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Effects of various additives on the formation of heterocyclic amines in fried fish fibre

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Abstract

The effects of various additives on the formation of heterocyclic amines (HAs) in fried fish fibre (*Trachinooephlus myops*) were studied. Fried fish fibre was prepared by boiling raw snake fish, followed by deboning, eviscerating, separating of fish meat and pressing. The fish meat was subjected to frying, during which treatment the additives, such as sugar, monosodium glutamate (MSG), antioxidants and edible oil, were added. The HAs were analyzed by high-performance liquid chromatography (HPLC) with diode-array detection. Results showed that the formation of HAs was retarded after the addition of a high level of sugar (19%), and the amount of 9H-pyrido-[4,3-b]indole (Norharman), 1-methyl-9H-pyrido-[4,3-b]indole (Harman), 2-amino-9H-pyrido[2,3-b]indole (AαC) or 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC) also decreased to a minimum. The total amount of HAs rose with increasing levels of MSG, and the individual HAs, Norharman, Harman, AαC and MeAαC showed the same trend. Antioxidants, such as vitamin C, α-tocopherol and BHT (Butylated hydroxytoluene), did not show any consistent effect of concentration on HAs formation. Coconut oil contributed to the highest levels of HAs formation, followed by lard and soybean oil.

Keywords: Heterocyclic amines; Fried fish fiber; HPLC

1. Introduction

Heterocyclic amines (HAs), formed through heating of several precursors, such as single amino acids or amino acids together with creatine or creatinine, represent an important class of food mutagens in cooked meat products (Abdulkarim & Smith, 1998; Chiu, Yang, & Chen 1998; Jägerstad, Skog, Grivas, & Olsson 1991; Johansson & Jägerstad, 1994; Kinze et al., 1998). Epidemiological evidence appears to imply that consumption of the HAs-containing meat products, in excess, may induce colon and other cancers (Gerhardsson de Verdier, 1995; Steineck, Hagman, Gerhard de verdier, & Norell 1990; Sugimura, 1997; Willett, Stampfer, Colditz, Rosner, & Speizer 1990). Thus, it is important to learn more about the formation and inhibition of HAs in cooked foods.

The amount and variety of HAs formed in cooked meat products can be dependent on many factors, among which processing methods and conditions are the most important (Chiu et al., 1998; Skog, Steinech, Augustsson, & Jägerstad 1995). Of the various processing methods, frying and broiling were reported to be related to HA formation (Chiu et al., 1998; Sinha et al., 1998). Starvic (1994) reviewed the effects of cooking methods, frying, broiling, smoking, grilling and roasting on HA formation in meat products and showed that several HAs, including IQ, MeIQx, MeIQ, 4,8-DiMeIQx, 7,8-DiMeIQx, Trp-P-1, Trp-P-2, AαC, PhIP, were present.

Fried fish fibre is a popular oriental-style meat commodity, consumed in Asian countries. It is often prepared by boiling raw fish, followed by eviscerating, separating of fish flesh, pressing, grinding or frying at 120°C for 1–2 h. During frying, the various ingredients, such as salt, sugar, soybean sauce, monosodium glutamate, edible oil, soybean flour and antioxidants, were added together or at intervals. It has been reported that the addition of oils and antioxidants may promote or retard the HAs formation (Chen, Pearson, & Gary 1992; Johansson & Jägerstad, 1996; Johansson, Skog, & Jägerstad 1993). In a previous study, we reported the

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formation of HAs in fried fish fibre during processing and storage (Chen, Leee, & Tai 2000). However, the effects of various additives on the HAs formation in fried fish fibre remain unknown. The objective of this study was to determine the formation or inhibition of HAs in fried fish fibre as affected by various additives.

2. Materials and methods

2.1. Materials

Snake fish (*Trachinooephlus myops*) was purchased from a local market in Keelung. Salt, sugar (sucrose) and monosodium glutamate (MSG) were obtained from Ding-Hau supermarket (Taipei, Taiwan). Edible oils, including lard, soybean oil, and coconut oil were from Tung-Chin Co. (Taipei, Taiwan). Antioxidants, including BHT, ascorbic acid and α-tocopherol were from Gemfont Co. (Taipei, Taiwan).

