

Research Article

Inhibitory activities of dietary phenolic compounds on heterocyclic amine formation in both chemical model system and beef patties

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The correlation between radical scavenging capacity and inhibitory activity in PhIP formation of 12 food-derived antioxidative phenolic compounds was investigated. Very poor correlation was found between their radical scavenging activity assessed by Trolox equivalent antioxidant capacity assay and PhIP formation inhibition in a model system. The effects of several of these polyphenols were further evaluated using beef patties. Remarkably, theaflavin 3,3'-digallate, epicatechin gallate, rosmarinic acid, and naringenin were capable of simultaneously reducing the levels of PhIP, MeIQx, and 4,8-DiMeIQx. Moreover, the inhibition of the formation of one of these HAs was not compromised by the inhibition of the formation of another HA. Naringenin, a flavonoid found in many citrus fruits, was found to be the most promising inhibitor in both chemical model system and beef patties, suggesting its great potential for practical application in daily cuisine.

Keywords: Beef patties / Dietary phenolic compounds / Heterocyclic amine formation

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1 Introduction

Heating, especially high temperature-prolonged processing of muscle-based foods has been demonstrated, since 30 years ago, to induce the formation of a group of genotoxic compounds called heterocyclic amines (HAs). HAs have been shown to be potently mutagenic in bacteria mutagenicity test [1]. Some of them have been classified by IARC as probably and some as possible human carcinogens [2]. This implies significant carcinogenic potential as the human populations are frequently exposed to this group of genotoxic compounds. Over decades, ample research efforts have been devoted both to unraveling the mechanisms of formation of HAs and to developing strategies which would effectively reduce or prevent their formation using chemical model systems [3, 4] and real food matrices [5, 6]. Several laboratory studies have investigated the effects of cook-

ing temperature and time on the formation of HAs, and a clear correlation has generally been observed between these determinants and HA contents [4]. Other factors such as pretreatment with marinades, microwave cooking, and addition of synthetic antioxidants have also been examined [7–9]. Addition of plant extracts/tissues to meats and fishes is a common practice in many cultures, and the selective use of certain extracts and tissues may help prevent the formation of HAs. Apart from using plant tissues/extracts, such as cherry tissue [10], and antioxidant spices [5], several studies also used pure phenolic compounds in an effort to identify potent inhibitors [6, 11, 12]. So far, green tea catechins (catechin and epigallocatechin gallate (EGCG)) have been among the most promising inhibitors of HA formation in model systems [11].

In the past years, the formation pathways of HAs have been partially elucidated and the unstable free radical Mailard reaction intermediates have been suggested to play an important role in the formation of IQ and IQx type HAs [13, 14]. Phenolic compounds are well known for their antioxidant/free radical scavenging activities. Therefore, their roles in inhibiting the formation of these HAs have frequently been related to their free radical scavenging activities. However, there have been only limited reports which demonstrated a correlation between free radical scavenging capacities and inhibitory activities of phenolic compounds

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Abbreviations: EGC, epigallocatechin; EGCG, epigallocatechin gallate; HA, heterocyclic amine

