Meat Science 94 (2013) 202–207

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effects of varying degrees of doneness on the formation of Heterocyclic Aromatic Amines in chicken and beef satay

S. Jinap [a,b,*], M.S. Mohd-Mokhtar [a], A. Farhadian [c], N.D.S. Hasnol [a], S.N. Jaafar [a], P. Hajeb [a]

[a] Food Safety Research Centre (FOSREC), Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[c] School of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia

ARTICLE INFO

Article history:
Received 7 May 2012
Received in revised form 8 January 2013
Accepted 23 January 2013

Keywords:
Heterocyclic Aromatic Amines (HAs)
Charcoal grilled
Chicken
Beef
Satay
Doneness

ABSTRACT

The study was carried out to determine the effect of cooking method on Heterocyclic Aromatic Amines (HAs) concentration in grilled chicken and beef (satay). Six common HAs were investigated: 2-amino-3-methylimidazo [4,5-f]quinolone (IQ), 2amino 3,4dimethylimidazo [4,5f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8 trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx), 2-amino-3,7,8trimethylimidazo [4,5-f]quinoxaline (7,8-DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Chicken and beef satay samples were grilled to medium and well done level of doneness. Charcoal grilled (treatment A), microwave pre-treatment prior to grilling (treatment B), and microwave-deep fried (treatment C) were applied to beef and chicken satay samples. The satay samples which were microwaved prior to grilling (B) showed significantly ($p<0.05$) lower HAs concentration as compared to those charcoal grilled (A). Both medium and well done cooked beef and chicken satay samples that were microwaved and deep fried (C) as an alternative method to grilling were proven to produce significantly lesser HAs as compared to charcoal-grilled (A) and microwaved prior to grilling (B).

1. Introduction

Heterocyclic amines (HAs) are mutagenic products when meat or fish are thermally processed (Khan, Bertus, Busquets, & Puignou, 2009). HAs form when meat and fish muscle products are exposed to temperatures greater than 150 °C during preparation and cooking (Oz & Kaya, 2011; Sugimura, 1997). International Agency for Research on Cancer classified several HAs as probable and possible human carcinogens (IARC, 1993). In a study by Totsuka, Nishigaki, Sugimura, and Wakabayashi (2006) the possible involvement of HAs in human cancer has been discussed. The relationship between dietary HAs exposure and cancer risk has been reviewed (Knize & Felton, 2005) and interests in this subject is growing. Higher exposure to HAs is more likely from consuming well done cooked meats.

Many studies have shown that cooking conditions are crucial in the formation of HAs (Janoszka, Błaszczyk, Damasiewicz-Bodzek, & Sajewicz, 2009; Liao, Wang, Xu, & Zhou, 2010; Oz, Kaban, & Kaya, 2007; Solyakov & Skog, 2002). According to Solyakov and Skog (2002) a variety of heterocyclic amines in heat-treated poultry products can be formed with different cooking methods and cooking conditions such as duration and temperature of cooking as well as the composition of the food (Jägerstad, Skog, Arvidsson, & Solyakov, 1998). Epidemiological studies examining the relation between cancer incidences and preference for well done meat have been shown in several cases to have positive correlations especially for colorectal (Sinha et al., 2000), and breast cancer (Sinha, Kulldorff, Chow, Denobile, & Rothman, 2001). In order to estimate the intakes and risks to human health, it is important to quantify HAs in different meat products prepared in different ways (Solyakov & Skog, 2002).

Satay is a popular grilled food delicacy in Southeast Asia (Malaysia, Indonesia, Thailand, and Singapore), and some western countries; it is similar to shish kebab, which is also a popular grilled food in middle-eastern countries. It consists of dice-sized chunks or slices of boneless meat (chicken, mutton, beef, pork, fish), on skewers made from the midrib of coconut leaf or bamboo. Satay is grilled over wood or charcoal fires, and then served with various spicy seasonings depending on the satay recipe. Open charcoal grilling is the common cooking method for satay prepared in Southeast Asian countries. Compared to other cooking practices such as prolonged heating or frying, this fast grilling method can produce a different mixture of HAs. The preparation of satay is simple; however, the combination of ingredients used varies. Turmeric is the basic ingredient used for marinating satay together with others such as shallot, cumin, coriander powder and lemongrass.

Wu, Lee, Wong, and Ong (1997) showed that HAs present in chicken satay range from 7.8 ng/g to as high as 84.0 ng/g. PhIP, abundantly found in cooked beef, chicken and fish, was also detected in mutton,

* Corresponding author at: Food Safety Research Centre (FOSREC), Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia. Tel.: +60 38946 8393; fax: +60 38942 3552.
E-mail addresses: jinap@food.upm.edu.my, sjinap@gmail.com (S. Jinap).

0309-1740/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2013.01.013

pork and chicken satays. In that study however, satay was purchased from different food stalls. As such, the grilling process may not be the same from stall to stall. In a study by Jahurul et al. (2010) the HAs concentration in chicken and beef satay has been investigated but the samples were purchased from different food stalls. The HAs concentration in satay (i.e. chicken and beef) under controlled conditions has not been fully investigated. Thus, the present study was designed to investigate the effect of cooking conditions and different meat (chicken and beef) on HAs formation in satay samples during cooking.