Chemicals, such as hydrochloric acid, sodium hydroxide, ammonium acetate, lead acetate, potassium oxalate and trichloroacetic acid, were purchased from Sigma Chemicals Co. (St. Louis, MO). 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). The propylsulfonic acid silica gel cartridge (500 mg) was from Varian Co. (Harbor, CA) and C18 cartridges (100 and 500 mg) were from J. T. Baker Co. (Philipsburg, NJ). A TSK-GEL ODS C18 column (250×4.6 mm I.D., 5μm) by Tosoh Co. (Tokyo, Japan) was used for separation of HAs by HPLC.

Fifteen HA standards, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3- methyl-imidazo[4,5-f]quinoxaline (IQx), 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-3,7,8-trimethylimidazo[4,5-f]quinoxaline (7,8-DiMeIQx), 2-amino-9H-pyrido [2,3-b]indole (AαC), 2-amino-3-methyl-9H-pyrido [2,3-b]indole (MeAαC), 2-amino-1-methyl-6-phenylimidazo[4,5-f]pyridine (PhIP), 2-amino-6-methyldipyrido-[1,2-a:3′,2′-d]imidazole (Glu-P-1), 2-amino-dipyrido-[1,2-a:3′,2′-d]imidazole (Glu-P-2) 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-5H-pyrido[4,3-b]indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) were from Wako Co. (Osaka, Japan); 1-methyl-9H-pyrido[4,3-b]indole (Harman) and 9H-pyrido-[4,3-b]indole (Norharman) were from Aldrich Co. (Steinheim, Germany).

2.2. Instrumentation

The HPLC system is composed of two Jasco PU-980 pumps, a Jasco MD-915 photodiode-array detector and a Jasco 821-FP fluorescence detector (Jasco Co., Tokyo, Japan). Borwin computer software was used to process data. The amino acid analyzer (model L8500) was from Hitachi Co. (Tokyo, Japan). The fish fibre fryer (model BA-3) was from Jin-Seng-Hau Co. (Taipei, Taiwan), The Sorvall high-speed centrifuge (model RC5C) was from Du Pont Co. (Wilmington, Delaware), and the homogenizer (model HG-2800) was from Hsiang-Tai Co. (Taipei, Taiwan).

2.3. Processing of fried fish fibre

A total of 152 snake fish was divided into 19 groups of eight each. Each group of eight fish, about 9 kg, was cooked in boiling water for 30 min for easy separation of flesh from skin. After cooling to room temperature, both head and tail of fish were removed, followed by deboning, eviscerating and pressing. Approximately 5 kg fish meat were obtained and chopped into pieces. About 1.5 kg chopped fish meat were collected for processing. The fish meat was placed in a fryer preheated to 120°C and fried for 30 min, during which a stirrer was used to help disperse the fish meat into a shredded form. After 30 min, the various ingredients, including salt, sugar, soy sauce, MSG and soybean flour or antioxidants in different proportions, were added either separately or together, to the fish meat. Some were not added, depending on the requirement of each treatment. The control treatment contained all the basal ingredients, including 1.5% salt (22.5 g), 14% sugar (210 g), 5% soy sauce (75 g), 1% MSG (15 g), 4% lard (60 g) and 10% soybean flour (150 g). After continuous frying for 30 min, 60 g of lard, soybean oil or coconut oil were added and frying continued for 5 min. The total frying time was 65 min and about 0.8 kg fried fish fibre was obtained. The fryer temperature was automatically controlled at 120°C all the time during heating.

To determine the effect of sugar on HA formation in fried fish fibre, one level of sugar was adjusted, while the levels of the other ingredients remained unchanged. Four levels of sugar, 0% (0 g), 9% (135 g), 14% (210 g) and 19% (285 g), were each mixed with 1500 g fish meat and the other ingredients in an appropriate amount (salt 22.5 g, soy sauce 75 g, MSG 15 g, lard 60 g, and soybean flour 150 g). Three treatments plus one control treatment were used.

