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Blueberry, raspberry, and strawberry extracts reduce the formation of carcinogenic heterocyclic amines in fried camel, beef and chicken meats

Mohammad Rizwan Khan [a], Rosa Busquets [b,*], Mohammad Azam [a]

[a] Department of Chemistry, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia
[b] School of Life Sciences, Pharmacy and Chemistry, Kingston University London, Penrhyn Road, KT1 2EE, Kingston Upon Thames, United Kingdom

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ABSTRACT

Heterocyclic amines (HCAs) are toxic products from the Maillard reaction that form from the reaction of sugars, amino acids and creatine/creatinine when cooking protein rich food. In this work, commonly consumed meats in Saudi Arabia (camel, beef and chicken) were fried under conditions resembling home cooking. The effect of marinades made of blueberry, raspberry and strawberry were tested separately on meat at different marinating times (1, 6, 12, 24h, at 4 °C) before frying. The marinades caused an overall reduction of HCAs. The decrease was more noticeable with long marination time ≥ 6h. The reduction of individual HCAs, after 24h marinades, was 91–100% for pyridines; 40–67% for β-carbolines; and 100% for quinoxalines, quinolines, α-carbolines and ɣ-carbolines, although the latter three were seldomly detected in this study. An increase, up to 2 times, on the formation of the studied quinoxalines was observed in every meat and marination for no more than 1h. Therefore, longer marinating times with berry extracts, from 6h, are recommended over those below (1h).

1. Introduction

In the last 30 years, the occurrence of the foodborne carcinogenic heterocyclic amines (HCAs) in various protein-rich cooked foods such as meat and fish has been extensively investigated (Barzegar, Kamankesh, & Mohammadi, 2019; Khan, Busquets, Saurina, Hernández, S., & Puignou, 2013, Lu, Kuhnle, & Cheng, 2017). Thus far, over 24 HCAs have been identified in cooked food and it is accepted that HCAs can form from reactions of amino acids, creatine/creatinine and sugar, although these 3 types of biomolecules are not essential for the formation of all HCAs (Gibis & Weiss, 2015; Murkovic, 1999; Skog, Johansson, & Jägerstad, 1998). Structurally, HCAs found in food are in the form of aminocarbolines and aminoimidazoazaarenes. While aminocarbolines are described to form from amino acids and protein pyrolysis at high temperatures (>300 °C), aminoimidazoazaarenes form readily at lower temperatures via aldol condensation of pyrazines or pyridines with aldehydes and creatinine (Naushad & Khan, 2014; Oz & Kotan, 2016).

The relationship between the consumption of red meat and the likelihood of developing different types of cancer has been established in epidemiological studies (Oostindjer et al., 2014), however the link between exposure to HCAs and the onset of these cancers remains unclear (Bellamri & Turesky, 2019). Animals studies and clinical trials have been performed to elucidate the causative link between exposure to HCAs and alterations in DNA (Tang, Kassie, Qian, Ansha, & Turesky, 2013; Turesky & Vouros, 2004). However, many of the existing studies were carried out with HCAs concentrations and exposure-times that do not resemble those in a normal diet (Felton et al., 2007).

Recent studies have revealed a correlation between the intake of the HCA 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and the likelihood of developing cancer (Bellamri, Xiao, Murugan, Weight, & Turesky, 2018; Rogers et al., 2016). Indeed, several HCAs are categorized as possible or probable human carcinogens by the International Agency for Research on Cancer (IARC), and there is a recommendation for a reduction of their consumption (IARC, 1993). The US National Toxicology Program (NTP) also listed some HCAs as reasonably anticipated human carcinogens (NTP, 2004). The discovery of mutagenic forms of HCAs and their adducts with DNA in human tissues is indicative of their toxicity under common meat intake levels through diet (Bellamri et al., 2018; Busquets, Frandsen, Jönsson, Puignou & Galceran & Skog 2013; Guo et al., 2018).