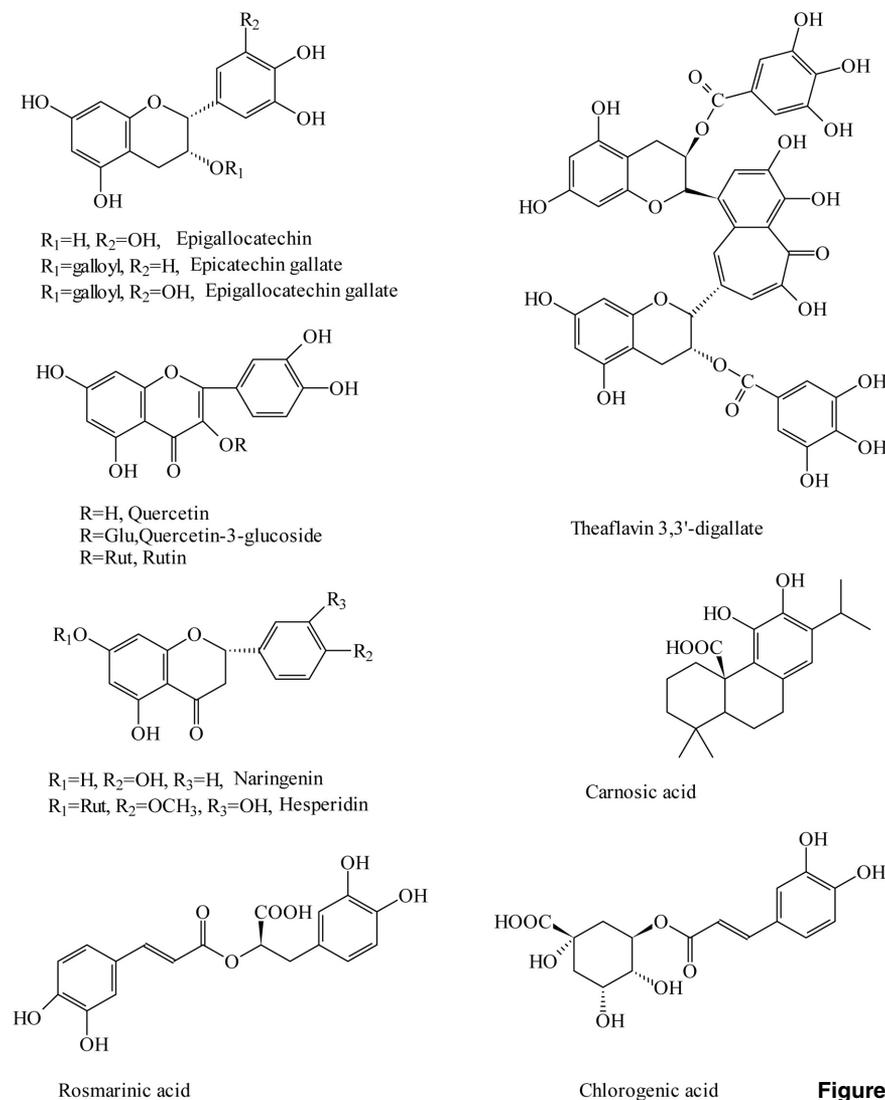


Figure 1. Structures of phenolic antioxidants.

in HA formation. The role of phenolic compounds in Maillard reaction related to HA formation should be more complex than just being free radical scavenging agents [15].

The aims of this study were to evaluate the relative activities of 12 food-derived dietary phenolic compounds (Fig. 1) in inhibiting HA formation; to research whether a correlation between their radical scavenging capacities and inhibitory activities in HA formation can be established; and to look for more powerful HA formation inhibitors of natural origins. Four of them, chlorogenic acid, quercetin, quercetin-3-glucoside, and rutin are common phenolic compounds found in various fruits and vegetables. Naringenin and hesperidin are mainly found in citrus fruits. Rosmarinic acid and carnosic acid are principal antioxidants in rosemary, which is a popular spice in household cooking. Others are four tea polyphenols, EGCG, epicatechin gallate (ECG), epigallocatechin (EGC), and theaflavin-3,3'-digallate, which are well known for their antioxidant properties.

2 Materials and methods

2.1 Materials

Phenylalanine, glucose, creatinine, diethylene glycol, NH_4OH , $NaOH$, silicone oil, ammonium acetate, triethylamine, HCl , dipotassium peroxosulfate, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), chlorogenic acid, hesperidin, naringenin, rosmarinic acid, rutin, and quercetin were purchased from Sigma–Aldrich Company (St. Louis, MO). Quercetin-3-glucoside, carnosic acid, EGC, ECG, and EGCG were obtained from Chromadex (Santa Ana, CA) and theaflavin 3,3'-digallate was purified by us from black tea using chromatographic methods. HA standards, 2-amino-3-methylimidazo[4,5-f]quinoline (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-trimethyl-

imidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-3,7,8-trimethyl-imidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx), and 2-amino-1-methyl-6-henylimidazo [4,5-*b*]pyridine (PhIP) were from Toronto Research Chemicals (Toronto, Canada). Propyl-sulfonic acid (PRS) Bond-Elut cartridges (500 mg), C-18 cartridges (100 mg), Bond-Elut reservoir, and packing materials (diatomaceous earth) were from Varian (Harbor City, CA). All solvents used were of analytical grade and were obtained from BDH Laboratory Supplies (Poole, UK). Fresh ground beef was purchased from a local supermarket. The Reacti-Therm III heating module (model 18840) and the screw cap Tuf-Bond Teflon fitted glass reaction vials (10 mL capacity) were purchased from Pierce (Rockford, IL).

2.2 Trolox equivalent antioxidant capacity (TEAC) of phenolic compounds

The assay was carried out according to the method of Re *et al.* [16] with a minor modification. Briefly, 7 mM ABTS salt solution was reacted with 2.45 mM potassium peroxy-sulfate solution (final concentration) and the reaction mixture was allowed to stand in the dark for 16 h at room temperature and was used within 2 days [17]. The resultant radical solution was diluted with deionized water to an absorbance of 0.7 ± 0.05 at 734 nm. Fifty microliters of diluted sample solution (in ethanol, except hesperidin which was dissolved in DMSO) or standard (different concentrations of Trolox) was added to 1.9 mL of diluted ABTS⁺ radical solution and absorbance was taken at 734 nm on a Shimadzu UV-1206 Spectrophotometer after 6-min incubation. Results were expressed as TEAC values (mmol trolox/mmol phenolic compound). Triplicate analyses were performed.

2.3 Effects of phenolic compounds on HA formation in PhIP-producing model system

The control model system contained 0.4 mmol phenylalanine, 0.4 mmol creatinine, and 0.2 mmol glucose, as described by others [18, 11]. 0.04 mmol of each tea polyphenol and 0.1 mmol of other polyphenols were added to the reaction vials in a powdery form. The reaction medium was 3 mL diethylene glycol containing 14% water. The vials were sealed with a screw cap fitted with Tuf-Bond Teflon. Three milliliters of silicone oil was added to each cavity of the heating block. The temperature meter of the heating block was then set to 125°C and preheated for 2 h before inserting the vials into the cavities. The total heating time was 2.5 h and the temperature was checked every half an hour. Fluctuation of temperature was within 2°C. The vials were immediately cooled in an ice–water mixture at the 2.5 h time point. The content of each vial was mixed with 57 mL of 2 M NaOH. Five milliliters of diluted aliquot was transferred into a 100-mL beaker. The aliquot was then

mixed thoroughly with diatomaceous earth before packing into a Bond-Elut reservoir fitted with a bottom frit (20 μm). Then elution was performed with 48 mL of dichloromethane directly into an attached PRS Bond-Elut cartridge. The PRS cartridges were dried under maximum vacuum for 5 min and were sequentially washed with 6 mL of 0.1 M HCl, 15 mL of 40% methanol in 0.1 M HCl, and 2 mL of water. The HAs were then eluted into Bond-Elut C-18 cartridges with 20 mL ammonium acetate solution (0.5 M, pH 8). Prior to use, the PRS and C-18 cartridges were conditioned according to a method suggested by Gross and Gruter [19]. The C-18 cartridges were washed with 2 mL of water and dried under positive pressure. The final elution was carried out with 1.2 mL of MeOH–NH₄OH (9:1 v/v) into microvials. The eluate was dried under nitrogen gas and the residue dissolved in 100 μL methanol for HPLC analysis.

The above procedure was also applied for investigating the dose-dependent effects (0.02, 0.04, 0.1, 0.2 mmol) of EGCG and naringenin on PhIP formation in model system.