To determine the effect of MSG on HA formation in fried fish fibre, four levels of MSG, 0% (0 g), 0.5%(7.5 g), 1.0%(15 g) and 1.5% (22.5 g) were mixed with 1500g fish meat and the other ingredients in an appropriate amount (salt 22.5 g, soy sauce 75 g, sugar 210 g, MSG 15 g, lard 60 g, and soybean flour 150 g). Three treatments plus one control treatment were used.

To determine the effect of antioxidant on HA formation in fried fish fibre, four levels of BHT, vitamin C or α-tocopherol, 0% (0 g), 0.01% (0.15 g), 0.05% (0.75 g) or 0.1% (1.5 g) were mixed with 1500g fish meat and the other ingredients in an appropriate amount (salt 22.5 g, soy sauce 75 g, sugar 210 g, MSG 15 g, lard 60 g and soybean flour 150 g). Nine treatments plus one control treatment were used.

To determine the effect of edible oil on HA formation in fried fish fibre, 60 g of lard, soybean oil or coconut oil were each mixed with 1500 g fish meat during frying, while the other ingredients were added together in an appropriate amount (salt 22.5 g, soy sauce 75 g, sugar 210 g, MSG 15 g, lard 60 g and soybean flour 150 g). Three treatments plus one control treatment were used.

A total of 19 treatments of eight fish samples each, were used as raw material for processing fried fish fibre. After frying, the fried fish fibre from each treatment was analyzed for the contents of glucose, amino acid and HAs.

2.4. Analysis of HAs in fried fish fibre

The HAs were extracted from the samples of fried fish fibre, based on a modified solid-phase extraction and HPLC method, as described by Chen and Yang (1998). This method was originally developed by Gross and Gruter (1992). The identification of HAs was carried out by comparing retention times of unknown peaks with those of reference standards and cochromatography with added standards. The identity of each HA was further confirmed by comparing UV spectra with those of reference standards. The HAs were quantified by comparing peak areas of samples with area of internal standard, as described by Chen et al. (2000).

2.5. Analysis of glucose in fried fish fibre

The glucose content in samples of fried fish fibre was determined according to a procedure described in a previous study (Chen et al., 2000).

2.6. Analysis of amino acids in fried fish fibre

The contents of free amino acids in fried fish fibre were analyzed using a method described in a previous report (Chen et al., 2000).

2.7. Statistical analysis

All the data for the contents of HAs, glucose, and amino acids were subjected to analysis of variance and Duncan's multiple range test (SAS, 1985). Duplicate analyses were performed and mean values were calculated. The correlations (r values) between glucose and/or amino acid and HAs formation were measured.

3. Results and discussion

3.1. HPLC analysis of HAs

The HPLC condition used in this study was found to provide adequate resolution of 16 HAs standards, as reported in a previous paper (Chen & Yang, 1998). The average recoveries for AαC, MeAαC, Harman and Norharman were 73.6 ± 2.8, 75.4 ± 3.6, 82.3 ± 5.1 and 85.7 ± 3.2 respectively, which were slightly different from those shown in a report by Chen and Yang (1998). This difference is probably due to the variety of impurities in the samples used. In the current study, samples of fried fish fibre were used, and the presence of impurities may account for the recovery difference. The detection limits based on signal-to-noise ratio of three and UV detection at 258 nm were 0.1, 0.3, 0.1 and 0.1 ng for AαC, MeAαC, Harman and Norharman, respectively.

