

## Heterocyclic aromatic amines in commercial Chinese dried meat products

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### Summary

Heterocyclic aromatic amines (HAAs) are mutagenic and/or carcinogenic compounds formed in meat during processing. Dry meat products are known to have a high level of HAAs, while only few studies focused on the factors affecting the formation of HAAs. The aim of this study was to investigate the impact of different factors (manufacture methods, spices, breed and production place) on formation of HAAs in Chinese dried meat products. The results revealed that the manufacture methods impacted the contents of HAAs, roast non-deep frying production > natural air-drying method > deep frying method. Analysis of air-dried beef with different spices confirmed that spices inhibited HAAs formation. However, spices seemed to increase HAAs levels in air-dried yak samples. The total content of HAAs in dried product from Chinese yellow cattle was higher than that of other selected breeds (Aberdeen Angus cattle and Chinese yak). Beef jerky from Sichuan had higher HAAs contents than that from Inner Mongolia. Yak jerky from Sichuan had lower HAAs amounts than that from Qinghai. Overall, this study indicates that processing methods, spices, the breed and the place affected the HAAs levels in dried beef jerky products. However, additional studies are needed to determine the interaction of several factors.

### Keywords

heterocyclic aromatic amines; dried meat; breed; spice; beef jerky

Heterocyclic aromatic amines (HAAs) are potent mutagens and/or carcinogens, which contain nearly 25 compounds generated in the cooked meat products [1]. Depending on chemical characteristic, HAAs are divided into two categories, polar and non-polar HAAs [2, 3]. The latter category was shown to be responsible for most of the HAAs-associated mutagenic activities detected in food [4]. Polar HAAs, also known as thermic HAAs or 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-type HAAs, including IQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amin-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-(1,6-dimethylfuro[3,2-e]imidazo[4,5-b])pyridine (IFP), are formed at 150–200 °C.

Several studies showed that they could also be formed at higher temperatures, up to 300 °C [2, 5]. Non-polar HAAs, also known as pyrolytic HAAs or non-IQ-type HAAs, including 2-amino-9H-pyrido[2,3-b]indole (AαC), 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC), 9H-pyrido[3,4-b]indole (Norharman), 1-methyl-9H-pyrido[3,4-b]indole (Harman), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), and 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu-P-1), are generated over 300 °C [2, 5]. Epidemiological experiments showed that dietary intake of HAAs through consumption of cooked meat products increased the risk of breast, stomach, pancreas and colon cancers in humans [1, 6]. In October 2015, 22 scientists from ten countries convened by the International Agency for Research on Cancer

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(IARC) Monographs Programme classified the consumption of processed meat as carcinogenic to humans (Group 1) [7].

Numerous studies revealed that, when cooking at high temperatures, HAAs can be formed in all types of meat, such as beef, pork, lamb and chicken [8–10]. The type and quantity of HAAs formation are dependent upon several parameters, including type of meat, type of cooking, cooking temperature, the spices used, the antioxidants used and pH [3, 10–17]. To estimate the risk of HAAs in human health, it is important to evaluate the impact of these factors on HAAs formation in meat products.

Dried meat product is a nutrient-dense meat product because of high protein (> 35%) and iron level, and is low in fat (< 10%). Traditional air-drying technology for dried meat products is an ancient technology, which has been used for thousands of years [18]. Meat snack products (e.g. beef jerky) are widely consumed in modern society, due to their convenience for eating and offer of variable flavours. However, only few studies focused on the safety of dried meat products, besides microbiological quality [19–21].

In our previous study, we showed that a dry meat product had the highest level of HAAs among other popular meat products in China (smoked meat, sauced meat, roasting meat, dry meat, fried meat, sausage and ham) [8]. The objective of this study was to estimate the impact of different factors on HAAs levels in dried meat products. The factors included the breed (beef and yak), the processing methods (natural air drying, drying and frying, drying and roasting), the spices added (original, spicy, spiced) and the production place (Inner Mongolia, Qinghai, Sichuan).

## MATERIALS AND METHODS

### Chemicals

All solvents and chemicals used were of high performance liquid chromatography (HPLC) or analytical grade. Ultrapure water was obtained from a Milli-Q Advantage water purification system (Millipore, Bedford, Massachusetts, USA). Bond Elut cartridges, Oasis MCX 3cc cartridge (60 mg, particle size 30  $\mu$ m) and diatomaceous earth were obtained from Agilent Technologies (Santa Clara, California, USA), Waters (Milford, Massachusetts, USA) and Sinopharm Chemical Reagent (Beijing, China), respectively. The nine heterocyclic aromatic amine standards used (IQ, MeIQx, 4,8-DiMeIQx, PhIP, Harman, Norhar-

man, 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), A $\alpha$ C, and MeA $\alpha$ C) were provided by Toronto Research Chemicals (Toronto, Canada).

### Meat products

The dried meat products were purchased from local supermarkets in Beijing, China, and some specific ones were bought online (Tab. 1). Sixteen types of samples (three samples per type) were collected and stored at  $-18^{\circ}\text{C}$  until analysis. Four different factors were studied: the manufacturing method, the spices, the breed and the production place.

### Extraction of heterocyclic aromatic amines

HAAs were extracted according to the methods of PAN et al. [8]. Sample preparation was as follows: dried meat samples were ground with a grinder (SZ-22; Chendong Xuzhong, Sichuan, China) and 2 g of each ground sample were digested in 10 ml of an alkaline solution (2 mol $\cdot$ l $^{-1}$  NaOH). The suspension was homogenized by stirring on a magnetic stirrer (EMS-19; Tianjin Honour Instrument, Tianjin, China) for 20 min at room temperature and then submitted to ultrasonic extraction (Ameritech Scientific, Laguna Hills, California, USA) for 30 min at  $21^{\circ}\text{C}$ . After this step, the sample was mixed with 12 g of diatomaceous earth and the powdery mixture was filled into empty solid phase extraction (SPE) cartridges. HAAs were extracted 2 times with 40 ml of dichloromethane and the eluent was poured into an Oasis MCX cartridge, which had been washed with 2 ml of dichloromethane. The cartridge was then succinctly rinsed with 2 ml of different solvents: dichloromethane, methyl alcohol (MeOH)-0.1 HCl mixture (chromatographic pure; Fisher Scientific, Waltham, Massachusetts, USA), MeOH (chromatographic pure; Fisher Scientific) and ultrapure water (Direct-Q 3; Millipore). HAAs were eluted with 2 ml of MeOH-ammonia (chromatographic pure; Fisher Scientific) (15 : 85, v/v) and the extracts were concentrated with an evaporator (UGC-12C; Usun Technologies, Beijing, China) under nitrogen and stored at  $4^{\circ}\text{C}$  until used for analysis, where they were re-dissolved in 200  $\mu$ l of MeOH.