2.4 Effects of phenolic compounds on HA formation in beef patties

An accurately weighed amount (30 ± 0.2 g) of ground beef was formed into a disk shape with the aid of a glass Petri dish (6.2 cm × 1.2 cm). Powders of selected phenolic compounds ($0.1 \pm 0.01\%$) were thoroughly mixed into batches of ground beef before forming into patties. The patties were covered with cling wrap and incubated in a refrigerator for 1 h. The patties were fried on a Teflon coated frying pan at a surface temperature of 200°C for 6 min on each side (3 min × 2 for each side). Three samples were processed for each treatment in each experiment and triplicate analyses were performed.

The three beef patties for each treatment were combined and homogenized in 60 mL of 1 M NaOH for 2 min to a dense paste. Four portions (each equivalent to 5 g beef patties) were weighed into separate beakers. Eighteen milliliters of 1 M NaOH was added to each beaker and the content stirred to form a suspension. These were then thoroughly mixed with diatomaceous earth and packed into separate reservoirs. The subsequent steps were the same as for the analysis of samples from PhIP model system, except that the extract from these four portions were combined and dissolved in 100 μL methanol for HPLC analysis.

2.5 HPLC analysis of HAs

HPLC analysis was performed on a Waters Alliance HPLC system with a 2695 separation module, an autosampler and a photodiode array detector (model 2996). Separation of HAs was carried out on an YMC-Pack Pro C-18 column (5 μm, 150 × 4.6 mm). The elution program was adopted from Shin *et al.*'s [20] study with a slight modification. The

mobile phase was water (0.01 M triethylamine, pH was adjusted to 3.2 using phosphoric acid)/ACN gradient with a flow rate of 1 mL/min. The initial ratio of water (0.01 M triethylamine, pH was adjusted to 3.2 using phosphoric acid)/ACN was 95:5, which was increased to 83:17 during the first 10 min. The ACN concentration continued to linearly increase to 75:25 for the next 10 min, and then linearly increase to 45:55 in the following 10 min and finally increased to 20:80 in 5 min. The total running time was 35 min and the postrunning time was 15 min for equilibration of the column. The HAs were monitored at two wavelengths, 265 and 312 nm. Before commencing an analytical run, the column was conditioned with the initial mobile phase composition for 30 min and the LC-PDA system tested for system stability using blank and standard HA solutions. Peak identification was accomplished by comparing the retention times and UV spectral characteristics of the HPLC peaks with those obtained from standard solutions of HA mixture analyzed under the same conditions. Quantitative determination was performed using an external calibration curve. Correlation coefficients (r^2) for HA standard curves were: 0.997 for PhIP, 0.999 for MeIQx, and 0.991 for 4,8-MeIQx. Triplicate analyses were performed for each treatment.

2.6 Statistical analysis

Statistical analyses were performed using the SPSS statistical package (SPSS, Chicago, IL). Paired samples *t*-test was applied to determine whether a particular treatment of the sample would result in a significantly different content of HAs compared with the control. $p < 0.05$ was selected as the level decision for significant differences.

3 Results and discussion

3.1 TEAC of phenolic antioxidants and their effects on the formation of PhIP in model system containing creatinine, glucose, and phenylalanine

The radical scavenging capacities of the 12 phenolic compounds are presented in Table 1. Among them, the four tea phenolics represent the top four free radical scavengers. In contrast, naringenin and hesperidin, the two flavonoids found in many citrus fruits, were the two least active ones. From the structure–activity point of view, the free radical scavenging capacities of phenolic compounds in *in vitro* assays such as ABTS free radical scavenging assay, mainly depend on the number of hydroxyl group on the aromatic rings of the tested compounds. The more hydroxyl groups the higher antioxidant activity is expected; moreover, the presence of a second hydroxyl group on the *ortho* or *para* position is going to increase the antioxidant activity [21–23]. Our results highly agree with this rule. Using EGC and

Table 1. Radical scavenging capacities of phenolic antioxidants determined by the TEAC assay

