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Food and Chemical Toxicology 40 (2002) 989–1000

www.elsevier.com/locate/foodchemtox

Research Section

Effects of soy sauce and sugar on the formation of heterocyclic amines in marinated foods

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Accepted 7 November 2001

Abstract

The effects of soy sauce and sugar on the formation of heterocyclic amines (HAs) in marinated pork, eggs, and bean cakes were studied. Food samples were immersed in water in the presence of various levels of soy sauce and sugar, and the mixtures were subjected to simmering at 98±2 °C for 1 h in a closed saucepan. The various HAs in marinated food samples were analyzed by HPLC with photodiode–array detection. Results showed that seven HAs: 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ); 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx); 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ); 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx); 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1); 2-amino-1-methyl-6-phenylimidazo[4,5-*f*]pyridine (PhIP); and 2-amino-9*H*-pyrido[2,3,-*b*]indole (AαC) were detected in marinated pork, while five HAs: IQ, MeIQx; 4,8-DiMeIQx; PhIP; and AαC in bean cakes, as well as four HAs, MeIQx, 4,8-DiMeIQx, Trp-P-1 and PhIP in eggs. In most samples PhIP was formed in largest amount, followed by MeIQx, 4,8-DiMeIQx, IQ, AαC, Trp-P-1 and MeIQ. The amounts of HAs produced in marinated food samples followed an increased order for each increasing level of soy sauce or sugar. Marinated juice was found to contain a higher content of HAs than marinated foods.

Keywords: Soy sauce; Sugar; Heterocyclic amines; HPLC; Marinated foods

1. Introduction

Heterocyclic amines (HAs) represent an important class of toxicological compounds that are widely distributed in cooked fish and meat products (Chiu et al., 1998; Felton et al., 1998; Wakabayashi and Sugimura, 1998; Chen et al., 2000). Epidemiological studies have shown a possible relationship between consumption of HAs and occurrence of human cancer such as colorectal and pancreatic cancer (Gerhardsson de Verdier, 1995). The modulation of mutagenicity and carcinogenicity of HAs by dietary factors was thoroughly reviewed by Wakabayashi and Sugimura (1998). Felton et al. (1998) determined the calculated risk of consumption of HAs and concluded that the risk should be equivalent to that for many carcinogens that are regulated. Thus, it is important to learn more about the HAs contents in different varieties of meat products on the market. The types and amounts of HAs formed in cooked meat products could be attributed to time, temperature and method of cooking (Abdulkarim and Smith, 1998; Knize et al., 1998). Of these parameters, cooking temperature has been reported to be the most important (Kinze et al., 1985; Skog et al., 1997, 1998). In several reviews dealing with the effect of processing methods on HAs formation in meat products, both broiling and frying were reported to be the two major processing methods causing formation of high amount of HAs (Stavric, 1994; Skog et al., 1998). Interestingly, in a recent study Chiu et al.(1998) reported that the IQ type of HAs could be inhibited by microwave cooking, while

Abbreviations: AαC, 2-amino-9*H*-pyrido[2,3,-*b*]indole (CAS No. 26148-68-5); 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (CAS No. 95896-78-9); 7,8-DiMeIQx, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (CAS No. 92180-79-5); Glu-P-1, 2-amino-6-methyldipyrido[1,2-a:3′,2′-*d*]imidazole (CAS No. 67730-11-4); Glu-P-2, 2-amino-dipyrido[1,2-a:3′,2′-*d*]imidazole (CAS No. 67730-10-3); Harman, 1-methyl-9*H*-pyrido[4,3-*b*]indole (CAS No. 486-84-0); HAs, heterocyclic amines; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline (CAS No. 76180-98-6); IQx, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (CAS No. 108354-47-8); MeAαC, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (CAS No. 68806-83-7); MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (CAS No. 77094-11-2); MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (CAS No. 77500-04-0); Norharman, 9*H*-pyrido[4,3-*b*]indole (CAS No. 244-63-3); PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*f*]pyridine (CAS No. 105650-23-5); 4,7,8-TriMeIQx, 2-amino-3,4,7,8-tetramethylimidazo[4,7,8-*f*]quinoxaline; Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (CAS No. 62450-06-0); Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (CAS No. 62450-07-1).

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0278-6915/02/$ - see front matter
PII: S0278-6915(02)00013-3

both IQ and Carboline types of HAs could not be inhibited by frying.

Marinated foods such as ground pork, bean cakes and eggs are traditional Chinese food commodities consumed in both Taiwan and China. "Marinated" means immersing the food items in a marinade for 1 h or longer at about boiling temperature. They are often prepared by mixing food samples with various kinds of ingredients such as soy sauce, sugar, salt, spice and seasonings, and marinated in the presence of water at about 100 °C for an extensive period of time. However, no information is available as to the presence of HAs in these marinated foods. The objectives of this study were to determine the HAs contents in Chinese marinated foods in the presence of various levels of soy sauce and sugar.

2. Materials and methods

2.1. Materials

Raw pork, eggs and bean cakes as well as ingredients such as soy sauce and rock candy, consisting mainly of sucrose, were purchased from a local supermarket in Taipei. Chemicals such as hydrochloric acid, sodium hydroxide, ammonium acetate, lead acetate, potassium oxalate and trichloroacetic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Solvents including methanol, methylene chloride and acetonitrile were from Merck Co. (Darmstadt, Germany). The HPLC–grade solvent acetonitrile was degassed by sonication and filtered through a 0.2-μm membrane filter prior to use. Deionized water was produced using a water purification system by Millipore Co. (Bedford, MA, USA). The propylsulfonic acid silica gel cartridge (500 mg) was from Varian Co. (Harbor, CA, USA) and C18 cartridge (100 and 500 mg) was from J. T. Baker Co. (Philipsburg, NJ, USA). A TSK-CEL ODS C18 column (250×4.6 mm I.D, 5 μm) by Tosoh Co. (Tokyo, Japan) was used for separation of HAs by HPLC.