### Preparation of mobile phase

Two solvents composed the mobile phase. Solvent A was prepared by adding 1 g of triethylamine in 1000 ml of water and pH was adjusted to 3.6 with phosphoric acid. Solvent B was a HPLC-grade acetonitrile. Both solvents were filtered with a vacuum pump through nylon filters (pore size 0.45  $\mu$ m) and degassed for 15 min before using.

**Tab. 1.** Ingredients in dried meat products.

Type of sample	Ingredients	Packaging information
Deep fried beef jerky	Beef, salt, white granulated sugar, mixed spices, sodium glutamate, potassium sorbate, disodium 5'-ribonucleotide, ethyl maltol	Vacuum-packed
Natural air-dried beef jerky	Beef, salt, white granulated sugar	Vacuum-packed
Roast-non deep fried beef jerk	Beef, salt, monosodium glutamate, white granulated sugar, soybean sauce, mixed spices	Vacuum-packed
Original beef jerky	Beef, salt, white granulated sugar, vegetable oils, monosodium glutamate	Vacuum-packed
Spicy beef jerky	Beef, seasoning wine, salt, mixed spices, monosodium glutamate, nisin	Vacuum-packed
Non-flavour beef jerky	Beef, salt, monosodium glutamate	Vacuum-packed
Spiced yak jerky	Yak meat, salt, white granulated sugar, chicken essence seasoning, mixed spices	Vacuum-packed
Spicy yak jerky	Yak meat, salt, monosodium glutamate, mixed spices	Vacuum-packed
Original yak jerky	Yak meat, salt, monosodium glutamate, mixed spices	Vacuum-packed
Beef jerky used Aberdeen Angus cattle meat	Beef, salt, white granulated sugar	Vacuum-packed
Beef jerky used Chinese yellow cattle meat	Beef, salt, white granulated sugar, vegetable oils, monosodium glutamate, mixed spices	Vacuum-packed
Beef jerky used Chinese yak	Beef, salt, monosodium glutamate, white granulated sugar, mixed spices, d-sodium erythorbate, potassium sorbate, sodium nitrite	Vacuum-packed
Beef jerky from Sichuan	Beef, salt, monosodium glutamate, white granulated sugar, mixed spices, chicken essence seasoning, d-sodium erythorbate, sodium nitrite	Vacuum-packed
Beef jerky from Inner Mongolia	Beef, salt, white granulated sugar, vegetable oils, monosodium glutamate	Vacuum-packed
Yak jerky from Qinghai	Beef, white granulated sugar, vegetable oils, salt, soybean sauce, mixed spices, chicken essence seasoning, monosodium glutamate, edible essence	Vacuum-packed
Yak jerky from Sichuan	Yak meat, salt, white granulated sugar, chicken essence seasoning, mixed spices	Vacuum-packed

### Preparation of standard solutions

Nine different standards were used: IQ (CAS No. 76180-98-6), MeIQ (CAS No. 77094-11-2), 4,8-DiMeIQx (CAS No. 95896-78-9), Norharman (CAS No. 244-63-3), Harman (CAS No. 485-84-0), Trp-P-2 (CAS No. 62450-07-1), PhIP (CAS No. 105650-23-5), A $\alpha$ C (CAS No. 26148-68-5) and MeA $\alpha$ C (CAS No. 68806-83-7). One milligram of each standard was weighed and mixed with 1 ml of MeOH. Taking 50  $\mu$ l of each solution to 950  $\mu$ l of MeOH, this represented the most concentrated solution (5  $\mu$ g·ml<sup>-1</sup>), which was diluted to obtain different standard solutions: 1000, 500, 200, 100, 50, 20, 10, 5, 2  $\mu$ g·ml<sup>-1</sup> and 1  $\mu$ g·ml<sup>-1</sup>.

### HPLC analysis

Analyses were carried out using the high performance liquid gradient chromatography technique by using an Agilent 1200 series system (Agilent Technologies) equipped with a degasser (G1322A), a quaternary pump (G1311A), automatic liquid sampler (ALS, G1329A), temperature control column oven (TCC, G1316A) and diode array detector (DAD, G1315D). The

analytical column used was a reversed-phase TSK-gel ODS 80TM (250 mm  $\times$  4.6 mm, particle size 5  $\mu$ m; Tosoh, Tokyo, Japan). The injection volume was 30  $\mu$ l. The separation of HAAs was achieved under gradient conditions using solvent A and solvent B as the mobile phases at a flow rate of 1.0 ml·min<sup>-1</sup>. The running time was 35 min. The gradient program for HAAs quantification was: 0–15.0 min 5% A, 15.0–25.0 min 25% A, 25.0–30.0 min 45% A, 30.0–30.1 min 30% A, and 30.1–35.0 min 5% A. The UV detector was set to different wavelengths for different compounds during the entire experiment, the detection being carried out at 228 nm for A $\alpha$ C and MeA $\alpha$ C, at 263 nm for MeIQx, 4,8-DiMeIQx and Trp-P-2, at 253 nm for IQ, Norharman and Harman, and at 321 nm for PhIP.

### Moisture content

The moisture content of samples was determined by rapid microwave drying using MJ33 Infrared Moisture Analyzer (Mettler Toledo, Columbus, Ohio, USA).

### Statistical analyses

Data were analysed by SPSS for Windows version 21.0 (IBM, Armonk, New York, USA). The results were expressed as mean  $\pm$  standard deviation (*SD*). Data were examined by analysis of variance (ANOVA) followed by Duncan's test and means were considered significant at  $P < 0.05$ . All pairwise comparisons of data for the beef jerky from different places were examined using an unpaired Student's two-tailed *t*-test. The level of significance was set at  $P < 0.05$ . Contents were calculated based on standard curves with different contents of standards. Quantitative determination was performed using an external calibration curve method. Coefficients of the regression line ( $r^2$ ) for HAAs standard curves were all between 0.997 and 0.999.