However, the exposure to HCA in not unavoidable. The intake of HCAs' through the consumption meat and fish can be reduced by adopting particular cooking practices such as reducing cooking temperature and time, decreasing superficial cooking temperature with

* Corresponding author.
E-mail address: r.busquets@kingston.ac.uk (R. Busquets).

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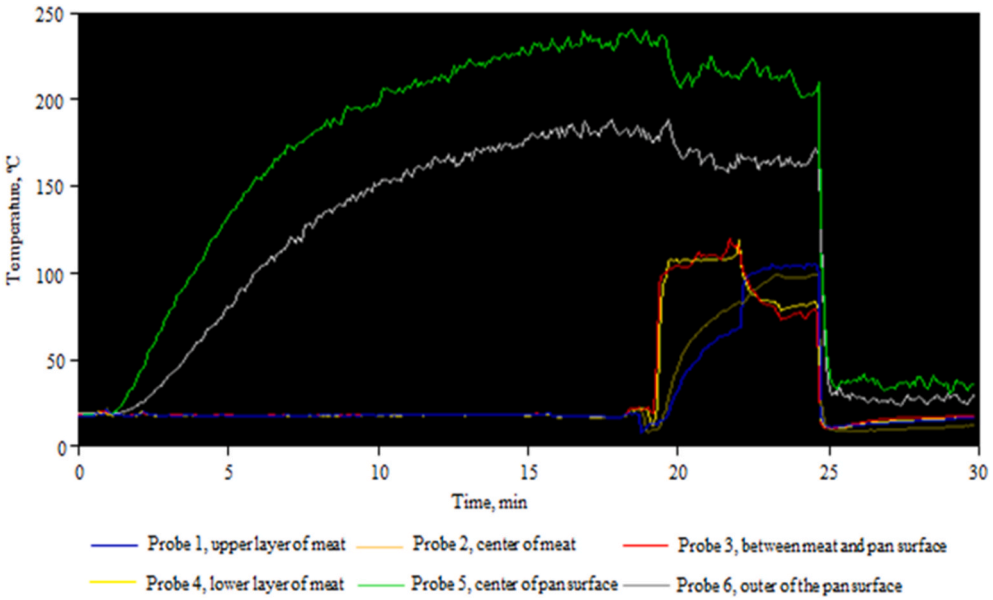


Fig. 1. Temperature profile obtained with type-K proves. Specifically probe 1 was located the upper surface (~2 mm) of meat; probe 2 was at center of meat; probe 3, was located between meat and pan surface; probe 4 was inserted within the lower layer of meat; probe 5 was located at center of pan surface; and probe 6 indicated the temperature at outer of the pan surface.

water (e.g. stews) and using ingredients that affect the transport of HCAs' precursors to the food surface, where temperature will be greater. In this regard, the addition of ingredients with water-holding capacity or marinating methods have been shown to be effective at reducing the formation of HCAs (Oz & Kaya, 2011; Persson, Sjöholm, & Skog, 2003; Vitaglione & Fogliano, 2004).

In Saudi Arabia, HCAs have been reported in camel (Khan, Naushad, & Zeid, 2017) and chicken items from local restaurants (Alsohaimi, Khan, Ali, & Azam, 2019), with some chicken dishes presenting relatively high levels of MeIQx (2–3 ng/g) and PhIP (7–36 ng/g) compared to their levels reported in other items (Busquets, 2012). Recipes including marinades could have an important impact on the formation of HCAs due to the presence of radical scavengers but also the effect of sugars, pH and the aqueous environment that will affect the transport of HCA's precursors within meat. The main hypothesis of this study is that fruit-based marinades (blueberry, raspberry and strawberry) can be effective at reducing the formation of HCAs during the cooking of camel, beef and chicken, three types of meat that are highly consumed in Saudi Arabia but also elsewhere.