Sixteen HAs standards: IQ (CAS No. 76180-98-6); 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQx; CAS No. 108354-47-8); 2-amino-,3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx; CAS No, 77500-04-0); 2-amino-3,4-dimethylimidzo[4,5-*f*]quinoline (MeIQ; CAS No. 77094-11-2); 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx; CAS No. 95896-78-9); 2-amino-3,7,8-trimethyl imidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx; CAS No. 92180-79-5); 2-amino-9*H*-pyrido[2,3,-*b*]indole (AαC; CAS No. 26148-68-5); 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]-indole (MeAαC; CAS No. 68806-83-7); 2-amino-1-methyl-6-phenyl imidazo[4,5-*f*]pyridine (PhIP; CAS No. 105650-23-5); 2-amino-6-methyldipyrido[1,2-a:3′,2′-*d*] imidazole (Glu-P-1; CAS No. 67730-11-4); 2-amino-dipyrido[1,2-a:3′,2′-*d*]imidazole (Glu-P-2; CAS No. 67730-10-3); and internal standard 2-amino-3,4,7,8-tetramethylimidazo[4,7,8-*f*]quinoxaline (4,7,8-TriMeIQx) were from Toronto Research Chemical Co. (Downsview, Ontario, Canada); 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1; CAS No. 62450-06-0) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2; CAS No. 62450-07-1) were from Wako Co. (Osaka, Japan); 1-methyl-9*H*-pyrido[4,3-*b*]indole (Harman; CAS No. 486-84-0) and 9*H*-pyrido[4,3-*b*]indole (Norharman; CAS No. 244-63-3) were from Aldrich Co. (Steinheim, Germany).

2.2. Instrumentation

The HPLC instrument is composed of two Jasco PU-980 pumps (Jasco Co., Tokyo, Japan), a Rheodyne 7161 injector (Rheodyne Co., Rohnert Park, CA), a Phenomenex DG-440 degassing system (Phenomenex Co., Torrance, CA, USA), a Jasco MD-915 photodiode–array detector and a Borwin computer software for processing data. The Sorvall RC5C high speed centrifuge was from Du Pont Co. (Wilmington, Delware). The homogenizer (model HG-2800) was from Hsiang-Tai Co. (Taipei, Taiwan). The Beckman 6300 amino acid analyzer was from Beckman Co. (Fullerton, CA, USA).

2.3. Processing of marinated foods

Raw ground pork, bean cakes and eggs were used as raw materials for marinating. To study the effect of soy sauce on the formation of HAs in marinated foods, the unmarinated juice was prepared by mixing one level (1%) of sugar, various levels (5, 10 and 20%) of soy sauce and different amounts of deionized water. A total of 2 l unmarinated juice was thus obtained by mixing the following ingredients in three treatments: (1) 20 g sugar, 100 ml soy sauce and 1880 ml deionized water; (2) 20 g sugar, 200 ml soy sauce and 1780 ml deionized water; (3) 20g sugar, 400 ml soy sauce and 1580 ml deionized water. Each unmarinated juice was poured into a 4-l stainless-steel saucepan with a lid on the top and heated by gas. After boiling, 500 g raw ground pork was poured into the saucepan and heating was continued for 1 h with a small fire. The temperature was controlled at 98±2 °C and monitored with a thermometer all the time during heating. Raw eggs and bean cakes were also marinated separately in the same way as raw ground pork, with the exception that the raw eggs were boiled at 100 °C for 5 min and then shelled before marinating. After marinating, 50 g ground pork was collected for HAs analysis. Prior to analysis, the ground pork was freeze dried and ground into a fine powder and 10-g sample was obtained for HAs determination. Both eggs and bean cakes were cut into small pieces and freeze dried before grinding into fine powder, and 10-g sample of each was collected for

HAs analysis. Twenty millilitres of marinated juice each of pork, eggs and bean cakes were also collected separately and filtered through a 0.2-μm membrane filter for HAs analysis. Two eggs with an average weight of about 15 g each and four bean cakes with an average weight of about 25 g were used for each treatment.

To study the effect of sugar on the formation of HAs in marinated foods, the unmarinated juice was prepared by mixing one level (10%) of soy sauce, various levels (0.5, 1.0 and 5.0%) of sugar and different amounts of deionized water. A total of 2-l unmarinated juice was thus obtained by mixing the following ingredients in three treatments: (1) 200 ml soy sauce, 10 g sugar and 1790 ml deionized water; (2) 200 ml soy sauce, 20 g sugar and 1780 ml deionized water; (3) 200 ml soy sauce, 100 g sugar and 1700 ml deionized water. Each unmarinated juice was poured into a saucepan and food samples were added for heating in the same way as described above, after which a portion of both food and juice samples were collected for HPLC analysis of the HAs.

2.4. Analysis of HAs in marinated food samples and juice

A method developed by Gross and Gruter (1992) and modified by Chen and Yang (1998) was used to analyze the HAs contents in marinated food samples and juice. A standard addition method was used to determine the extraction efficiency of HAs. A mixture of 50 ng each of 15 HAs was added to ground pork samples for extraction. After extraction and collection of both the apolar and polar HA extracts, the mixture was filtered through a 0.2-μm membrane filter and evaporated to dryness. Then the HA extract was dissolved in 100 μl of methanol for recovery determination by HPLC. The recovery of each HA was obtained based on the ratio of the calculated concentration to the added concentration. The recovery of HAs in food samples such as eggs and bean cakes were assessed to be equivalent to that of ground pork. Although peaks 6 and 7 were partially overlapped, the recovery could still be determined because the straight baseline could be extended using our software system and the actual area of the triangles for both peaks 6 and 7 could be calculated. In addition, eight concentrations of each HA ranging from 3 ppm to 5 ppb and internal standard 1 ng/ml were mixed and injected into HPLC for preparation of standard curves as described by Chen and Yang (1998). Quantitative measurements of HAs in food samples were carried out using the standard curves. Duplicate analyses were performed and the standard deviations of data were calculated.

2.5. Analysis of free amino acids in soy sauce and marinated food samples

The levels of free amino acids in soy sauce and marinated food samples were analyzed using a method described in a previous study (Chen et al., 2000).

2.6. Analysis of reducing sugars (glucose) in marinated juice and food samples

The levels of glucose in soy sauce, marinated juice and food samples were measured based on a method in a previous report (Chen et al., 2000).