Compound	TEAC (mmol trolox per mmol phytochemical)
Theaflavin 3,3'-digallate	8.18 ± 0.240
EGCG	4.61 ± 0.040
ECG	4.58 ± 0.050
EGC	2.71 ± 0.020
Quercetin	2.34 ± 0.040
Rosmarinic acid	1.99 ± 0.030
Rutin	1.11 ± 0.010
Quercetin-3-glucoside	1.10 ± 0.005
Chlorogenic acid	0.96 ± 0.010
Carnosic acid	0.92 ± 0.010
Hesperidin	0.46 ± 0.004
Naringenin	0.42 ± 0.009

Each value is expressed as mean ± standard error of three replications.

EGCG as an example, EGCG showed much higher free radical scavenging capacity than EGC, due to the presence of one extra galloyl group which has three additional adjacent aromatic hydroxyl groups.

In this research, a chemical model system containing phenylalanine, glucose, and creatinine was chosen for evaluating the relative inhibitory activities of these phenolic compounds on HA formation as this model system was reported to be capable of producing PhIP as the dominant HA [20, 11] and PhIP is the most abundant HA formed in many meat products [24, 25]. The yield of PhIP was 28.3 ± 5.91 nmol/mmol creatinine (recovery = $72 \pm 18\%$) in the model system processed at $125 \pm 2^\circ\text{C}$ for 2.5 h. This is slightly lower than that (34.4 ± 8.37 nmol/mmol creatinine) reported in Shin *et al.*'s [20] study which had carried out the heating process at $180 \pm 5^\circ\text{C}$ for 30 min; however, much higher than that (8.36 nmol/mmol creatinine) of Oguri *et al.*'s [11] analysis which was conducted at 128°C for 2 h.

The first test using this chemical model was carried out with the four tea phenolic compounds. For them, only 0.04 mmol was used because of two considerations. These were the limited availability; and the findings in previous study that EGCG (0.2 mmol) demonstrated the most potent inhibitory activity on PhIP formation (96.8%) in a similar model system [11]. Adding too much of them to the model system may not facilitate the comparison of their relative activities. Figure 2 shows the percentages of the amounts of PhIP formed with the addition of these compounds, relative to the control. By preadding 0.04 mmol into the reaction mixture, all the four tea phenolic compounds clearly suppressed the formation of PhIP, with three of them, theaflavin 3,3'-digallate, EGCG, and ECG, being significantly different from the control ($p < 0.05$). This observation agrees well with our expectation that all these tea phenolic compounds should be good HA formation inhibitors as they are very active antioxidants.

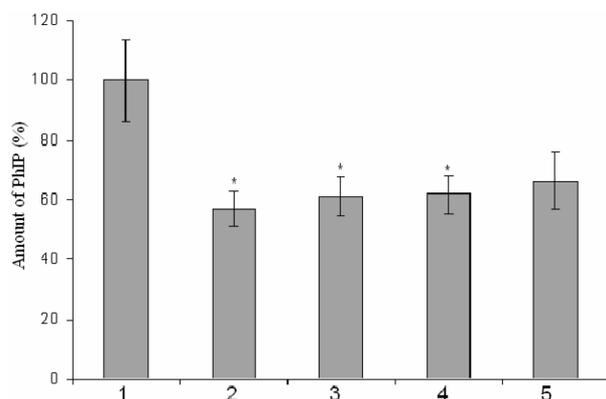


Figure 2. Relative percentages of the amounts of PhIP formed in a chemical model system containing creatinine, glucose, and phenylalanine with four tea phenolic compounds (0.04 mmol each). All treatments were made in triplicate. Bars with an asterisk indicate significant difference from the control ($p < 0.05$). 1, Control; 2, EGCG; 3, theaflavin-3,3'-digallate; 4, ECG; 5, EGC.