3.2. Effect of sugar on the formation of HAs in fried fish fibre

Table 1 shows the contents of HAs (ng/g), glucose (mg/100 g) and amino acid (mg/100 g) in fried fish fibre in the presence of various levels of sugar. Only four HAs, Norharman, Harman, AαC and MeAαC, were detected. The total amount of HAs showed an inconsistent change for each increasing level of sugar. With 9 and 14% sugar, the HAs content increased by 85 and 35%, respectively. However, with 19% sugar, the HAs level declined by 43%. Similar trends were found for each HAs; i.e. the content of Norharman, Harman, AαC or MeAαC decreased to a minimum after addition of 19% sugar. This result implied that the formation of HAs may be retarded after incorporation of a high level of sugar. This phenomenon may be explained as follows: The Maillard reaction products are more readily formed at a high sugar level than at a low sugar level; these in turn react with creatine or creatinine and thus the amount of glucose is greatly diminished (Skog & Jägerstad, 1990). In addition, the Maillard reaction products may react with the mutagenic substance and result in a decrease of HAs (Skog & Jägerstad, 1990).

The glucose contents in fried fish fibre in the presence of 0, 9, 14 and 19% sugar were 2.64, 1.03, 2.54 and 3.10 mg/100 g, respectively. Theoretically, the glucose content in fried fish fibre should be greatly increased because of hydrolysis of sugar during processing. However, with addition of 9 and 14% sugar, the glucose content in fried fish fibre was lower than that for the control treatment. Apparently, this result can be attributed to the participation of glucose in the non-enzymatic browning reaction or caramelization.

Without sugar, the total amounts of amino acids and HAs were 930 mg/100 g and 45.8 ng/g, respectively. With

the additions of 9 and 14% sugar, the amounts of amino acids dropped by 25 and 15%, respectively, while the HAs rose by 85 and 35%. However, with 19% sugar, the amino acids showed an increase of 8% while the HAs showed a decrease of 43%. This result revealed that, at a lower level of sugar, the non-enzymatic reaction may proceed more rapidly because of the formation of a high yield of HAs. However, at a higher level of sugar, the amino acid may not participate in the non-enzymatic browning reaction. Instead, the caramelization reaction may proceed rapidly, because of the formation of a low yield of HAs. In our study we also observed that the HA formation is minimized with the presence of a high level of sugar.

In the presence of various levels of sugar, the contents of free amino acids in fried fish fibre, i.e. taurine, serine, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, threonine and phenylalanine, were found to decrease with increasing yields of HAs (Table 2). Of the various amino acids, a high correlation was found

Table 1
Contents of HAs, glucose and amino acids in fried fish fibre in the presence of various levels of sugars

Sugar (%)	HAs (ng/g)[a,b]				Total amount[a,b]	glucose (mg/100 g)[a,b]	amino acid (mg/100g)[a,b]
	Norharman	Harman	AαC	MeAαC			
0	23.8 a	13.7 a	1.8 a	6.5 ac	45.8 a	2.64 a	930 a
9	41.6 b	24.4 b	3.1 b	15.5 b	84.6 b	1.03 b	695 b
14	35.2 c	16.7 c	2.0 a	7.9 a	61.8 c	2.54 c	789 c
19	10.5 d	8.80 d	0.9 c	5.9 c	26.1 d	3.10 d	1009 d

[a] Average of duplicate analyses.
[b] Symbols (a–d) bearing different letters in the same column are significantly different ($P<0.05$)

Table 2
Levels of amino acids (mg/100 g) in fried fish fibre in the presence of various levels of sugar