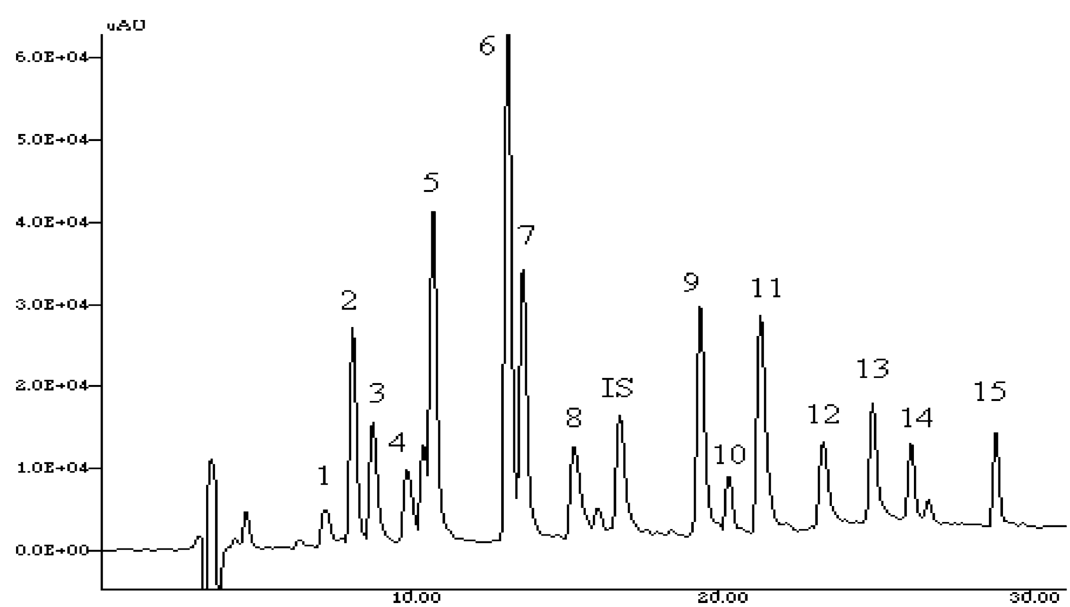

Fig. 1. HPLC chromatogram of 15 HAs containing 1 internal standard with photodiode–array detection at 258 nm. Peaks: 1, Glu-P-2: 2, IQx; 3, IQ; 4, Glu-P-1; 5, MeIQx; 6, MeIQ; 7, 7,8-DiMeIQx; 8, 4,8-DiMeIQx; I.S, internal standard; 9, Harman; 10, Norharman; 11, Trp-P-1; 12, PhIP; 13, Trp-P-2; 14, AαC; 15, Me AαC.

2.7. Analysis of creatinine in marinated juice and food samples

The contents of creatinine in marinated juice and food samples were determined using a method described by Henry (1974).

3. Results

3.1. Analysis of 16 HAs by HPLC

Fig. 1 shows the HPLC chromatogram of 16 HAs including one internal standard. Most peaks were adequately resolved within 30 min with the exception of peaks 6 and 7, which were partially overlapped. Nevertheless, it would not interfere with identification of HAs. The detection limits of 15 HAs standards based on signal–to–noise ratio of 3 were found to be: Glu-P-2 (0.2 ng), IQx (0.2 ng), IQ (0.1 ng), Glu-P-1 (0.1 ng), MeIQx (0.2 ng), MeIQ (0.1 ng), 7,8-DiMeIQx (0.1 ng), 4,8-DiMeIQx (0.05 ng), Norharman (0.1 ng), Harman (0.1 ng), Trp-P-1 (0.05 ng), PhIP (0.02 ng), Trp-P-2 (0.1 ng), AαC (0.1 ng) and MeAαC (0.3 ng). However, in real food samples, the detection limits for IQ, MeIQx, MeIQ, 4,8-DiMeIQx, Trp-P-1, PhIP and AαC were 0.13, 0.30, 0.15, 0.06, 0.12, 0.03 and 0.21 ng, respectively. The detection limits of most HAs were found to be higher than those in a previous report (Chen and Yang, 1998), mainly because only single wavelength (258 nm) was used for simultaneous detection in this study. The recoveries of 15 HAs based on duplicate determinations were as follows: Glu-P-2 ($92.1\pm5.2\%$), IQx ($91.6\pm3.1\%$), IQ ($88.6\pm2.7\%$), Glu-P-1($85.7\pm3.0\%$), MeIQx ($89.7\pm6.2\%$), MeIQ ($88.7\pm6.2\%$), 7,8-DiMeIQx ($84.6\pm5.1\%$), 4,8-DiMeIQx ($86.4\pm5.3\%$), Norharman ($90.3\pm3.2\%$), Harman ($87.4\pm1.8\%$), Trp-P-2 ($84.0\pm3.1\%$), PhIP ($62.1\pm4.3\%$), Trp-P-1 ($70.9\pm3.6\%$), AαC ($74.8\pm6.1\%$) and MeAαC ($73.2\pm2.5\%$). These data were different from a study by Chen and Yang (1998), which may be accounted for by the variety of sample used. The identity of HAs was further confirmed by comparing UV spectra of unknown peaks with reference standards. Figures 2, 3 and 4 show the UV spectra of several HAs in marinated pork, eggs and bean cakes, respectively. Surprisingly, there is no strong maxim around 310–330 nm for the spectra of both PhIP and AαC standards, as well as those in food samples, probably because of interference by the presence of impurities both in HAs standards and food samples.

3.2. Effect of soy sauce on the HAs formation in marinated foods and juice

Table 1 shows the contents of HAs in marinated foods and juice in the presence and absence of various levels of soy sauce and 1% rock candy. Seven HAs, including IQ, MeIQx, MeIQ, 4,8-DiMeIQx, Trp-P-1, PhIP and AαC, were formed in all the marinated pork and juice samples in the presence of soy sauce, however, in the absence of soy sauce, only 4,8-DiMeIQx was present in pork, and both 4,8-DiMeIQx and PhIP in juice. In most pork and juice samples, 4,8-DiMeIQx was formed in largest amount, followed by MeIQx, PhIP, IQ, Trp-P-1, AαC and MeIQ. A smaller variety of HAs was found in marinated eggs and juice, namely, MeIQx, 4,8-DiMeIQx, Trp-P-1 and PhIP, after addition of soy sauce, and, in most samples, PhIP showed the highest level, followed by MeIQx, Trp-P-1 and 4,8-DiMeIQx. Without soy sauce, no HAs were detected in marinated eggs, while both 4,8-DiMeIQx and PhIP were in juice. In the marinated bean cake and juice, IQ, MeIQx, 4,8-DiMeIQx, PhIP and AαC were present. The greatest concentration occurred for 4,8-DiMeIQx, followed by PhIP, MeIQx, AαC and IQ, in most samples and presence of soy sauce. Without soy sauce, only PhIP was formed in both bean cake and juice. For all the food samples, marinated juice was found to contain a higher amount of HAs than marinated food, and the content of each HA increased with increasing levels of soy sauce.

In the presence of 1% rock candy and 10% soy sauce, the glucose contents in raw ground pork, eggs and bean cake were found to be 0.57, 0.48 and 0.57 mg/g, respectively, however, it dropped to 0.48, 0.39 and 0.54 mg/g, after 1 h marinating (Table 2). On the contrary, only 0.01 and 0.02 mg/ml were present in the unmarinated juice of pork and bean cake, respectively, and then increased to 0.86 and 0.60 mg/ml, after marinating for 1 h (Table 2). No glucose was detected in the egg juice before heating probably because of sample variation, as the unmarinated juices of pork, bean cake and eggs were prepared separately. Apparently the large increase of glucose level could be attributed to decomposition of rock candy (95% sucrose) during heating. No fructose was analyzed because we assume both glucose and fructose should react in the same way for HAs formation. The creatinine content in pork samples decreased after marinating, but a concentration of 0.15 mg/ml was found in the marinated juice of pork, which may be explained by leaching of creatinine from pork into juice during marinating.