## RESULTS AND DISCUSSION

### Recovery of heterocyclic aromatic amines

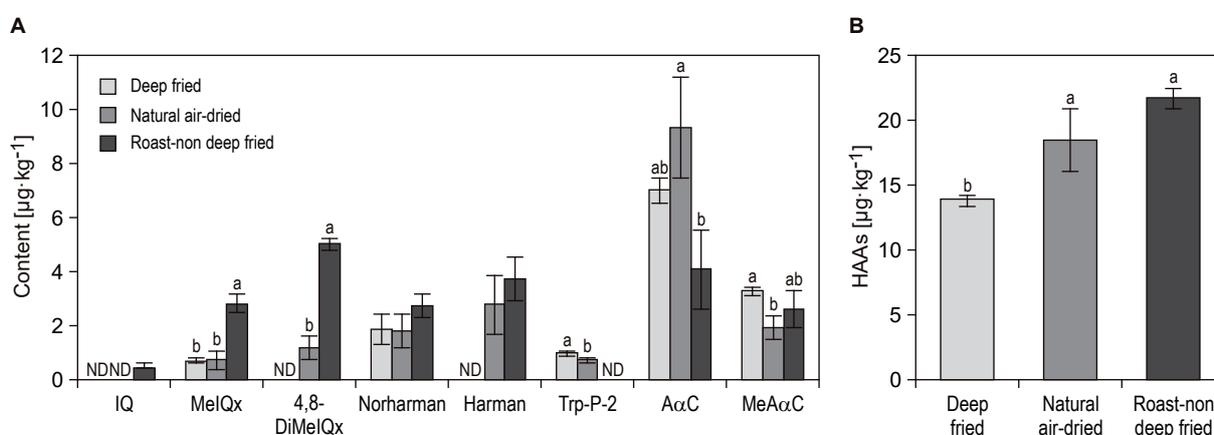
The method used in this study was developed in our laboratory [8]. Recoveries of 9 HAAs were  $54.0 \pm 0.7\%$  for IQ,  $73.2 \pm 0.4\%$  for MeIQx,  $74.0 \pm 0.3\%$  for 4,8-DiMeIQx,  $90.8 \pm 0.7\%$  for PhIP,  $93.4 \pm 0.4\%$  for Harman,  $90.5 \pm 0.8\%$  for Norharman,  $53.3 \pm 1.0\%$  for Trp-P-2,  $84.3 \pm 0.5\%$  for A $\alpha$ C and  $70.3 \pm 0.7\%$  for MeA $\alpha$ C. These were generally in agreement with the results of Oz et al. [22]. Limits of detection (*LOD*) were 0.03, 0.02, 0.03, 0.06, 0.06, 0.10, 0.02,  $0.41 \mu\text{g}\cdot\text{kg}^{-1}$  and  $0.23 \mu\text{g}\cdot\text{kg}^{-1}$  for IQ, MeIQx, 4,8-DiMeIQx, PhIP, Norharman, Harman, TrpP-2, A $\alpha$ C and MeA $\alpha$ C, respectively. Limits of quantification (*LOQ*) were 0.10, 0.05, 0.09, 0.18, 0.33, 0.18, 0.06,  $1.40 \mu\text{g}\cdot\text{kg}^{-1}$

and  $0.70 \mu\text{g}\cdot\text{kg}^{-1}$  for IQ, MeIQx, 4,8-DiMeIQx, PhIP, Harman, Norharman, Trp-P-2, A $\alpha$ C and MeA $\alpha$ C, respectively.

### Effects of the production method on the content of heterocyclic aromatic amines

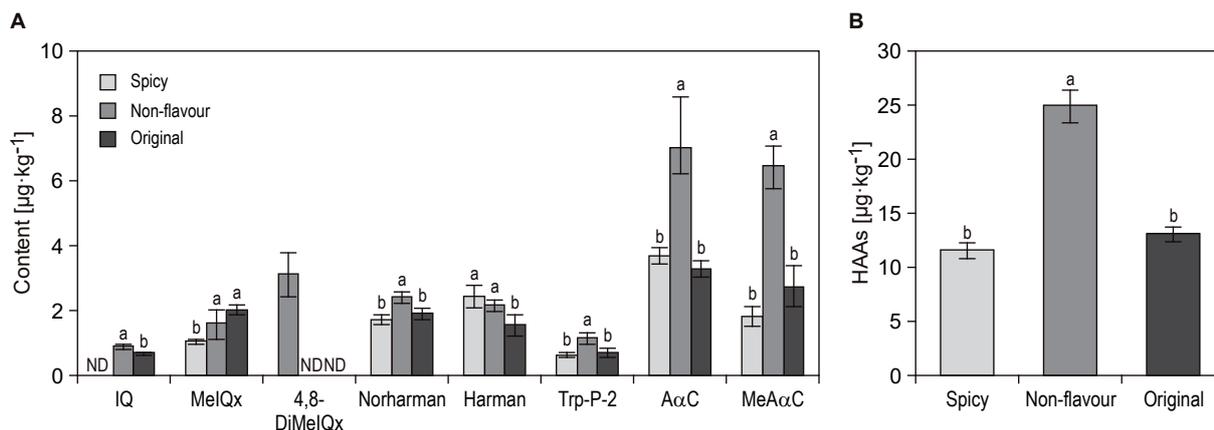
Seven HAAs were found in beef dried by roasting and by natural air drying, whereas 5 different HAAs were found in beef dried by cooking using the deep frying method (Fig. 1A). A $\alpha$ C was the most abundant HAA no matter what cooking method was used, its content ranging from  $4.1 \mu\text{g}\cdot\text{kg}^{-1}$  to  $7.0 \mu\text{g}\cdot\text{kg}^{-1}$ . No significant differences were noticed in Norharman contents among the three processing method. Harman was not detected in deep fried beef jerky and Trp-P-2 was not detected in roasted beef jerky. IQ was only detected in beef dried using the roasting method ( $0.5 \mu\text{g}\cdot\text{kg}^{-1}$ ). Roasting led to an increase in the contents of MeIQx, 4,8-DiMeIQx and in the total HAAs content ( $21.8 \mu\text{g}\cdot\text{kg}^{-1}$ ), which was followed by natural air-drying method ( $18.6 \mu\text{g}\cdot\text{kg}^{-1}$ ) and the deep frying method ( $13.9 \mu\text{g}\cdot\text{kg}^{-1}$ ) (Fig. 1B).

