



## Effect of selected local spices marinades on the reduction of heterocyclic amines in grilled beef (satay)



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### ABSTRACT

The present study focused to investigate the effect of some selected spices (turmeric, torch ginger, lemongrass and curry leaves) on the formation of heterocyclic amines (HCAs, IQx, MeIQ, MeIQx, DiMeIQx, IQ, norharman, harman and AzC) in grilled satay (beef). Satay samples were marinated with 1–4 g/100 g concentration of turmeric and 2.5–10 g/100 g concentration of torch ginger, lemongrass and curry leaves, at different medium (70 °C) and well done (80 °C) doneness cooking temperatures. The concentration of HCAs in grilled satay samples were analysed using LC/MS technique. The results have shown that turmeric reduced maximum 82 ng/100 g level of IQ (11.43 ± 0.53 to 2.05 ± 0.18 ng/g) at 4 g/100 g concentration at medium doneness and 44.4 ng/100 g level was reduced of IQ, when satay was marinated with 10 g/100 g lemongrass concentration at medium doneness. The highest level of MeIQx was reduced to 83 ng/100 g of satay meat marinated in 10 g/100 g torch ginger at medium doneness. Curry leaves with 10 g/100 g concentration marinated beef meat was reduced the level of IQ 78.5 at medium doneness. The use of local spices in marinating of grilled beef will certainly inhibit/reduce the level of toxic and harmful HCAs.

*Significance of results for industry:* The use of native spices during grilling of meat will minimize the formation of heterocyclic amines and therefore will help to avoid toxic and carcinogenic effects from these toxins.

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### 1. Introduction

Studies have proved that different kind of harmful components, including various mutagens and carcinogens, may be produced, when meat is heat-treated using traditional procedures such as frying, barbecuing and smoking (Jägerstad & Skog, 2005). It has been observed that all types of meat such as beef, pork, goat and lamb, may produce heterocyclic aromatic amines (HCA) when cooked at high temperatures. HCAs are recognized as mutagenic and carcinogenic compounds (Damašius, Venskutonis, Ferracane, & Fogliano, 2011).

Currently, more than 25 HCAs have been isolated and recognized in cooked foods, since their discovery (Alaejos & Afonso, 2011; Murkovic, 2007) and divided in two main families:

aminoimidazo-azaarenes or thermic HCAs and amino-carbolines or pyrolytic HCAs. These HCAs includes 2-amino-3-methylimidazo [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), and 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) (Knize, Dolbeare, Carroll, Moore, & Felton, 1994). Thermic HCAs are produced as a result of complex reaction between creatine/creatinine, free amino acids and sugars through the Maillard reaction at temperatures between 150 and 250 °C (Jägerstad, Skog, Arvidsson, & Solyakov, 1998; Nagao, Honda, & Seino, 1977). However, the pathway of pyrolytic HCAs production was not so clear but suggested that it may be produced as a result of pyrolysis of proteins or amino acids heated at higher temperatures i.e. > 250 °C (Matsumoto, Yoshida, & Tomita, 1981).

In Southeast Asia (Malaysia, Indonesia, Thailand, and Singapore), satay is a very popular grilled food similar to shish kebab which is also popular in western and middle-eastern countries. It consists of dice-sized chunks or slices of boneless meat

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(chicken, beef, mutton, pork, fish), on skewers made from the midrib of coconut leaf or bamboo. It is usually grilled over wood or charcoal fires, and then served with various spicy seasonings depending on the satay recipe. Mostly 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 HCAs.

The HCAs (IQ, MeIQ, MeIQx and PhIP) are identified as compounds reasonably anticipated to be a human carcinogen. However, the International Agency for Research on Cancer has classified MeIQx, MeIQ and PhIP as reasonably anticipated to be a human carcinogen, while IQ is placed in the list of a probable human carcinogen (IARC, 1993). Epidemiological studies (Knutsen, Binderup, Vikse, & Øvrebø, 2007; Norat et al., 2005) have shown an increased risk of cancer in the intestine, bladder, prostate, breast and pancreas after high consumption of fried, well-done and barbecued meat.

Previous studies have revealed that by adding ingredients such as tomatoes to marinades before cooking, is effective to reduce the concentration of HCA (Persson, Graziani, Ferracane, Fogliano, & Skog, 2003), tart cherry tissue (Britt, Gomaa, Gray, & Booren, 1998), and spices (such as onion and garlic) (Shin, Gomaa, Strasburg, & Gray, 2002). The present study is focused to investigate the effect of local Malaysian spices including turmeric, lemongrass, torch ginger and curry leave on the reduction of HCAs in grilled beef (satay) at different doneness cooking.

## 2. Materials and methods

### 2.1. Chemicals

Standards of HCAs used, 2-amino-3-methylimidazo[4,5-f]quinolone (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]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), norharman, harman and A $\alpha$ C were purchased from Toronto Research Chemicals (Toronto, Canada). A stock solution of 100  $\mu$ g/g in methanol was prepared for each HCAs. Diatomaceous earth (Extrelut 20) was obtained from the International Sorbent Technology (Hengoed Mid Gleam, UK) and Oasis MCX cartridges (3 cm<sup>3</sup>/60 mg) were purchased from Waters (Milford,

Massachusetts, USA). MCX cartridges were preconditioned with ethyl acetate (2 mL).