A small amount of creatineine was found in the egg before but not after marinating. No creatinine was detected in egg juice or in bean cake samples. The amount of creatinine showed a declined trend in pork and egg juices,. No creatinine was detected in bean cake juice, because it is normally found only in tissues from muscles. A slight amount of creatinine was present in egg juice without or with 5% soy sauce. As soy sauce was found to contain 5% sucrose, the increase of glucose concentration could be due to sucrose decomposition during marinating. Theoretically a reduction of glucose in marinated juice is supposed to occur because of its participation in the Maillard reaction. However, the substantial increase of glucose level observed in this study revealed that the degradation rate of rock candy may be greater than the formation rate of HAs. A slight variation of the glucose concentration in the marinated juice of food samples containing 10% soy sauce and 1% rock candy was found in Tables 2 and 3, mainly because this treatment was conducted repeatedly for each food item at different time intervals. The creatinine concentration showed the same phenomenon.

Table 4 shows the levels of amino acids in soy sauce, ground pork, eggs and bean cake before and after cooking for 1 h. The levels of most amino acids in food samples increased during marinating, which may be due to leaching from soy sauce into pork, eggs or bean cake.

3.3. *Effects of sugar on the HAs formation in marinated foods and juice*

Table 5 shows the HAs contents in marinated foods in the presence and absence of various levels of rock candy

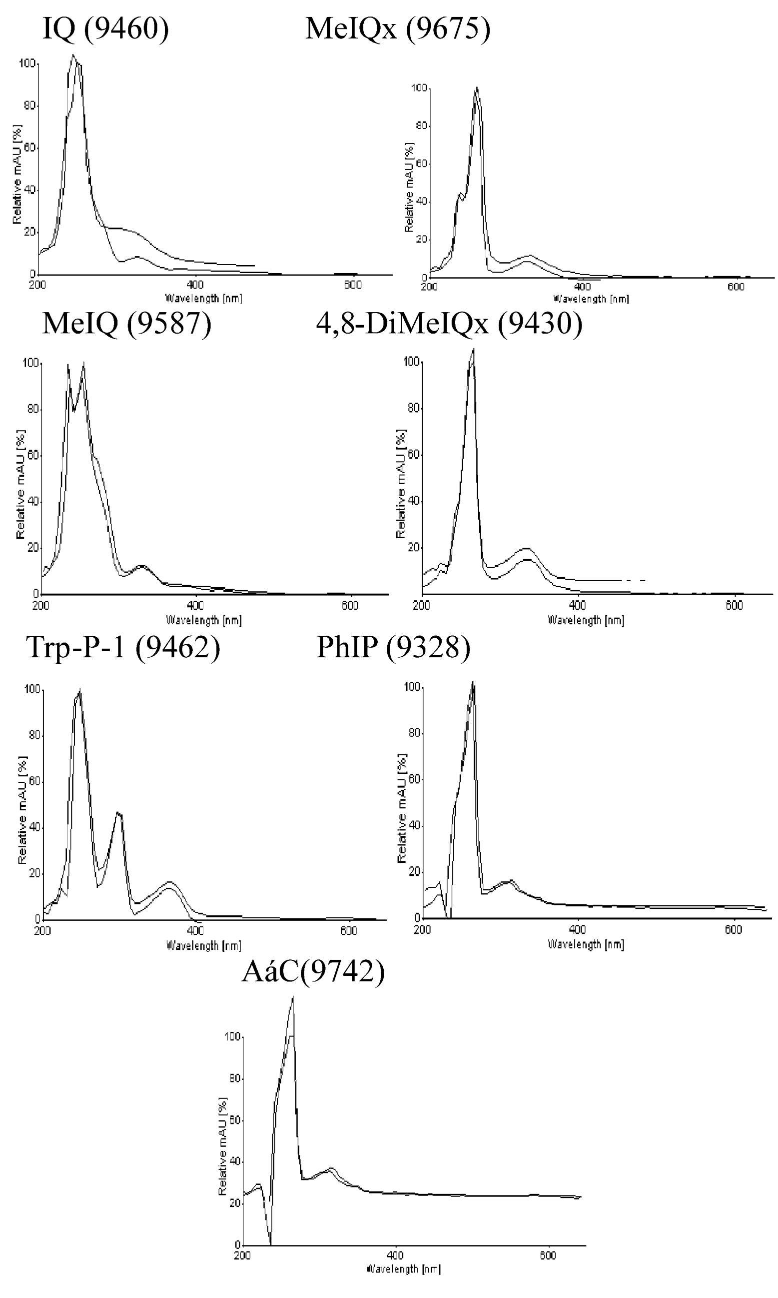

Fig. 2. UV spectra of IQ, MeIQx, MeIQ, 4,8-DiMeIQx, Trp-P-1, PhIP, AαC in marinated pork. Detected by a photodiode–array detector.

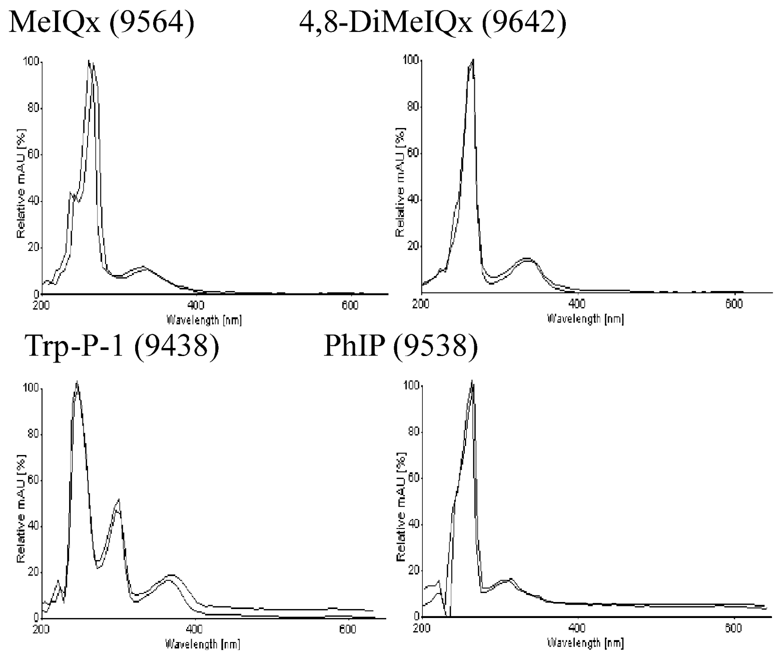

Fig. 3. UV spectra of MeIQx, 4,8-DiMeIQx, Trp-P-1, PhIP in marinated eggs. Detected by a photodiode–array detector.