KEŞKEKOĞLU and ÜREN [23] reported that the contents of Norharman and Harman in deep-fried and barbecued beef meatballs ranged from  $1.9 \mu\text{g}\cdot\text{kg}^{-1}$  to  $6.9 \mu\text{g}\cdot\text{kg}^{-1}$  and from “not detected” to  $1.3 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively, which were similar to the quantities found in deep-fried and roasted beef jerky. However, KEŞKEKOĞLU and ÜREN [23] found high contents of IQ,  $303.1 \mu\text{g}\cdot\text{kg}^{-1}$  and  $122.8 \mu\text{g}\cdot\text{kg}^{-1}$ , for charcoal-frying and deep frying methods, and of MeIQx,  $35.2 \mu\text{g}\cdot\text{kg}^{-1}$  and  $29.7 \mu\text{g}\cdot\text{kg}^{-1}$ , for charcoal-frying and deep frying method. Although the contents of these substances detected in that study were higher than the results



**Fig. 1.** Levels of heterocyclic aromatic amines in dried beef products produced by different methods.

A – contents of 8 types of heterocyclic aromatic amines, B – total heterocyclic aromatic amines content for 3 samples. Values with different letters differed significantly ( $P < 0.05$ ). HAAs – heterocyclic aromatic amines, ND – not detected.



**Fig. 2.** Levels of heterocyclic aromatic amines in dried beef products of various flavours.

A – contents of 8 types of heterocyclic aromatic amines, B – total heterocyclic aromatic amines content for 3 samples. Values with different letters differ significantly ( $P < 0.05$ ). HAAs – heterocyclic aromatic amines, ND – not detected.

of our study, both of them had similar trends, in which the roasting method produced more HAAs than the deep frying method. SUN et al. [24] noticed that the heating surface influenced the HAAs contents. Sliced mutton had the lowest content of HAAs and it increased with enlarging the heating surface [24]. Similarly, KEŞKEKOĞLU and ÜREN [23] found, under the same conditions using minced beef, stimulated HAAs formation similar to that in beef jerky.

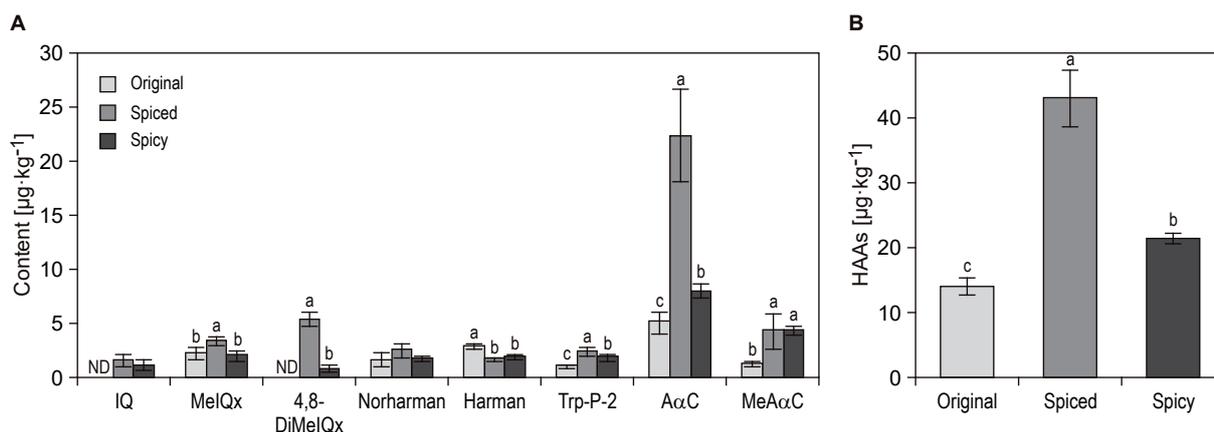
LIAO et al. [25] found the content of HAAs in chicken breast to be  $21.3 \mu\text{g}\cdot\text{kg}^{-1}$  for deep frying (oil at  $180^\circ\text{C}$  for 10 min) and  $112.0 \mu\text{g}\cdot\text{kg}^{-1}$  for charcoal grilling ( $200^\circ\text{C}$  for 20 min). PAN et al. [8] reported that the total content of HAAs was  $18.8 \mu\text{g}\cdot\text{kg}^{-1}$  in a roasted meat product,  $36.6 \mu\text{g}\cdot\text{kg}^{-1}$  in a dried meat product and  $10.3 \mu\text{g}\cdot\text{kg}^{-1}$  in a fried meat product. Both this study and the previous studies illustrate a similar trend that the roasting method could increase the total HAAs content in meat products stronger than the deep frying method. One possible reason for the results could be that the charcoal grilling used a longer cooking time (20 min) and a higher temperature ( $200\text{--}280^\circ\text{C}$ ) than the deep frying method ( $1\text{--}10$  min,  $150\text{--}180^\circ\text{C}$ ). For natural air drying, the results were more complex because the meat was in a solar drying system at a low temperature ( $30\text{--}55^\circ\text{C}$ ) for several days.

#### Effects of spices on the content of heterocyclic aromatic amines

Six types of HAAs were found in spiced air-dried beef, whereas in original and unspiced air-dried beef, 7 and 8 types of HAAs were detected, respectively (Fig. 2A). 4,8-DiMeIQx was only de-

tected in unspiced jerky ( $3.1 \mu\text{g}\cdot\text{kg}^{-1}$ ). Both AαC and MeAαC could be found in all three products, with the highest contents found in dried-beef jerky,  $3.3\text{--}7.0 \mu\text{g}\cdot\text{kg}^{-1}$  and  $2.8\text{--}6.5 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively. Unspiced air-dried beef jerky had significantly higher contents of IQ, Norharman, Trp-P-2, AαC and MeAαC than the other air-dried beef jerkies. Fig. 2B illustrates the average contents of total HAAs in air-dried beef jerky with different flavours. Non-flavoured dried jerky had the highest content of total HAAs ( $24.9 \mu\text{g}\cdot\text{kg}^{-1}$ ), followed by original taste ( $13.0 \mu\text{g}\cdot\text{kg}^{-1}$ ) and spicy taste ( $11.5 \mu\text{g}\cdot\text{kg}^{-1}$ ).