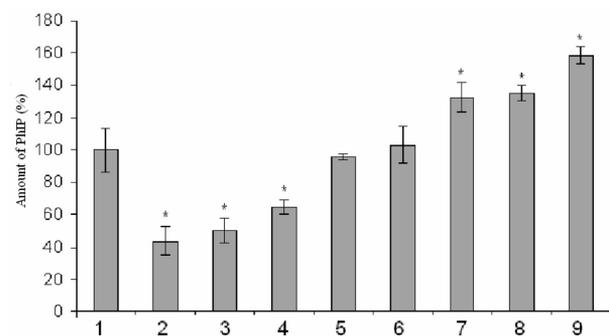


Figure 3. Relative percentages of the amounts of PhIP formed in a chemical model system containing creatinine, glucose, and phenylalanine with eight phenolic antioxidants (0.1 mmol each). All treatments were made in triplicate. Bars with an asterisk indicate significant difference from the control ($p < 0.05$). 1, Control; 2, naringenin; 3, quercetin-3-O-glucoside; 4, quercetin; 5, hesperidin; 6, rutin; 7, chlorogenic acid; 8, carnosic acid; 9, rosmarinic acid.

The experiment was further extended to include a wider range of phenolic compounds mentioned in the previous sections. As these compounds showed much weaker radical scavenging capacities in the TEAC assay, a larger amount (0.1 mmol) was added to the model in order to observe their effects on PhIP formation. For this group of compounds, interesting results were observed (Fig. 3). Naringenin, quercetin, and quercetin-3-glucoside were found to be capable of significantly ($p < 0.05$) reducing the level of PhIP in the model and the inhibitory activity of naringenin also was significantly better ($p < 0.05$) than that of quercetin. Quercetin has been shown to be a very effective inhibitor of the formation of PhIP in a similar model system by other group [11]. Using the same parameter, the mean inhibitory activity of

quercetin from replicate experiments, was found to be in good agreement with that reported in this previous study which used 0.2 mmol quercetin to produce a 77% reduction in PhIP level, relative to the control. However, the inhibitory effect observed with naringenin was surprising as naringenin showed the weakest free radical scavenging activity among the tested compounds. Another interesting observation was that rutin, the diglycoside of quercetin, although it is a good free radical scavenging agent, did not demonstrate the obvious suppressing effect on the formation of PhIP. In contrast, its addition slightly promoted PhIP formation. In addition, hesperidin, the diglycoside of hesperetin, was also ineffective against PhIP formation. Finally, all the three phenolic acids (rosmarinic, carnosic, and chlorogenic acids) examined strongly enhanced PhIP formation although they are good free radical scavenging agents in the ABTS free radical scavenging assay. The observation regarding the effect of phenolic acids on the formation of PhIP in model system is in agreement with that reported by Oguri *et al.* [11]. In their study, only caffeic acid showed mild inhibitory activity in PhIP formation while the other three acids (ellagic acid, ferulic acid, and syringic acid) were either ineffective or strongly promoted the formation of PhIP (~100 to ~380% of the control). Apart from the probable involvement of some reactive groups derived from these acids in PhIP formation [11], the pH lowering effect upon the addition of these phenolic acids to the model system, and the probable reaction of the carboxylic acid group with the amino group of phenylalanine may also influence its formation. In summary, our data clearly showed the inhibitory activity of a few phenolic compounds on PhIP formation in model system, but a good positive correlation with their free radical scavenging activities was not established.

The mechanism of formation of PhIP has been controversial. While it was proposed that radical involving reactions are not the dominant steps leading to PhIP formation [26], a very recent study attributed the inhibitory effects of rosemary extracts on PhIP formation to their antioxidant activities [6]. In Zochling *et al.*'s [26] study, conclusion was drawn based on the use of crude spice flavors which might contain many other components whose activities in the model system might overwhelm that of the target antioxidative compound(s). On the other hand, Tsen *et al.* [6] studied only a limited number of antioxidants without structural diversity. Our data tentatively suggested that radical scavenging may not be the principal mechanism of intervention executed by these phenolic compounds, or radical reaction is not the rate-limiting step for PhIP formation in model system. Many other factors might contribute to the inhibitory activities of these phenolic compounds.