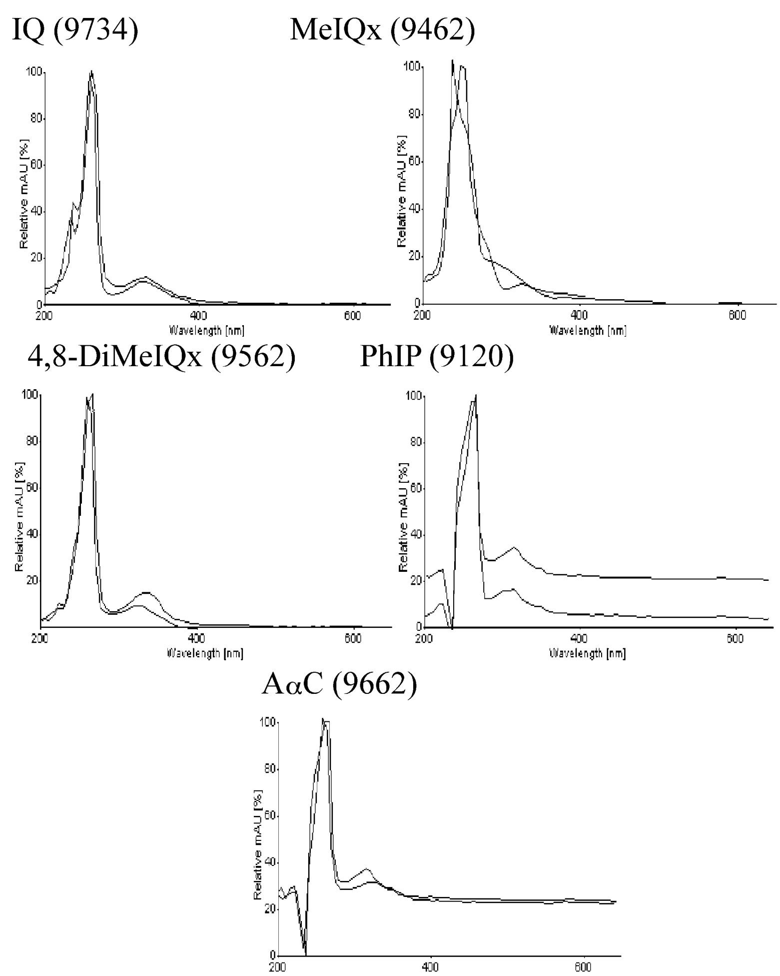

Fig. 4. UV spectra of IQ, MeIQx, 4,8-DiMeIQx, PhIP, AαC in marinated bean cake. Detected by a photodiode–array detector.

Table 1
Contents of HAs in marinated foods (ng/g) and juice (ng/ml) in the presence and absence of various levels of soy sauce and 1% rock candy

Compound	Soy sauce (%)[a]																							
	0						5						10						20					
	Pork	Juice	Egg	Juice	Bean cake	Juice	Pork	Juice	Egg	Juice	Bean cake	Juice	Pork	Juice	Egg	Juice	Bean cake	Juice	Pork	Juice	Egg	Juice	Bean cake	Juice
IQ	N.D.[c]	N.D.	N.D.	N.D.	N.D.	N.D.	0.71 (0.14)	0.93 (0.32)	N.D.	N.D.	0.13 (0.08)	0.53 (0.37)	0.74 (0.27)	1.45 (0.63)	N.D.	N.D.	0.22 (0.14)	0.66 (0.36)	0.82 (0.47)	1.73 (0.76)	N.D.	N.D.	0.43 (0.25)	0.74 (0.38)
MeIQx	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.24 (0.26)	1.93 (0.61)	0.32 (0.23)	0.63 (0.38)	0.52 (0.24)	0.84 (0.25)	1.34 (0.45)	1.85 (1.02)	0.44 (0.22)	0.82 (0.54)	0.63 (0.35)	1.13 (0.54)	1.44 (0.62)	3.03 (2.15)	0.53 (0.25)	1.25 (0.84)	0.73 (0.16)	1.82 (1.25)
MeIQ	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.24 (0.08)	0.74 (0.27)	N.D.	N.D.	N.D.	N.D.	0.33 (0.13)	0.83 (0.63)	N.D.	N.D.	N.D.	N.D.	0.44 (0.23)	0.93 (0.53)	N.D.	N.D.	N.D.	N.D.
4,8-DiMeIQx	0.06 (0.02)[b]	0.07 (0.03)	N.D.	0.06 (0.02)	N.D.	N.D.	1.93 (0.57)	2.83 (1.12)	0.15 (0.14)	0.53 (0.26)	1.53 (0.86)	2.04 (1.36)	1.95 (0.85)	1.46 (0.87)	0.25 (0.16)	0.66 (0.28)	1.63 (0.68)	1.04 (0.87)	2.04 (1.25)	3.75 (2.68)	0.43 (0.18)	1.17 (0.36)	1.82 (1.54)	2.83 (1.67)
Trp-P-1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.53 (0.12)	0.82 (0.43)	0.14 (0.08)	0.63 (0.45)	N.D.	N.D.	0.56 (0.12)	0.95 (0.26)	0.26 (0.32)	0.75 (0.26)	N.D.	N.D.	0.62 (0.28)	1.36 (0.98)	0.52 (0.37)	1.04 (0.58)	N.D.	N.D. .
PhIP	N.D.	0.04 (0.01)	N.D.	0.03 (0.01)	0.03 (0.02)	0.05 (0.04)	1.22 (0.36)	1.47 (0.38)	0.33 (0.16)	0.91 (0.65)	0.82 (0.53)	1.82 (0.58)	1.27 (0.54)	2.32 (1.53)	0.43 (0.15)	0.82 (0.37)	0.93 (0.45)	1.83 (1.23)	1.34 (1.23)	2.66 (1.87)	0.84 (0.56)	1.45 (1.23)	1.04 (0.35)	2.83 (0.87)
AαC	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.43 (0.28)	0.77 (0.26)	N.D.	N.D.	0.23 (0.18)	0.71 (0.26)	0.52 (0.14)	0.85 (0.63)	N.D.	N.D.	0.33 (0.13)	0.91 (1.12)	0.62 (0.35)	1.05 (0.45)	N.D.	N.D.	0.62 (0.27)	1.83 (0.56)
Total	0.06	0.11	0.00	0.09	0.03	0.05	6.30	9.49	0.94	2.70	3.23	5.94	6.71	9.71	1.38	3.05	3.74	5.57	7.32	14.51	2.32	4.91	4.64	10.05

[a] Average of duplicate analyses.
[b] Values in parentheses represent ± standard deviation.
[c] N.D. = not detected.