JINAP et al. [26] studied the impact of local spices marinade in beef satay, which showed that the addition of torch ginger reduced the level of AαC and MeIQx, from  $4.6 \mu\text{g}\cdot\text{kg}^{-1}$  to  $0.7 \mu\text{g}\cdot\text{kg}^{-1}$  and from  $12.0 \mu\text{g}\cdot\text{kg}^{-1}$  to  $2.0 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively. In the present study, the reduction of MeIQx content between non-flavoured and spicy dried beef was not that evident, only from  $1.6 \mu\text{g}\cdot\text{kg}^{-1}$  to  $1.1 \mu\text{g}\cdot\text{kg}^{-1}$ . However, a clear reduction could be seen in AαC and MeAαC contents between non-flavoured and spicy samples, from  $7.0 \mu\text{g}\cdot\text{kg}^{-1}$  to  $3.7 \mu\text{g}\cdot\text{kg}^{-1}$  and from  $6.5 \mu\text{g}\cdot\text{kg}^{-1}$  to  $1.8 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively. Meanwhile, a clear reduction could also be seen in AαC and MeAαC contents between non-flavoured and original samples, from  $7.0 \mu\text{g}\cdot\text{kg}^{-1}$  to  $3.3 \mu\text{g}\cdot\text{kg}^{-1}$  and from  $6.5 \mu\text{g}\cdot\text{kg}^{-1}$  to  $2.8 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively. In this study, the reduction of the total HAAs content between spicy and non-flavoured beef jerky was approximately by 46%. These results were consistent with the previous study, which showed that black pepper (1% w/w) reduced HAAs by 12–100% [27]. In the present study, the ingredients used in original and



**Fig. 3.** Levels of heterocyclic aromatic amines in dried yak meat products of various flavours.

A – contents of 8 types of heterocyclic aromatic amines, B – total heterocyclic aromatic amines content for 3 samples. Values with different letters differ significantly ( $P < 0.05$ ). HAAs – heterocyclic aromatic amines, ND – not detected.

spicy beef jerky included white granulated sugar, seasoning wine and mixed spices, which could prevent the formation of HAAs.

Six types of HAAs were found in original taste air dried yak samples and 8 types of HAAs were found in spiced dried yak samples (Fig. 3A). All the values of HAAs were low (under  $5.3 \mu\text{g}\cdot\text{kg}^{-1}$ ) except for A $\alpha$ C. Spiced dried yak jerky had the highest content of MeIQx, 4,8-DiMeIQx, Trp-P-2 and A $\alpha$ C. Furthermore, Trp-P-2 and A $\alpha$ C contents were significantly higher in spicy and spiced flavour samples than in original sample. Norharman content was similar in all flavours, but Harman was higher in original flavour sample than in other samples. IQ was only found in spicy dried yak sample. The average contents of total HAAs in air-dried yak samples is shown in Fig. 3B. Spiced dried yak samples had the highest content of total HAAs ( $43.2 \mu\text{g}\cdot\text{kg}^{-1}$ ), followed by spicy taste ( $21.4 \mu\text{g}\cdot\text{kg}^{-1}$ ) and original taste ( $13.9 \mu\text{g}\cdot\text{kg}^{-1}$ ).

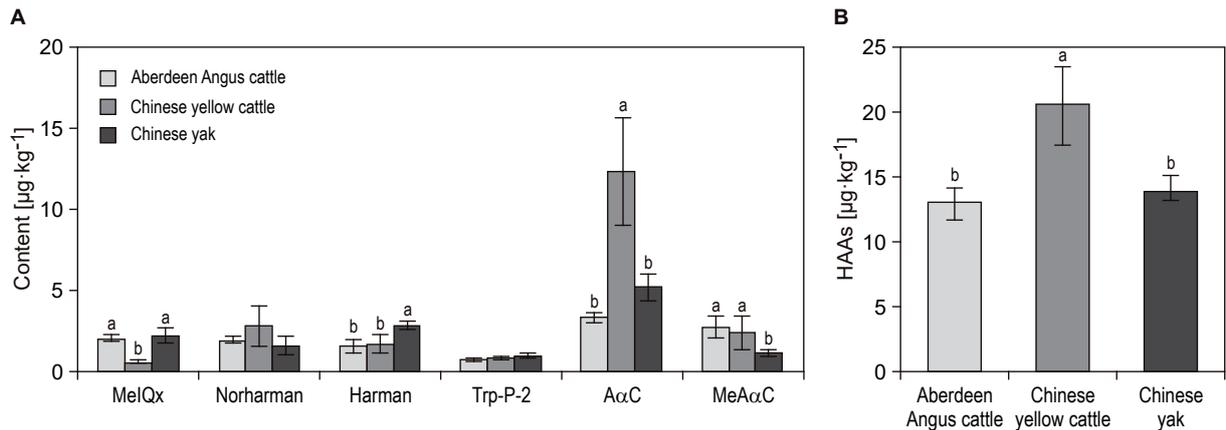
On one hand, spices decreased the contents of HAAs in meat products like beef satay, high fat meatballs or dried beef. However, spices could also increase the contents of HAAs in dried yak meat products. The different types of spices used in these two experiments might have contributed to the adverse results. SUN et al. [24] noticed that salt increased Trp-P-2, MeIQx and IQ contents, whereas sugar, white pepper, cumin and red pepper increased the contents of Norharman and Harman. Moreover, the mixture of these spices increased 4,8-DiMeIQx, PhIP and MeA $\alpha$ C contents. The authors suggested that spices allowed transport of the water-soluble precursors to the surface of the meat, which stimulated HAAs formation. The differences of these results could have been

affected by many factors. Further studies should be conducted on the role of spices and the mechanism they could be implicated in.