The samples (n = 3) of fresh beef (sirloin) 5 kg of local origin were purchased from local markets of Selangor, Malaysia. Visible connective tissues and external fat were removed before storage in freezer at  $-20^{\circ}\text{C}$ . The samples of beef were thawed overnight in a cold room ( $4 \pm 1^{\circ}\text{C}$ ). The pH of meat samples was determined prior to analysis. The meat was then cut into big pieces again the grain, in which each piece has weight about  $100 \pm 0.5\text{g}$ . All the cut meat samples were put into polyethylene containers and stored in the refrigerator ( $4 \pm 1^{\circ}\text{C}$ ).

### 2.2. Local spices preparation

The fresh turmeric, torch ginger flower, curry leaves and lemongrass were obtained from local market, Selangor, Malaysia. The skins of fresh turmeric samples were removed and the curry leaves were stripped off from their stalks. The lemongrass was separated from its stalks and leaves. All the cleaned spices were brought to washing, wiped with paper towel and chopped into small pieces separately. The weight of each spice was noted and kept in polyethylene container. The samples were then kept in freezer ( $-20^{\circ}\text{C}$ ) for overnight and then brought to freeze drying. The samples were freeze-dried for two days, weighed and then grounded using a dry grinder. The homogenized powder samples were kept in polyethylene containers and stored at  $-20^{\circ}\text{C}$ . The moisture content of each powdered sample was determined prior to analysis.

### 2.3. Preparation of marinades formulation

A range of each individual spice was developed prior to this analysis based on the acceptable maximum level in satay recipes and these levels are optimised. The optimised range for turmeric was 0, 1, 2, 3, and 4 g/100 g, while other spices have ranges 0, 2.5, 5, 7.5 and 10 g/100 g of meat. Each spice concentration was applied (2 replicates) on meat samples, then mixed and left for 24 h. The meat samples having no treatment of spices were control samples. Percentage of marinade uptake for each treatment was determined just after marination (0 h) and after 24 h (Table 1). The weight of sample before marination ( $W_0$ ) and after marination ( $W_1$ ) was recorded. The percentage weight gain was calculated based on formulation.

**Table 1**  
Marinate uptake of meat at 0 and 24 h duration.

Spices	Concentration of spices (g/100 g)	Marinate uptake at 0 h (g/100 g)	Marinate uptake after 24 h (g/100 g)	Marinate uptake loss 0–24 h (g/100 g)
Turmeric	1	0.95 $\pm$ 0.17 <sup>aA</sup>	0.38 $\pm$ 0.18 <sup>aA</sup>	60.00 <sup>a</sup>
	2	1.82 $\pm$ 0.12 <sup>bA</sup>	1.21 $\pm$ 0.08 <sup>bB</sup>	33.52 <sup>b</sup>
	3	2.79 $\pm$ 0.05 <sup>cA</sup>	2.08 $\pm$ 0.04 <sup>cB</sup>	25.45 <sup>b</sup>
	4	3.69 $\pm$ 0.13 <sup>dA</sup>	3.00 $\pm$ 0.16 <sup>dA</sup>	18.70 <sup>b</sup>
Lemongrass	2.5	2.15 $\pm$ 0.19 <sup>aA</sup>	0.91 $\pm$ 0.30 <sup>aB</sup>	57.67 <sup>a</sup>
	5.0	4.05 $\pm$ 0.27 <sup>bA</sup>	2.24 $\pm$ 0.11 <sup>bB</sup>	44.69 <sup>b</sup>
	7.5	6.69 $\pm$ 0.28 <sup>cA</sup>	3.77 $\pm$ 0.44 <sup>bcB</sup>	43.65 <sup>b</sup>
	10	8.22 $\pm$ 0.35 <sup>dA</sup>	4.66 $\pm$ 0.32 <sup>cB</sup>	43.30 <sup>b</sup>
Torch ginger flowers	2.5	2.20 $\pm$ 0.02 <sup>aA</sup>	1.05 $\pm$ 0.04 <sup>aB</sup>	52.27 <sup>a</sup>
	5.0	2.34 $\pm$ 0.06 <sup>aA</sup>	1.35 $\pm$ 0.08 <sup>abB</sup>	42.31 <sup>b</sup>
	7.5	2.97 $\pm$ 0.12 <sup>bA</sup>	1.85 $\pm$ 0.08 <sup>abB</sup>	37.71 <sup>c</sup>
	10	3.31 $\pm$ 0.16 <sup>bA</sup>	2.39 $\pm$ 0.52 <sup>bA</sup>	27.79 <sup>d</sup>
Curry leaves	2.5	2.29 $\pm$ 0.03 <sup>aA</sup>	1.32 $\pm$ 0.06 <sup>Ab</sup>	42.36 <sup>a</sup>
	5.0	2.42 $\pm$ 0.03 <sup>abA</sup>	1.40 $\pm$ 0.04 <sup>abB</sup>	42.15 <sup>a</sup>
	7.5	2.71 $\pm$ 0.06 <sup>bcA</sup>	1.57 $\pm$ 0.00 <sup>abB</sup>	42.07 <sup>a</sup>
	10	2.91 $\pm$ 0.16 <sup>cA</sup>	1.69 $\pm$ 0.09 <sup>bb</sup>	41.92 <sup>a</sup>

Values with same English lowercase within row of each species are not significant ( $p \geq 0.05$ ).

Values with same English uppercase letter within column of marinate uptake (0 and 24 h) are not significant ( $p \geq 0.05$ ).

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