Table 2
Contents of glucose and creatinine in marinated foods and juice before and after marinating for 1 h in the presence of 1% rock candy and 10% soy sauce[a]

Sample	Marinated sample (mg/g)				Marinated juice (mg/ml)			
	Glucose		Creatinine		Glucose		Creatinine	
	Before marinating	After marinating for 1 h	Before marinating	After marinating for 1 h	Before marinating	After marinating for 1 h	Before marinating	After marinating for 1 h
Ground pork	0.57±0.16	0.48±0.09	2.84±0.76	0.92±0.23	0.01±0.01	0.86±0.37	N.D.	0.15±0.04
Egg	0.48±0.23	0.39±0.14	0.03±0.01	N.D.[b]	N.D.	0.81±0.23	N.D.	N.D.
Bean cake	0.57±0.18	0.54±0.12	N.D.	N.D.	0.02±0.01	0.60±0.15	N.D.	N.D.

[a] Average of duplicate analyses ± standard desiation.
[b] N.D. = not detected.

Table 3
Contents of glucose and creatinine in marinated juice of food samples after cooking for 1 h in the presence of various levels of soy sauce and 1% rock candy

Marinated juice	Soy sauce (%)[a]							
	Glucose (mg/ml)				Creatinine (mg/ml)			
	0	5	10	20	0	5	10	20
Ground pork	0.28±0.08	0.42±0.15	0.89±0.15	1.42±0.56	0.41±0.12	0.23±0.08	0.18±0.05	0.06±0.03
Egg	0.32±0.14	0.54±0.09	0.82±0.23	0.73±0.14	0.02±0.02	0.01±0.02	N.D.	N.D.
Bean cake	0.48±0.23	0.26±0.12	0.57±0.09	1.43±0.25	N.D.[b]	N.D.	N.D.	N.D.

[a] Average of duplicate analyses±standard deviation.
[b] N.D. = not detected.

Table 4
Levels of amino acids (mg/100g) in soy sauce, ground pork, egg and bean cake before and after cooking for 1 h

Amino acid	Soy sauce	Ground pork		Egg		Bean cake	
		Before cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking
Aspartic acid	4.21	0.46	13.76	1.01	3.60	0.11	2.00
Hydroxyproline	0.04	1.28	N.D.	N.D.	N.D.	N.D.	N.D.
Threonine	1.39	2.28	5.65	0.22	0.71	0.04	0.23
Serine	2.05	0.08	6.58	0.26	0.89	0.04	0.13
Asparagine	0.04	N.D.	N.D.	0.25	0.19	N.D.	0.12
Glutamic acid	11.00	29.85	30.78	2.40	11.16	0.33	2.44
Glutamine	0.06	0.14	0.25	0.02	0.22	0.27	0.10
Proline	2.21	0.05	7.10	0.26	1.83	0.01	0.27
Glycine	1.49	12.72	5.19	0.23	0.84	0.03	0.10
Alanine	2.35	25.32	8.18	0.89	3.82	0.04	0.30
Citrulline	0.05	N.D.	0.62	N.D.	0.49	0.01	0.36
Valine	1.53	8.64	5.13	0.14	2.38	0.05	0.11
Cystine	0.01	0.03	0.08	1.21	0.58	0.03	0.01
Methionine	0.49	N.D.	N.D.	N.D.	N.D.	N.D.	0.02
Isoleucine	1.46	5.64	4.08	0.08	1.52	0.01	0.46
Leucine	3.05	10.29	9.52	0.19	3.67	0.05	0.18
Tyrosine	0.85	0.07	3.07	0.32	2.04	0.10	0.06
Phenylalanine	1.74	5.78	6.21	0.40	2.77	0.06	0.14
Anserine	N.D.[a]	3.34	0.18	0.15	0.16	0.09	0.18
Tryptophan	0.09	N.D.	N.D.	0.03	0.24	0.01	0.35
Lysine	2.18	0.82	6.65	0.33	1.94	0.15	0.33
Histidine	0.41	6.06	2.05	0.29	1.07	0.05	0.17
3-Methyl-His	N.D.	0.56	N.D.	N.D.	N.D.	N.D.	N.D.
Arginine	1.57	0.08	5.99	3.61	1.97	0.07	0.09
Total	38.27	113.49	121.07	12.29	42.09	1.55	8.15

[a] N.D. = not detected.

and 10% soy sauce. With or without sugar, the same variety of HAs was observed in all the marinated pork and juice samples as described above. With sugar, in most samples PhIP was present in largest amount, followed by MeIQx, 4,8-DiMeIQx, IQ, AαC, Trp-P-1 and MeIQ. Similar phenomenon was found in the absence of sugar, with the exception that the level of 4,8-DiMeIQx was greater than MeIQx.

Similarly, in marinated eggs and juice, four HAs were found both in the presence and absence of sugar. In most cases, the highest concentration of PhIP was generated, followed by MeIQx, 4,8-DiMeIQx and Trp-P-1. However, the difference between PhIP and MeIQx was minor. Likewise, five HAs were present in the marinated bean cake and juice. PhIP also showed the highest amount, followed by MeIQx, 4,8-DiMeIQx, AαC and IQ (Fig. 5). For all the marinated food samples, an increased order was found for the total amount of HAs after addition of sugar, and the HAs were more susceptible to formation in juice than in food samples.

The glucose content in marinated juices of food samples showed the same trend as HAs as described above (Table 6). For the creatinine content change, the higher level of the rock candy was, the less amount of creatinine

Table 5
Contents of HAs in marinated foods (ng/g) and juice (ng/ml) in the presence and absence of various levels of rock candy and 10% soy sauce