#### Effects of breeds on the content of heterocyclic aromatic amines

When the deep fried beef jerky was in original flavour, no matter what kind of breed the beef was (Aberdeen Angus cattle, Chinese yellow cattle or Chinese yak), they all formed 6 types of HAAs (Fig. 4A). The content of each substances, including MeIQx, Trp-P-2, Norharman and Harman, was not higher than  $2.85 \mu\text{g}\cdot\text{kg}^{-1}$ , ignoring the breed. However, the content of A $\alpha$ C in Chinese yellow cattle jerky was significantly higher than in products made from meat of other breeds. Meanwhile, the level of MeA $\alpha$ C in yak jerky sample was lower than in other breed samples. The total contents of HAAs in products made from meat of different breeds are shown in Fig. 4B. The total content of HAAs was quite similar for Aberdeen Angus cattle beef jerky and Chinese yak jerky samples ( $13.0 \mu\text{g}\cdot\text{kg}^{-1}$  and  $13.9 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively), while Chinese yellow cattle had the highest HAAs total content ( $20.6 \mu\text{g}\cdot\text{kg}^{-1}$ ).

In the present study, yellow cattle beef jerky was produced in Inner Mongolia and yak was from Qinghai. The Aberdeen Angus cattle meats were imported from Argentina, which were also used to produce the beef jerky in Inner Mongolia. Thus, it was difficult to conclude whether the breed had an impact on HAAs formation, because the manufacture place might affect the HAAs content. Moreover, other factors, such as ingredient composition, cutting method, aging time, meat shape, gender and thickness, could also contribute to the HAAs



**Fig. 4.** Levels of heterocyclic aromatic amines in dried beef products from different breeds.

A – contents of 6 types of heterocyclic aromatic amines, B – total heterocyclic aromatic amines content for 3 samples. Values with different letters differ significantly ( $P < 0.05$ ). HAAs – heterocyclic aromatic amines.

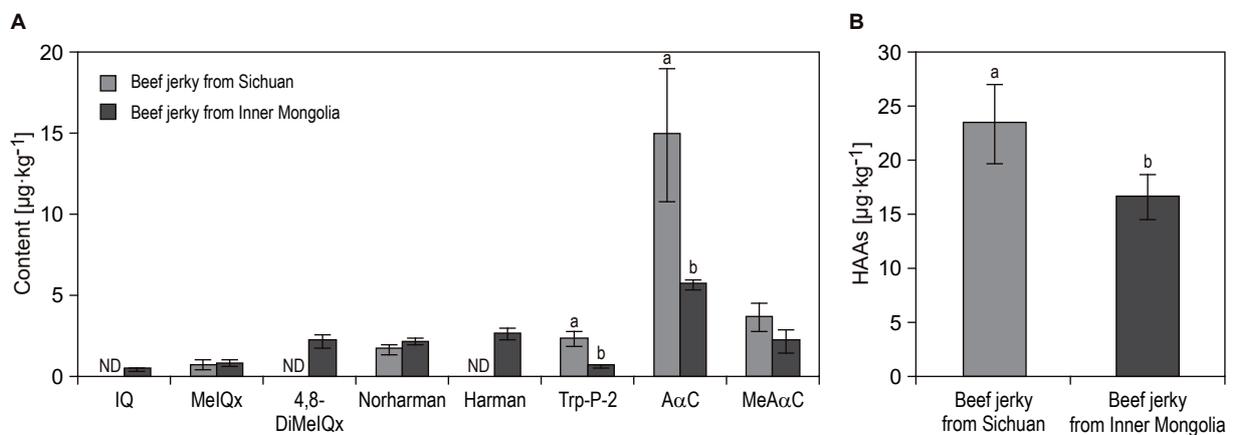
formation. Nevertheless, it could be indicated that the breeds affected HAAs formation in products that underwent deep frying process. Their influence on HAAs formation in meat products that underwent thermal processing requires further research in order to expand knowledge on the processing suitability of raw meat.

#### Effects of the production place on the content of heterocyclic aromatic amines

Beef jerky manufactured in Sichuan and Inner Mongolia contained 5 and 6 types of HAAs, respectively (Fig. 5A). AαC was the most abundant type of HAAs. No significant difference was detected for MeIQx, MeAαC and Norharman between the two samples. AαC and TrP-P-2

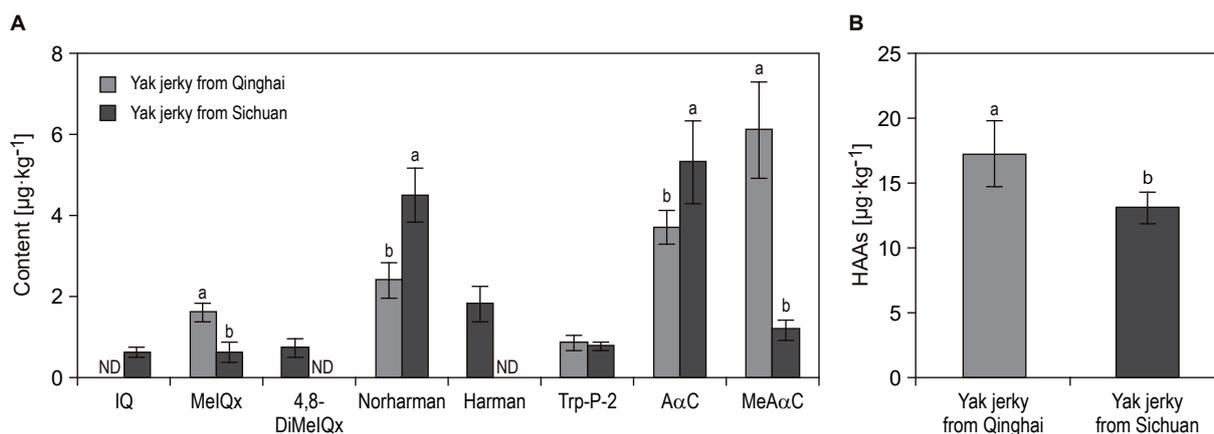
contents were higher in beef jerky from Sichuan ( $15.0 \mu\text{g}\cdot\text{kg}^{-1}$  and  $2.4 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively) compared to beef jerky from Inner Mongolia ( $3.3 \mu\text{g}\cdot\text{kg}^{-1}$  and  $0.7 \mu\text{g}\cdot\text{kg}^{-1}$ , respectively). Contents of other HAA types were lower than  $3.7 \mu\text{g}\cdot\text{kg}^{-1}$ . The total HAAs content of beef jerky produced in Sichuan was higher than in Inner Mongolia ( $23.4 \mu\text{g}\cdot\text{kg}^{-1}$  vs  $12.3 \mu\text{g}\cdot\text{kg}^{-1}$ ) (Fig. 5B).