Amino acids	Sugar[a,b]			
	0%	9%	14%	19%
Phosphoserine	3.17 a	2.69 b	3.33 ac	3.64 c
Taurine	63.28 a	41.2 b	47.0 c	74.4 d
Phosphoethanolamine	0.56 a	1.13 b	1.79 c	2.11 c
Aspartic acid	6.93 a	5.04 b	4.56 c	7.40 a
Threonine	18.8 a	12.8 b	16.1 c	20.6 d
Serine	13.5 a	9.73 b	12.7 c	15.5 d
Glutamic acid	390 a	322 b	328 c	395 d
Proline	19.8 a	11.5 b	14.1 c	21.5 d
Glycine	14.4 a	9.75 b	13.1 c	16.1 d
Alanine	23.7 a	17.8 b	21.7 c	35.8 d
Citrulline	2.44 ab	2.02 a	2.89 bc	3.94 c
Valine	9.39 a	6.82 b	8.67 c	10.4 d
Cystine	0.70 a	2.22 b	2.90 c	3.79 d
Cysteine	0.00 a	0.00 a	0.00 a	0.00 a
Isoleucine	8.03 a	6.04 b	7.51 c	9.87 d
Leucine	15.14 a	11.1 b	14.5 c	17.7 d
Tyrosine	5.13 a	3.81 b	4.93 a	7.69 d
Phenylalanine	8.05 a	5.54 b	7.39 c	10.9 d
β-alanine	4.19a	3.25 bc	2.92 b	3.59 c
γ-Aminobutyric acid	3.19 a	5.10 b	5.64 bc	6.07 c
Tryptophan	2.16 a	2.53 a	3.15 b	4.66 c
Ornithine	4.14 a	3.66 b	4.20 a	4.83 c
Lysine	43.6 a	27.2 b	30.9 c	40.2 d
1-Methylhistidine	2.12 a	1.38 b	0.73 c	1.55 b
Histidine	0.00 a	1.40 b	1.79 b	2.26 c
Anserine	245 a	161 b	206 c	264 d
Carnosine	4.96 a	2.87 bc	3.44 c	2.46 b
Arginine	17.52 a	16.1 b	19.1 c	22.5 d
Total	930	695	789	1009

[a] Average of duplicate analyses.
[b] Symbols (a–d) bearing different letters in the same row are significantly different ($P<0.05$)

between the consumption of threonine ($r = 0.99$), serine ($r = 0.99$), leucine ($r = 0.99$) or phenylalanine ($r = 0.98$) and the amounts of HAs formed. Likewise, a high correlation ($r = 0.93$) was found between the consumption of glucose and the amounts of HAs formed. It may be postulated that amino acids may play a more significant role in HA formation in fried fish fibre than that of glucose. In addition to reacting with glucose, several studies have shown that amino acids may degrade by themselves to form HAs (Nagao, Sato, & Sugimura 1983; Jägerstad et al., 1991). In most cooked products, the IQ-type HAs were reported to occur. However, in our study only the carboline-type HAs were found. This is probably because the various ingredients added during processing of fried fish fibre can make the reaction of HAs formation more complex. It is quite possible that the formation of the carboline-type HAs was favoured (Chen et al., 2000). In addition, the non-enzymatic browning reaction should proceed slowly because of the low moisture content (1%) of fried fish fibre. Instead, the caramelization reaction should proceed rapidly, because of formation of the deep-brown colour of fried fish fibre (Chen et al., 2000).

3.3. Effect of MSG on the formation of HAs in fried fish fibre

Fig. 1 shows the HPLC chromatogram of HA extracts of boiled fish with MSG additions of 0.5, 1.0 and 1.5%. Likewise, only four HAs, Norharman, Harman, AαC and MeAαC were present. Table 3 shows the levels of HAs, glucose and total free amino acids in fried fish fibre in the presence of various levels of MSG. The total amount of HAs showed an increased trend for each increasing level of MSG. The same phenomenon was observed for the content change of each HAs, i.e. Norharman, Harman, AαC and MeAαC. Without MSG, the amounts of glucose and HAs were 2.98 mg/100 g and 34.5 ng/g, respectively. With the addition of 0.5% MSG, the amount of glucose decreased by 0.25 mg/100 g (8.4%) while the HAs increased by 7.6 ng/g (21.5%). Then an increased order was followed afterwards for 1.0 and 1.5% MSG. This result implied that the higher the MSG level, the more the consumption of glucose and the greater the formation of HAs. A high correlation (0.86) was found between the consumption of glucose and the formation of HAs. In the absence of MSG, the total amount of amino acid followed a decreased order, while a reverse trend occurred for the HAs. Similarly, the higher level of MSG resulted in a higher consumption of amino acid and a larger formation of HAs.

