes. The marinated chicken cubes were then skewered onto a bamboo skewer (4 pieces per skewer) and then kept in sealed polyethene bags (4 skewers per bag) at 4 °C for 24 h.

2.2. Grilling conditions

The 100 g of chicken cubes skewered onto bamboo skewers were grilled for 4 min per side (total duration for both sides was 8 min), using an electric grill (model R-360 J(S), Sharp Electronics Corp., Mahwah, NJ), in which the temperature was set at 300 °C. The internal temperature of the chicken breast was measured during grilling at 0, 4, and 8 min, using a thermocouple thermometer (A type-K, Fluke Corporation, Everett, WA). Unmarinated chicken cube (control) samples were also treated identically as the treatment samples, except that they were not mixed with marinating ingredients. All samples were prepared separately in triplicate.

2.3. Chemicals and reagents

D-(+)-Glucose (99.5%) and D-(−)-fructose (99%) were obtained from Sigma (St Louis, MO). Dowex 50WX4 and Anion WGR-2 were purchased from Supelco (Bellefonte, PA). D-(+)-Maltose monohydrate (99%) and triethylamine (TEA) were purchased from Fluka Chemie AG (Buchs, Switzerland). α-Aminobutyric acid (AABA) and phenylisothiocyanate (PITC) were obtained from Sigma–Aldrich (Steinheim, Germany). Nine HCA standards, 2-amino-3-methyl-3*H*-imidazo[4,5-f]quinoline (IQ), 2-amino-3-methyl-3*H*-imidazo[4,5-f]quinoxaline (IQx), 2-amino-3,4-dimethyl-3*H*-imidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethyl-3*H*-imidazo[4,5-f]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-9*H*-pyrido[2,3-b]indole (AαC), 1-methyl-9*H*-pyrido-[4,3-b]indole (harman), and 9*H*-pyrido-[4,3-b]indole (norharman) were obtained from Toronto Research Chemicals (Toronto, ON, Canada). Acetonitrile, ethyl acetate, hexane, hydrochloric acid, methanol, sodium hydroxide, acetic acid, ammonium acetate and ammonium hydroxide (25%) were purchased from Merck (Darmstadt, Germany). All chemicals were of HPLC grade. Ultra-pure water was used throughout the experiment (Purelab Classic UV, Elga Lab Water, Lane End, UK). Isolute diatomaceous earth extraction cartridges (Isolute HM-N) and refill materials were purchased from International Sorbent Technology Ltd. (Hengoed, UK). The Oasis MCX cartridges (3 cm^3/60 mg) were supplied by Waters Corp (Milford, MA).

For each HCA standard, 100 μg/g stock standard solutions were prepared using methanol as the solvent. Working standards were mixed and prepared by diluting the stock solutions of HCA to concentrations of 1, 3, 5, 7, 10, 25, 50, 75, 100 ppb with methanol. All stock solutions and working standards were stored at 4 °C for a maximum of 3 months.

2.4. Weight Loss

The percentage weight loss for each control and marinated sample was calculated by the difference of the sample weight before and after grilling with the subtraction of the sample weight before grilling as described by Sinha et al. (1998). The determinations of weight loss were performed in triplicate.

2.5. Analysis of fructose, glucose and sucrose

Fructose, glucose and sucrose were analysed by the modified method of Aliani and Farmer (2002). The sample was ground in a