Regarding yak jerky, 7 types of HAAs were found in the sample made in Qinghai, which was by one HAA type more than in a sample made in Sichuan. (Fig. 6A). 4,8-DiMeIQx and Harman were not detected in yak jerky sample from Sichuan, and IQ was not detected in the sample from Qinghai. As shown in Fig. 6B, the total HAAs content was different in the two



**Fig. 5.** Levels of heterocyclic aromatic amines in dried beef products from different regions.

A – contents of 6 types of heterocyclic aromatic amines, B – total heterocyclic aromatic amines content for 2 samples. Values with different letters differ significantly ( $P < 0.05$ ). HAAs – heterocyclic aromatic amines, ND – not detected.



**Fig. 6.** Levels of heterocyclic aromatic amines in dried yak meat products from different regions.

A – contents of 8 types of heterocyclic aromatic amines, B – total heterocyclic aromatic amines content for 2 samples. Values with different letters differ significantly ( $P < 0.05$ ). HAAs – heterocyclic aromatic amines, ND – not detected.

samples ( $17.3 \mu\text{g}\cdot\text{kg}^{-1}$  in yak jerky from Qinghai and  $12.5 \mu\text{g}\cdot\text{kg}^{-1}$  in yak jerky from Sichuan) and the contents of MeIQx, Norharman, A $\alpha$ C and MeA $\alpha$ C were significantly different between the two samples.

In the present study, the selected beef jerky products were manufactured in Sichuan, Qinghai and Inner Mongolia, and were produced from local meat. SZTERK [12] showed that free amino acids, glucose, free nitrogenous bases and their nucleosides in raw meat influenced HAAs formation during grilling process. Due to differences in feed type, environment, weight, slaughter time and other factors, the cattle from different regions, even the same breed, had different chemical composition that influenced HAAs formation during the cooking process. In addition, the processing parameters including temperature and heating time also played significant roles in HAAs formation [5, 25, 28]. Thus, it is necessary to study more samples from different regions to draw a conclusion whether production place could have an impact on the formation of HAAs.

## CONCLUSIONS

Results of this study show that the processing methods, the species, the breed and the production place affected the HAAs levels in Chinese dried beef jerky products.

Results in this study showed that the production method influenced the quantity of HAAs in final dried-meat products. Dried beef jerky had the highest contents of HAAs using the roasting method. The total content of HAAs was lowest in

deep fried beef samples. HAAs contents were very high when the meat was processed by air-drying method, which used a low temperature.

The results for air-dried beef jerky products confirmed that spices inhibited HAAs formation. However, spices seemed to increase HAAs levels in air-dried yak jerky samples.

HAAs contents in dried beef jerky products varied in different breeds (Aberdeen Angus cattle, Chinese yellow cattle and Chinese yak). The total content of HAAs in beef jerky from Chinese yellow cattle was higher than in the other two selected breeds.

Beef jerky product from Sichuan had higher HAAs content than that of from Inner Mongolia. However, yak jerky product from Sichuan had a lower HAAs content than that from Qinghai.

Eight selected HAAs compounds were determined in the beef jerky and yak jerky samples, in which A $\alpha$ C content was the highest among other compounds in two samples.

Four different factors were studied in the present study. Interactions can occur since several factors can occur at the same time and this aspect will be taken into consideration in the future experiments.