In the presence of various levels of MSG, the contents of amino acid in fried fish fibre, i.e. taurine, serine, threonine, glutamic acid, glycine and β-alanine, were found to decrease with increasing amounts of HAs. Of the various amino acids, a high correlation was observed between the consumption of taurine ($r = 0.97$),

Table 3
Contents of HAs, glucose and amino acids in fried fish fibre in the presence of various levels of monosodium glutamate (MSG)

MSG (%)	HAs[ab]				Total amount[ab]	glucose (mg/100 g)[ab]	amino acids (mg/100 g)[ab]
	Norharman	Harman	AαC	MeAαC			
0	17.0 a	15.1 a	1.0 a	1.4 a	34.5 a	2.98 a	914 a
0.5	20.7 b	15.3 ab	1.6 ab	4.5 b	42.1 b	2.73 b	891 b
1.0	35.2 c	16.7 b	2.0 b	7.9 c	61.8 c	2.54 c	790 c
1.5	36.7 c	18.4 c	5.1 c	9.7 d	69.9 d	1.40 d	735 d

[a] Average of duplicate analyses.
[b] Symbols (a–d) bearing different letters in the same column are significantly different ($P < 0.05$)

Table 4
Contents of HAs (ng/g) in fried fish fibre in the presence of various levels of antioxidants

Compound	Control[a,b]	Ascorbic acid (%)[a,b]			α-Tocopherol (%)[a,b]			BHT (%)[a,b]		
	0	0.01	0.05	0.1	0.01	0.05	0.1	0.01	0.05	0.1
Norharman	35.2 a	103 b	60.9 c	40.2 d	12.4 b	38.5 c	39.6 c	87.8 b	107 c	22.0 d
Harman	16.7 a	69.2 b	84.7 c	18.8 a	9.0 b	14.7 c	15.4 ac	57.5 b	60.1 b	14.7 a
AαC	2.0 a	8.3 b	ND[c] c	ND[c] c	1.6 a	3.5 b	3.6 b	2.0 a	2.6 a	5.0 b
MeAαC	7.9 a	7.2 a	4.2 b	ND[c] c	5.4 b	2.1 c	1.5 c	9.8 b	9.8 b	1.6 c
Total	61.8	187.7	149.8	59.0	28.4	58.8	60.1	157.1	178.9	43.3

[a] Average of duplicate analyses.
[b] Symbols (a–d) bearing different letters in the same row are significantly different ($P < 0.05$)
[c] ND, not detected