2. Materials and methods

2.1. Materials

The HA standards used, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethyl-imidazo[4,5-*f*] quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 4,7,8-TriMeIQx were obtained from Toronto Research Chemicals (Toronto, Canada). For each HAs standard, a stock solution of 100 μg/g in methanol was prepared and used for further dilution. 4,7,8-TriMeIQx was used as an internal standard (250 ng/g methanolic solution). Acetonitrile, ethyl acetate, methanol, sodium hydroxide, acetic acid and ammonium hydroxide (25%) were purchased from Merck (Darmstadt, Germany). All chemicals and solvents were of High Performance Liquid Chromatography (HPLC) or analytical grade and water was purified (Elga LabWater Ltd, Marlow, UK).

Diatomaceous earth (Extrelut 20) was obtained from the International Sorbent Technology; Hengoed Mid Gleam (UK) and Oasis MCX cartridges (3 cm^3/60 mg) were purchased from Waters (Milford, Massachusetts, USA). MCX cartridges were preconditioned with ethyl acetate (2 mL). A microwave oven (model R-360J/S, Sharp Electronic Corp., Mahwah NJ, USA) with a power rate of 1100 W, and a thermocouple thermometer type-K (Fluke, Everett WA, USA) were used.

Fresh beef, chicken and marinade ingredients were purchased from Pasar Borong Selangor, Malaysia and were stored at −20 °C prior to marinating. Marinating ingredient amounts used are shown in Table 1.

2.2. Preparation of satay

Frozen beef and chicken (1 kg for each replicate) were thawed at 4 °C overnight and then cut into small cubes (1 cm × 1 cm dimension). All the marinating ingredients were blended in a Waring blender for 1 min before being mixed by hand with the beef and chicken cubes. The marinated beef and chicken cubes were then skewered with bamboo skewers, by hand and were kept in polyethylene bags at 4 °C overnight. The experiment was carried out three times.

Table 1
Amount of ingredients in the satay marinades.

Ingredient[a]	Amount
Cumin	50 g
Shallots	150 g
Coriander powder	100 g
Lemongrass	100 g
Turmeric powder	50 g
Sugar	100 g
Salt	10 g
Cooking oil (palm oil)	10 mL

[a] Marinating ingredients were stored at 4 °C before marinating.

2.3. Grilling conditions

Three cooking treatments were applied and the grilling process was monitored by a qualified chef. For treatment A, satay samples were grilled using charcoal. For treatment B, satay samples were pre-heated in microwave oven for 30 s before grilling using charcoal. For treatment C, satay samples were preheated in microwave oven for 30 s prior to deep frying in palm oil at 160 °C. The samples were cooked to two different degrees of doneness; medium and well-done. For medium cook, chicken and beef satay samples (treatment A) were charcoal grilled for 5 and 7 min respectively, chicken and beef satay samples (treatment B) were charcoal grilled for 2 and 3 min respectively, and chicken and beef satay samples (C) were deep fried for 2 and 3 min respectively. For well done cook, chicken and beef satay samples (treatment A) were charcoal grilled for 6 and 8 min respectively, chicken and beef satay samples (treatment B) were charcoal grilled for 3 and 4 min respectively, and chicken and beef satay samples (C) were deep fried for 3 and 4 min respectively. External temperatures for charcoal grilling were 270–300 °C and 300–350 °C for medium and well done samples respectively. The degrees of doneness of all samples were noted by visual inspection. The level of surface browning was judged to be one of the following categories; moderately browned (medium) and well browned (well done). For treatment C, degrees of doneness of samples that had been microwaved were determined by time: medium (30 s) and well-done (40 s). A preliminary study on microwave grilling was conducted to determine the appropriate time to grill satay. During grilling, the internal temperatures of samples were measured in duplicate.

2.4. Clean-up and extraction

The HAs were extracted from the samples using the method developed and validated by Gross and Grüter (1992) and, modified by Messner and Murkovic (2004). One gram of ground meat was dissolved in 12 mL 1 M NaOH. The suspension was homogenized by shaking for 3 h using a shaker (Memmert, Schwabach, Germany). Samples were mixed with 13 g of Extrelut diatomaceous earth, and the mixture filled into an Extrelut 20 column. A volume of 50 mL ethyl acetate was used as extraction solvent and the eluate was passed through Oasis MCX cartridges and then the MCX cartridges were washed with 0.1 M HCL (2 mL) followed by MeOH (2 mL). The analytes were then eluted with 2 mL of MeOH-concentrated ammonia (19/1, v/v). The samples were then evaporated to dryness under a stream of gaseous nitrogen and the final extracts were dissolved in 100 μL methanol containing 250 μg 4,7,8-TriMeIQx as an internal standard.

2.5. Instrumentation

HPLC analysis was performed on a Waters 600 HPLC system (Milford, Massachusetts, USA) equipped with a photodiode array detector (Waters 2996, Photo Diode Array). The analytical column used was a reversed phased column TSK-gel ODS 80-TM (4.6 mm id × 25.0 cm L, 5 μm) from Tosoh Bioscience GmBH (Stuttgart, Germany). Separation of HAs was performed at a flow rate of 1.0 mL/min by gradient elution with 0.01 M triethylamine at pH 3.3 (adjusted with acetic acid) as solvent A and acetonitrile as solvent B. The gradient was programmed for solvent A and solvent B (95/5%) from 0 to 17 min, (77/23%) for 17–24 min, (70/30%) for 24–26 min and (95/5%) for 26–42 min retention time.

2.6. Linearity Test, Limit of Detection (LOD) and Limit of Quantification (LOQ) and Recovery

The identity of peaks was established by comparing retention times of the peaks with standard peaks from standard HAs solution. Prior to HPLC separation of extracts, a series of HAs standard mixtures ranging from 1 ng/mL to 1000 ng/mL containing 250 μg internal