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## REFERENCES

- Turesky, R. J.: Heterocyclic aromatic amines: potential human carcinogens. In: Penning, T. M. (Ed.): Chemical Carcinogenesis. Totowa : Humana Press, 2011, pp. 95–112. ISBN: 978-1-61737-995-6. DOI: 10.1007/978-1-61737-995-6\_5.
- John, K. – Beedanagari, S.: Heterocyclic aromatic amines. In: Wexler, P. (Ed.): Encyclopedia of Toxicology. 3rd edition. Oxford : Academic Press, 2014, pp. 855–863. ISBN: 978-0-12-386455-0. DOI: 10.1016/B978-0-12-386454-3.01127-1.
- Gibis, M.: Heterocyclic aromatic amines in cooked meat products: causes, formation, occurrence, and risk assessment. *Comprehensive Reviews in Food Science and Food Safety*, 15, 2016, pp. 269–302. DOI: 10.1111/1541-4337.12186.
- Gross, G. A. – Grütter, A.: Quantitation of mutagenic/carcinogenic heterocyclic aromatic amines in food products. *Journal of Chromatography A*, 592, 1992, pp. 271–278. DOI: 10.1016/0021-9673(92)85095-B.
- Alaejos, M. S. – Afonso, A. M.: Factors that affect the content of heterocyclic aromatic amines in foods. *Comprehensive Reviews in Food Science and Food Safety*, 10, 2011, pp. 52–108. DOI: 10.1111/j.1541-4337.2010.00141.x.
- Alaejos, M. S. – Gonzalez, V. – Afonso, A. M.: Exposure to heterocyclic aromatic amines from the consumption of cooked red meat and its effect on human cancer risk: A review. *Food Additives and Contaminants Part A*, 25, 2007, pp. 2–24. DOI: 10.1080/02652030701474235.
- Bouvard, V. – Loomis, D. – Guyton, K. Z. – Grosse, Y. – Ghissassi, F. E. – Benbrahim-Tallaa, L. – Guha, N. – Mattock, H. – Straif, K.: Carcinogenicity of consumption of red and processed meat. *Lancet Oncology*, 16, 2015, pp. 1599–1600. DOI: 10.1016/S1470-2045(15)00444-1.
- Pan, H. – Wang, Z. – Guo, H. – Ni, N. – Zhang, D.: Heterocyclic aromatic amines in meat products consumed in China. *Food Science and Biotechnology*, 23, 2014, pp. 2089–2095. DOI: 10.1007/s10068-014-0284-0.
- Puangsoombat, K. – Gadgil, P. – Houser, T. A. – Hunt, M. C. – Smith, J. S.: Heterocyclic amine content in commercial ready to eat meat products. *Meat Science*, 88, 2011, pp. 227–233. DOI: 10.1016/j.meatsci.2010.12.025.
- Puangsoombat, K. – Gadgil, P. – Houser, T. A. – Hunt, M. C. – Smith, J. S.: Occurrence of heterocyclic amines in cooked meat products. *Meat Science*, 90, 2012, pp. 739–746. DOI: 10.1016/j.meatsci.2011.11.005.
- Wong, D. – Cheng, K. W. – Wang, M.: Inhibition of heterocyclic amine formation by water-soluble vitamins in Maillard reaction model systems and beef patties. *Food Chemistry*, 133, 2012, pp. 760–766. DOI: 10.1016/j.foodchem.2012.01.089.
- Szterk, A.: Heterocyclic aromatic amines in grilled beef: The influence of free amino acids, nitrogenous bases, nucleosides, protein and glucose on HAAs content *Journal of Food Composition and Analysis*, 40, 2015, pp. 39–46. DOI: 10.1016/j.jfca.2014.12.011.
- Szterk, A. – Waszkiewicz-Robak, B.: Influence of selected quality factors of beef on the profile and the quantity of heterocyclic aromatic amines during processing at high temperature. *Meat Science*, 96, 2014, pp. 1177–1184. DOI: 10.1016/j.meatsci.2013.11.019.
- Szterk, A. – Roszko, M. – Małek, K. – Kurek, M. – Zbieć, M. – Waszkiewicz-Robak, B.: Profiles and concentrations of heterocyclic aromatic amines formed in beef during various heat treatments depend on the time of ripening and muscle type. *Meat Science*, 92, 2012, pp. 587–595. DOI: 10.1016/j.meatsci.2012.06.004.
- Szterk, A. – Jesionkowska, K.: Influence of the cold storage time of raw beef meat and grilling parameters on sensory quality and content of heterocyclic aromatic amines. *LWT – Food Science and Technology*, 61, 2015, pp. 299–308. DOI: 10.1016/j.lwt.2014.12.005.
- Viegas, O. – Novo, P. – Pinto, E. – Pinho, O. – Ferreira, I. M. P. L. V. O.: Effect of charcoal types and grilling conditions on formation of heterocyclic aromatic amines (HAAs) and polycyclic aromatic hydrocarbons (PAHs) in grilled muscle foods. *Food and Chemical Toxicology*, 50, 2012, pp. 2128–2134. DOI: 10.1016/j.fct.2012.03.051.
- Viegas, O. – Ferreira, I. M. P. L. V. O.: Influence of beer marinades on the reduction of carcinogenic heterocyclic aromatic amines in charcoal-grilled pork meat. *Food Additives and Contaminants Part A*, 32, 2015, pp. 315–323. DOI: 10.1080/19440049.2015.1010607.
- Chen, X. D. – Mujumdar, A. S. (Eds.): *Drying technologies in food processing*. Oxford : Blackwell Publishing, 2008. ISBN: 978-1-4051-5763-6.
- Albright, S. N. – Kendall, P. A. – Avens, J. S. – Sofos, J. N.: Pretreatment effect on inactivation of *Escherichia coli* O157:H7 inoculated beef jerky. *LWT – Food Science and Technology*, 36, 2003, pp. 381–389. DOI: 10.1016/S0023-6438(03)00042-2.
- Yang, H.-S. – Hwang, Y.-H. – Joo, S.-T. – Park, G.-B.: The physicochemical and microbiological characteristics of pork jerky in comparison to beef jerky. *Meat Science*, 82, 2009, pp. 289–294. DOI: 10.1016/j.meatsci.2009.01.029.
- Plavsic, D. – Okanovic, D. – Gubic, J. – Njezic, Z.: Microbiological and chemical evaluation of dried smoked meat product. *Procedia Food Science*, 5, 2015, pp. 239–242. DOI: 10.1016/j.profoo.2015.09.061.
- Oz, F. – Kaban, G. – Kaya, M.: Effects of cooking methods and levels on formation of heterocyclic aromatic amines in chicken and fish with Oasis extraction method. *LWT – Food Science and Technology*, 43, 2010, pp. 1345–1350. DOI: 10.1016/j.lwt.2010.04.014.
- Keşkekoğlu, H. – Üren, A.: Inhibitory effects of pomegranate seed extract on the formation of heterocyclic aromatic amines in beef and chicken meatballs after cooking by four different methods. *Meat Science*, 96, 2014, pp. 1446–1451. DOI: 10.1016/j.meatsci.2013.12.004.

24. Sun, L. – Zhang, F. – Yong, W. – Chen, S. – Yang, M. – Ling, Y. – Chu, X. – Lin, J.: Potential sources of carcinogenic heterocyclic amines in Chinese mutton shashlik. *Food Chemistry*, 123, 2010, pp. 647–652. DOI: 10.1016/j.foodchem.2010.05.019.
25. Liao, G. Z. – Wang, G. Y. – Xu, X. L. – Zhou, G. H.: Effect of cooking methods on the formation of heterocyclic aromatic amines in chicken and duck breast. *Meat Science*, 85, 2010, pp. 149–154. DOI: 10.1016/j.meatsci.2009.12.018.
26. Jinap, S. – Iqbal, S. Z. – Talib, N. H. – Hasnol, N. D. S.: Heterocyclic aromatic amines in deep fried lamb meat: the influence of spices marination and sensory quality. *Journal of Food Science and Technology-Mysore*, 53, 2016, pp. 1411–1417. DOI: 10.1007/s13197-015-2137-0.
27. Oz, F. – Kaya, M.: The inhibitory effect of black pepper on formation of heterocyclic aromatic amines in high-fat meatball. *Food Control*, 22, 2011, pp. 596–600. DOI: 10.1016/j.foodcont.2010.10.010.
28. Puangsombat, K. – Jirapakkul, W. – Smith, J. S.: Inhibitory activity of Asian spices on heterocyclic amines formation in cooked beef patties. *Journal of Food Science*, 76, 2011, pp. T174–T180. DOI: 10.1111/j.1750-3841.2011.02338.x.

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