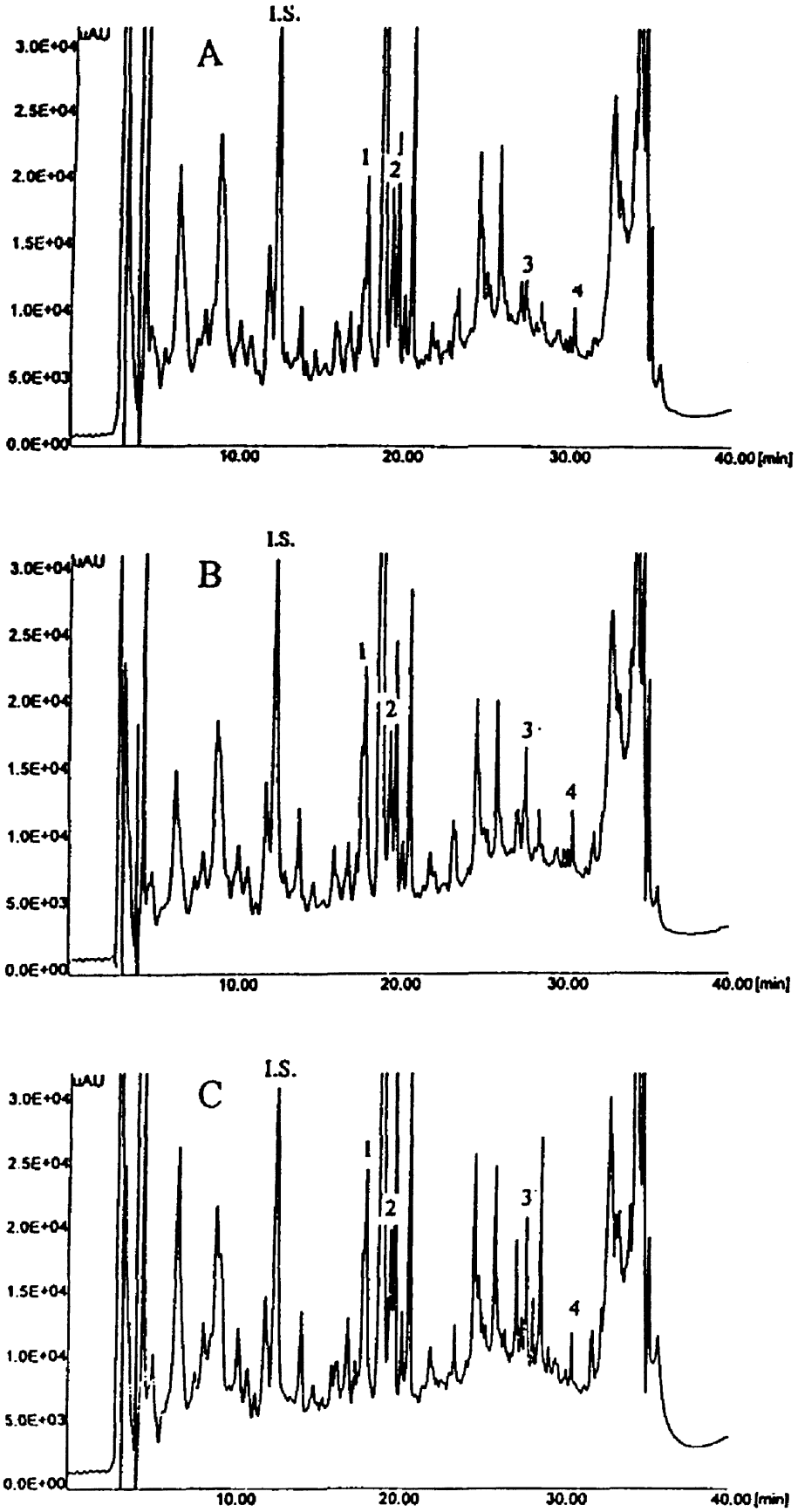

Fig. 1. HPLC chromatograms of HAs extracts of boiled fish with monosodium glutamate. (A) 0.5% (B)1.0% and (C) 1.5%. Chromatographic conditions described in text. Peak: I.S., Glu-P-1; 1, Norharman; 2, Harman; 3, AαC; 4, MeAαC.

threonine ($r = 0.98$), serine ($r = 0.99$) or glycine (0.94) and the formation of HAs. From the result shown earlier it may be postulated that amino acids may play a more significant role in HA formation than glucose because of the high correlation of the former.

3.4. Effect of antioxidant on the HAs formation in fried fish fibre

Table 4 shows the effects of various antioxidants on the HAs formation in fried fish fibre. Without vitamin C, the total amount of HAs was 61.8 ng/g, and Norharman was present in the largest amount, followed by Harman, AαC and MeAαC. With the addition of 0.15 g vitamin C, the HA content reached a plateau (187.7 ng/g). However, a higher amount of vitamin C reduced the HAs formation. The content changes of the individual HAs, Norharman, Harman, AαC or MeAαC, also followed a similar trend. Neither AαC nor MeAαC were not detected until the level of vitamin C increased to 0.75 and 1.5 g, respectively. With the exception of Harman, the amount of each HAs declined with increasing levels of vitamin C. This result indicated that a higher level of vitamin C was able to retard HA formation more effectively. The reduction of HAs by incorporation of vitamin C may be explained by the scavenging of free radicals and oxygen as reported by Johansson and Jägerstad (1996).

In contrast to the result shown earlier, the effect of α-tocopherol on the HAs formation in fried fish fibre is concentration-dependent. A low amount of α-tocopherol (0.15 g) was found to reduce the HAs formation more effectively than a high amount of α-tocopherol (0.75 or 1.5 g). With the exception of MeAαC, the amount of each HAs increased with increasing levels of α-tocopherol.