Compound	Rock candy (%)[a]																							
	0						0.5						1						5					
	Pork	Juice	Egg	Juice	Bean cake	Juice	Pork	Juice	Egg	Juice	Bean cake	Juice	Pork	Juice	Egg	Juice	Bean cake	Juice	Pork	Juice	Egg	Juice	Bean cake	Juice
IQ	0.54 (0.35)[b]	0.63 (0.58)	N.D.[c]	N.D.	0.14 (0.28)	0.15 (0.18)	0.63 (0.38)	0.96 (0.46)	N.D.	N.D.	0.19 (0.15)	0.53 (0.46)	0.72 (0.38)	1.19 (0.86)	N.D.	N.D.	0.25 (0.13)	0.66 (0.46)	0.97 (0.65)	1.52 (0.64)	N.D.	N.D.	0.44 (0.38)	0.75 (0.34)
MeIQx	0.85 (0.26)	0.92 (1.10)	0.33 (0.25)	0.36 (0.25)	0.52 (0.35)	0.63 (0.42)	0.94 (0.69)	1.63 (1.23)	0.44 (0.36)	0.74 (0.53)	0.65 (0.36)	0.86 (0.76)	1.24 (1.20)	1.83 (1.35)	0.55 (0.26)	0.82 (0.96)	0.74 (0.26)	1.22 (0.75)	1.51 (1.24)	2.02 (1.78)	0.63 (0.38)	0.94 (0.87)	0.83 (0.75)	1.55 (0.98)
MeIQ	0.07 (0.03)	0.17 (0.24)	N.D.	N.D.	N.D.	N.D.	0.17 (0.12)	0.42 (0.37)	N.D.	N.D.	N.D.	N.D.	0.22 (0.12)	0.55 (0.27)	N.D.	N.D.	N.D.	N.D.	0.35 (0.27)	0.70 (0.56)	N.D.	N.D.	N.D.	N.D.
4,8-DiMeIQx	1.02 (0.75)	1.14 (0.87)	0.15 (0.08)	0.17 (0.12)	0.53 (0.18)	0.56 (0.63)	1.15 (0.57)	1.41 (0.26)	0.17 (0.15)	0.52 (0.34)	0.63 (0.47)	0.93 (0.65)	1.23 (0.53)	1.44 (0.65)	0.25 (0.16)	0.72 (0.38)	1.66 (0.94)	1.05 (1.21)	1.33 (0.98)	1.83 (1.45)	0.44 (0.27)	0.85 (0.69)	0.83 (0.68)	1.13 (0.76)
Trp-P-1	0.14 (0.35)	0.43 (0.36)	0.14 (0.13)	0.15 (0.18)	N.D.	N.D.	0.45 (0.23)	0.72 (0.63)	0.17 (0.12)	0.44 (0.56)	N.D.	N.D.	0.53 (0.28)	1.02 (0.38)	0.25 (0.18)	0.75 (0.56)	N.D.	N.D.	0.63 (0.35)	0.96 (0.78)	0.36 (0.18)	0.63 (0.58)	N.D.	N.D.
PhIP	1.23 (0.58)	1.33 (0.75)	0.34 (0.26)	0.44 (0.18)	0.81 (0.24)	0.85 (0.54)	1.35 (0.87)	2.32 (1.54)	0.44 (0.36)	0.68 (0.48)	0.93 (0.62)	1.63 (0.87)	1.42 (0.86)	2.44 (1.86)	0.53 (0.47)	0.83 (0.68)	1.04 (1.21)	1.79 (1.38)	1.84 (1.36)	2.64 (1.89)	0.63 (0.38)	0.92 (0.76)	1.45 (0.87)	1.85 (1.45)
AαC	0.47 (0.26)	0.59 (0.45)	N.D.	N.D.	0.22 (0.16)	0.30 (0.28)	0.58 (0.32)	0.87 (0.92)	N.D.	N.D.	0.33 (0.24)	0.74 (0.38)	0.65 (0.38)	0.96 (0.38)	N.D.	N.D.	0.34 (0.17)	0.83 (0.65)	0.74 (0.47)	1.05 (1.23)	N.D.	N.D.	0.55 (0.23)	0.95 (0.85)
Total	4.53	5.21	0.96	1.12	2.22	2.49	5.27	8.33	1.22	2.38	2.73	4.69	6.01	9.43	1.58	3.12	4.03	5.55	7.37	10.72	2.06	3.34	4.10	6.23

[a] Average of duplicate analyses.
[b] Values in parentheses represent ±standard deviation.
[c] N.D. = not detected.

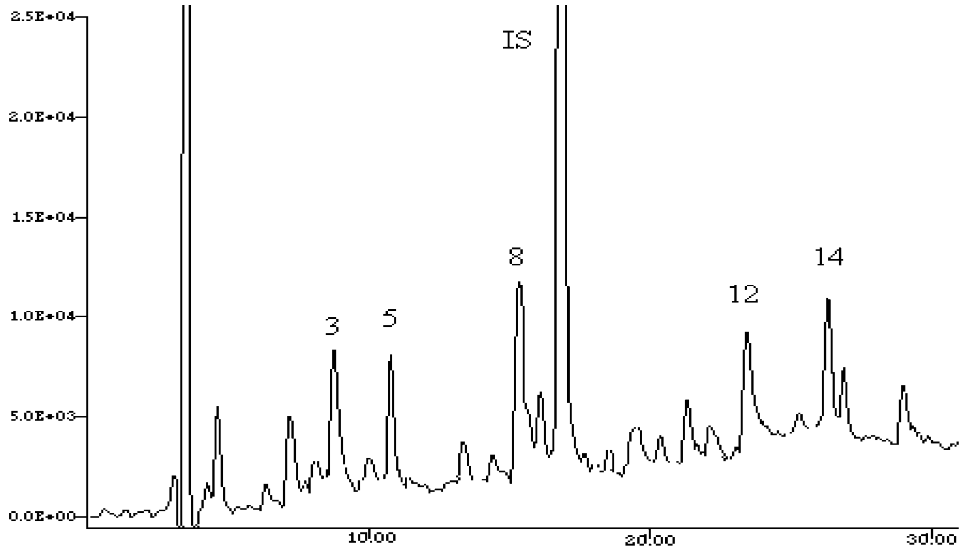

Fig. 5. HPLC chromatogram of the HAs extract of marinated bean cake in the presence of 5% rock candy. Peaks, 3, IQ; 5, MeIQx; 8, 4,8-DiMeIQx; IS, internal standard; 12, PhIP; 14, AαC.

Table 6
Contents of glucose and creatinine in marinated juice of food samples after cooking for 1 h in the presence of various levels of rock candy and 10% soy sauce

Marinated juice	Rock candy (%)[a]							
	Glucose (mg/ml)				Creatinine (mg/ml)			
	0	0.5	1	5	0	0.5	1	5
Ground pork	0.04±0.01	0.28±0.08	0.90±0.24	1.96±0.53	0.65±0.13	0.32±0.09	0.15±0.08	0.11±0.06
Egg	0.10±0.03	0.87±0.16	0.89±0.08	2.64±0.42	N.D.[b]	0.01±0.01	0.01±0.02	N.D.
Bean cake	0.09±0.05	0.36±0.13	0.61±0.16	2.66±0.98	N.D.	N.D.	N.D.	N.D.

[a] Average of duplicate analyses±standard deviation.
[b] N.D.=not detected.

was in pork juice (Table 6). With 0.5 or 1% rock candy, only a slight amount of creatinine was found in egg juice, while no creatinine was in bean cake juice.