Apart from identifying strong inhibitor(s), concentration-dependent effect of the inhibitor of interest was also examined. Based on the mean from replicate experiments and with reference to findings in previous studies [3, 11],

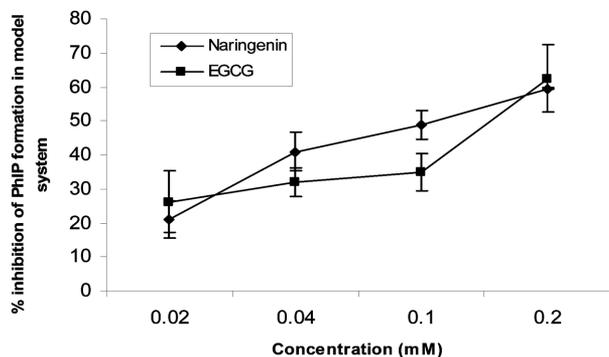


Figure 4. Effects of different concentrations of EGCG and naringenin on the formation of PhIP in the model system. Each data point represents the mean from three replicates and the vertical bars represent the standard errors of three replicates.

the activity of naringenin was compared with that of EGCG by using different molar quantities of these chemicals in the PhIP-producing model system. Both of them were capable of significantly reducing the level of PhIP formed at a level as low as 0.02 mmol. Despite the lack of a linear relationship between concentration and inhibitory activity in PhIP formation, the two phenolics showed a very similar trend (Fig. 4) over the concentration range examined in this study (0.02, 0.04, 0.1, and 0.2 mmol). From the above analyses, it can be concluded that the activity of naringenin is at least comparable to that of EGCG.

3.2 Effect of phenolic antioxidants on the formation of mutagenic HAs in beef patties

It should be noted that although rosmarinic acid and carnosic acid both greatly increased the level of PhIP formed in model system, it was recently reported that rosmarinic acid and a rosemary antioxidant powder of known carnosic acid content (16–18%) effectively reduced the formation of PhIP in beef patties [6]. On the other hand, naringenin, ECG, EGC, and theaflavin-3,3'-digallate, to the best of our knowledge, have not yet been investigated for their effects on HA formation. Therefore, the effects of ECG, rosmarinic acid, carnosic acid, and the most potent inhibitor identified, naringenin, were further tested using beef patties. Only the polar HA extract was of interest for evaluation since HAs

contained in this fraction account for most of the HA-associated mutagenic/carcinogenic activity in foods [27]. A frying temperature of 200°C was chosen considering previous findings that formation of PhIP may not be favored at low temperatures, whereas at temperatures higher than 200°C, degradation of HAs may become significant [4].

Three HAs were identified from the polar extract. These were PhIP, MeIQx, and 4,8-DiMeIQx (Fig. 5) with corresponding yield of 7.83 ± 0.78 , 7.80 ± 0.84 , and 2.69 ± 0.44 ng/g, respectively. The yield for PhIP was much higher than that reported by Tsen *et al.* [6]. Moreover, 4,8-DiMeIQx, which was usually absent from cooked beef [6, 28] or produced only after prolonged (30 min) period of heat treatment at a much higher temperature (>250°C) [25], was also produced. The much larger surface area-to-mass ratio of the patties used in this study may partly explain the variations as it was suggested that the high surface temperature and maintenance at such high temperature may promote the formation of HAs [29, 30]. The average extraction recoveries for the above HAs were $71 \pm 15\%$ for MeIQx, $55 \pm 16\%$ for 4,8-DiMeIQx, and $56 \pm 18\%$ for PhIP, similar to those reported in previous studies [6, 31].