Like that of α-tocopherol, the effect of BHT on HA formation in fried fish fibre is also concentration dependent. Without BHT, the total amount of HAs was 61.8 ng/g. With addition of 0.15 and 0.75 g BHT, the HA contents increased by 154% (95.3 ng/g) and 189% (117.1 ng/g), respectively. However, the addition of a higher amount of BHT (1.5 g) reduced the HA formation by 30%. A similar trend occurred for the content changes of Norharman, Harman, AαC and MeAαC. The addition of a low amount (0.15 and 0.75 g) of BHT significantly increased the formation of Norharman, Harman and MeAαC, while the AαC level showed an insignificant change. On the other hand, the addition of a high amount of BHT (1.5 g) increased the formation of AαC, while it decreased the formation of the other three HAs. In some previous studies, Barnes and Weisburger (1984) reported that the addition of BHA may promote the formation of IQ-type HAs in ground beef during cooking. In contrast, Chen (1988) found that both BHA (Butylated hydroxyanisole) and TBHQ (Tertiary butylhydroquinone) may reduce the formation of MeIQx in fried ground beef. Thus, the promoting or retarding effects of antioxidants toward HA formation should depend on many factors, such as cooking method, cooking condition, and variety and concentrations of antioxidants.

3.5. Effect of lipids on HAs formation in fried fish fibre

Table 5 shows the effect of various lipids on the HA formation in fried fish fibre. For the oil-free treatment, the level of HAs was 15.3 ng/g. With addition of lard, soybean oil and coconut oil, the HA contents were 61.8, 16.9 and 92.5 ng/g, respectively. For each HA, the content showed an inconsistent change. MeAαC was present in the largest amount for the oil-free treatment, followed by Harman, AαC and Norharman. After the addition of lard or coconut oil, Norharman was more readily formed than MeAαC or AαC. However, an opposite change occurred for soybean oil; MeAαC was the most susceptible to formation while Harman was the least. In a previous study, the addition of corn oil to a model system was found to significantly increase the formation of HAs such as MeIQx (Johansson et al., 1993). Several authors suggested that the formation of Maillard reaction products, such as pyridine and pyrazine, as well as the free radical reaction, may play significant roles in HA formation (Arnoldi, Arnoldi, Baldi, & Giffini 1987; Arnoldi, Arnoldi, Baldi, & Ghizzoni 1990; Barnes & Weisburger, 1984; Johansson & Jägerstad, 1996). This explanation seems to be contradictory to the result of this study, which showed that the highly saturated coconut oil was more prone to HAs formation than lard or soybean oil. This may be explained as follows: The soybean oil was found to contain antioxidant, vitamin E, at a concentration of 12.6 mg/100 g, and thus the stability of HAs was greatly enhanced. The highly saturated coconut oil may undergo hydrolysis to form a large amount of free fatty acid during heating, which in turn facilitates the degradation rate of lipid and thus

Table 5
Contents of HAs (ng/g) in fried fish fibre in the presence of various kinds of lipids

Compound	[Lipid[a–c]]			
	None	Lard (Control)	Soybean oil	Coconut oil
Norharman	ND[c]	35.2 a	5.3 b	51.9 c
Harman	6.0 a	16.7 b	ND	30.7 c
AαC	1.8 a	2.0 ab	2.2 b	1.8 a
MeAαC	7.5 a	7.9 a	9.4 b	8.1 c
Total	15.3	61.8	16.9	92.5

[a] Average of duplicate analyses.
[b] Symbols (a–d) bearing different letters in the same row are significantly different ($P < 0.05$)
[c] ND, not detected

promote formation of HAs. In conclusion, amino acids may play a more significant role than glucose in HA formation in fried fish fibre. The addition of a high level of vitamin C and BHT may create an inhibitory effect. A reverse trend occurs for α-tocopherol. Coconut oil is the most susceptible to HA formation in fried fish fibre during heating, followed by lard and soybean oil.

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