By comparison of the results shown above, both the creatinine and glucose contents in food samples showed a declined trend during marinating. Skog and Jagerstad (1990) postulated that creatinine should react with glucose in an appropriate amount for HAs formation. Interestingly, the authors also found that with glucose level in excess, the HAs formation could be retarded. However, in our study it was found that the addition of glucose during marinating could promote HAs formation. Chen and Meng (1999) studied the HAs formation in a model system during heating and reported that HAs could only be found in both systems of glucose and creatinine, and of glucose, phenylalanine and creatinine. No HAs were found when heating creatinine alone. This result further demonstrated that creatinine could result in formation of HAs only in the presence of glucose or amino acids. This is shown for liquid model systems, but dry heating produces HAs without sugars involved (Knize et al., 1988; Overvik et al., 1989; Skog et al., 1998). No creatinine was detected in marinated bean cake and juice in the absence and presence of various levels of soy sauce.

4. Discussion

Seven HAs were fomed in marinated pork while five and four HAs in marinated bean cakes and eggs, respectively. It has been reported that both IQ and PhIP could be formed through interaction between creatinine and amino acids such as phenylalanine or serine during heating (Knize et al., 1988; Felton and Knize, 1990). The incorporation of reducing sugars such as glucose or fructose, was found to facilitate formation of IQ (Grivas, 1986; Felton and Knize, 1990). Likewise, both MeIQx and 4,8-DiMeIQx may be formed through heating a mixture of reducing sugars and amino acids such as alanine and glycine with creatinine (Jagerstad et al., 1984; Skog and Jayerstrad, 1990). In most cooked meat products both MeIQx and PhIP were reported to occur more frequently than the other HAs (Abdulkarim and Smith, 1998; Knize et al., 1998). However, in our study some more varieties of HAs were produced, because the food samples investigated in this study were marinated in an environment full of amino acids, reducing sugars and creatinine. The varieties of HAs were found to be higher in marinated pork than in marinated eggs or bean cakes. This is probably because that ground pork was used as raw material for cooking and a larger surface area could be exposed, when compared to both eggs and bean cakes in solid forms. Also, a higher fat content (24.6%) in pork may facilitate the formation of IQ-type HAs through Maillard reaction products such as pyridine from lipid degradation products (Johansson and Jagerstad, 1993). Furthermore, the difference in levels of amino acids, creatinine and reducing sugars between food samples may account for this result. The HAs contents in marinated pork ranged from 0.24 ng/g for MeIQ to 1.34 ng/g for PhIP and 2.04 ng/g for 4,8-DiMeIQx in the presence of soy sauce and rock candy. This amount was substantially lower than pork fillet when fried at 150–225 °C for 3.5 min (Skog et al., 1997). Obviously temperature appears to be more important than time for HAs formation. No HAs were detected when minced beef were cooked at 100 °C for 20 min (Sinha et al., 1994).

The marinated juice investigated in this study could be regarded as some kind of model systems and the results compared with other model systems. Arvidsson et al. (1999) studied the kinetics for the formation of heterocyclic amines in meat juice and found after 150 min heating at 100 °C, only Harman and Norharman were detected, at 125 °C, some more IQ-type HAs such as MeIQx and 7,8-DiMeIQx were formed after 45 min heating, followed by 4,8-DiMeIQx and IQx after 120 and 150 min, respectively. However, no PhIP was detected at 125 °C. Compared to 100 °C heating, in our study we found some more varieties of HAs including PhIP in the marinated juice of food samples. Apparently this could be attributed to the addition of soy sauce and rock candy to the juice prior to cooking. As time and temperature are the most important parameters affecting HAs formation, the cooking conditions for juice have to be carefully controlled. For instance, Manabe et al. (1992) reported that PhIP was formed when heated a liquid mixture of creatinine, phenylalanine and sugar at 37 and 60 °C. When the temperature was increased from 180 to 225 °C, the yield of PhIP from a liquid model system of phenylalanine, creatinine and glucose was shown to increase (Skog and Jagerstad, 1991). However, a degradation of MeIQx and 4,8-DiMeIQx formed in a liquid model system was observed after 15–30 min of heating threonine, glucose and creatinine at 225 and 250 °C (Skog et al., 1998). Since the Chinese marinated foods are often cooked at about 100°C for an extensive period of time in the presence of juice, the degradation of HAs would be difficult to occur unless a long heating time was used.

Tikkanen et al. (1996) studied the effect of different marinades on the amount of HAs in grilled chicken and found that marinades could have a reducing effect on the mutagenicity of grilled chicken because the amounts of MeIQx, 4,8–DiMeIQx and PhIP were low. However, the authors failed to evaluate which factors inhibited the formation of HAs because the composition of the marinades was not known. In a later study Salmon et al. (1997) compared the amounts of HAs in marinated and unmarinated chicken during grilling, and a decrease of PhIP while an increase of MeIQx was observed for the former. The authors postulated that sugar may be involved in the increase of MeIQx. Nevertheless, it would be difficult to assess which component should be responsible for formation or inhibition of HAs because of the complex nature of the marinades. In our study the different levels of sugar were compared and a possible relationship between sugar and HAs formation in marinated food samples could be established. However, we have to point out here that the results presented by Tikkanen et al. (1996) and Salmon et al. (1997) were obtained in experiments where foods were first marinated and then cooked. In addition to sugar, the effects of soy sauce on HAs formation in marinated meat is extremely important because it is an abundant source of amino acids. By pyrolyzing single amino acids, several HAs such as Lys-P-1 and Glu-P-1 were produced in the smoke condensates of lysine and glutamic acid, respectively (Nagao at al., 1983). However, in our work no such HAs were found, probably because of low cooking temperature. Several authors revealed that the various HAs could be formed by heating amino acids with creatinine in model systems, with or without sugar (Johnson et al., 1995; Chen and Meng, 1999). This result clearly demonstrated that amino acids participated in HAs formation more actively than sugar, and the levels of both soy sauce and sugar have to be reduced in order to minimize HAs formation in Chinese marinated foods.

In conclusion, seven HAs could be formed in marinated ground pork, while five and four HAs could be formed in marinated bean cakes and eggs, respectively. In most marinated food samples PhIP was produced in highest amount, followed by MeIQx, 4,8 -DiMeIQx, IQ, AαC, Trp-P-1 and MeIQ. The addition of soy sauce and sugar enhanced the HAs formation in marinated foods. Further research is necessary to study the inhibition of HAs formation in Chinese marinated foods by incorporation of some other additives such as antioxidants.

Acknowledgements

This study was supported by a grant (DOH89-TD-1060) from Department of Health, Taiwan.

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