Table 2 presents the yields of HAs in beef patties with the addition of different phenolic compounds (0.1%) together with their percentage of inhibition relative to the control. Except for carnosic acid, all the compounds tested showed significant inhibition ($p < 0.05$) of the formation of polar HAs (Table 2). Based on the means from three replicate experiments, the relative inhibitory potency follows the same order (naringenin > theaflavin 3,3'-digallate > rosmarinic acid > ECG > carnosic acid) for all the three polar HAs identified. The opposite effects on the formation of PhIP in the model system and beef patties concerning rosmarinic acid and carnosic were in good agreement with those reported by previous studies [26, 6]. Apart from the consideration that the formation mechanisms of PhIP may differ in model system and in meat [26], the different components of these systems may have large impact on the effective interaction between the phytochemicals added and the purported intermediates of PhIP formation. This also points out that although chemical model systems are, in general good surrogates for investigating the chemistry of HAs encountered in foods, corroboration using real food system is critical before inferring the findings to actual applications in cooking practices.

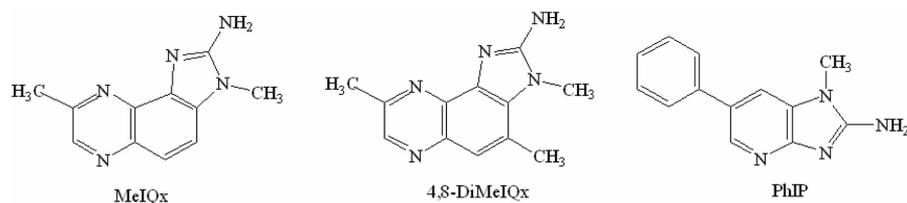


Figure 5. Structures of HAs identified from fried beef patties.

Table 2. Effect of phenolic antioxidants (0.1%) on the formation of HAs in beef patties fried at 200°C for 6 min on each side

Treatment	HAs (ng/g beef patties) and inhibition (%)			
	PhIP	4,8-DiMeIQx	MeIQx	Total (HAs)
Control	7.83 ± 0.78 ^c	2.69 ± 0.44 ^c	7.80 ± 0.84 ^c	18.3
Naringenin	2.05 ± 0.18 ^d (74)	1.00 ± 0.16 ^d (63)	2.58 ± 0.22 ^d (67)	5.6 (70)
Theaflavin 3,3'-digallate	3.82 ± 0.87 ^d (51)	1.34 ± 0.18 ^d (50)	3.58 ± 0.32 ^d (54)	8.7 (52)
ECG	5.50 ± 0.99 ^d (30)	1.51 ± 0.36 ^d (44)	4.32 ± 0.95 ^d (45)	10.3 (38)
Rosmarinic acid	5.21 ± 0.71 ^d (33)	1.46 ± 0.38 ^d (46)	4.03 ± 0.88 ^d (48)	10.7 (42)
Carnosic acid	6.39 ± 0.40 ^c (18)	1.81 ± 0.41 ^c (33)	5.72 ± 1.30 ^d (27)	13.9 (24)

Each value is expressed as mean ± SD ($n = 3$). Means with different letters in the same column are significantly different ($p < 0.05$).

4 Concluding remarks

Previous studies frequently attributed their findings for the inhibitory effects of phenolic compounds on HA formation to their free radical scavenging/antioxidant activities. However, the poor correlation demonstrated in this study between the TEAC of 12 phenolic antioxidants and their inhibitory activities in the formation of PhIP in model system suggests that antioxidation, more specifically, radical scavenging activity, may not be the principal mechanism of intervention of these phytochemicals. Alternatively, this provides evidence that a radical involving reaction may not be the rate-limiting step for the formation of PhIP. The observations that flavonoid diglycosides, and phenolic acids were generally ineffective in suppressing PhIP formation and that naringenin (a weak phenolic antioxidant), quercetin and tea phenolics were the promising inhibitors would require further study to clarify the detailed mechanism(s) of inhibition. In addition, our research based on both model system and beef patties studies identified naringenin as the most active inhibitor. Its capability of simultaneously suppressing the formation of three major HAs in beef patties, and its natural origin suggest a great potential for practical application in daily cuisine.

5 References

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