2.1. Materials and chemicals

Acetonitrile, ethyl acetate and methanol of LC grade were obtained from Merck (Darmstadt, Germany). Ammonium acetate (≥98%), ammonium formate (≥99%), ammonia solution (25%) formic acid (≥98%) and NaOH (≥97%) were purchased from Merck (Darmstadt, Germany). Fifteen HCAs (structures given in Fig. 1S) were studied: 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine (DMIP), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline (7,8-DiMeIQx) 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline (4,7,8-TriMeIQx, internal standard), 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), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2). These HCAs were obtained from Toronto Research Chemicals (Toronto, Canada). The co-mutagenic amines 1-methyl-9*H*-pyrido[3,4-*b*]indole (harman) and 9*H*-pyrido[3,4-*b*]indole (norharman) were purchased from Sigma-Aldrich (Missouri, USA). The HCAs purity was >99%. 4,7,8-TriMeIQx was added in standards and purified sample extracts as internal standard.

The HCAs stock standard solutions were prepared at 200 μg/mL in methanol and used for spiking samples in standard addition. Calibration curves with standard mixtures of fifteen HCAs between 0.001 μg HCAs/mL and 1.00 μg HCAs/mL were prepared to establish the linearity range. 4,7,8-TriMeIQx was added in every standard at constant concentration. Both standard and sample extracts were filtered using a 0.22 μm polytetrafluoroethylene (PTFE) syringe filters (Macherey-Nagel, Düren, Germany) before being injected into the ultra-performance liquid chromatography (UPLC) system.

2.2. Meat sample preparation and cooking

Fresh meat (camel loin, beef fillet and chicken breast) and cooking ingredients (blueberry, raspberry, strawberry and olive oil) were purchased in a local store (Riyadh, Saudi Arabia). The meat and oil were locally produced and berries, which trademark was Driscoll's, were imported: from Mexico (blueberries) and the US (raspberries and strawberries). The visible fat in the meat, including chicken skin, was removed and the meat was cut into fillets of nearly 1 cm in thickness. Blueberries, raspberries and strawberries, individually, were washed with water, cut into small pieces; blended with a juice extractor (Kenwood JE730, China) and filtered to remove pulps and fibres. Individual meat fillets (100 g) and fruit extracts (100 mL) were marinated at different time periods (1, 6, 12 and 24h), at 4 °C, to avoid any microbial contamination. A set of unmarinated samples were used as control samples. Both the marinated and unmarinated meat samples were pan-fried.

A gas cooker (Gibson, Cairo, Egypt) and a non-stick frying pan (Tefal, Durbase Technology, Paris, France) was used. The cooking temperature of the meat samples was measured with type K probes and TC6 software (Nomadics Inc., Stillwater, Oklahoma, USA). Prior to the cooking of meat samples, the probes were calibrated by submerging them in boiling water (Milli-Q) and readings adjusted to 100 °C. Cooking temperature was monitored and recorded every 5 s. The European Prospective

Table 1
Multiple reaction monitoring MS/MS conditions used for the quantification and confirmation of HCAs in meat samples[a].

HCAs	Precursor ion (*m/z*) tentative assignation	Quantification		Confirmation	
		Product ion (*m/z*) tentative assignation	Collision energy (eV)	Product ion (*m/z*) tentative assignation	Collision energy (eV)
DMIP	163 $[M+H]^+$	148 $[M+H-CH_3]^{+\bullet}$	25	147 $[M+H-CH_3-H]^+$	30
PhIP	225 $[M+H]^+$	210 $[M+H-CH_3]^{+\bullet}$	25	183 $[M+H-CH_3-HCN]^{+\bullet}$	30
Harman	183 $[M+H]^+$	115 $[M+H-CH_3CN-HCN]^+$	30	168 $[M+H-CH_3]^{+\bullet}$	30
Norharman	169 $[M+H]^+$	115 $[M+H-2HCN]^+$	30	142 $[M+H-HCN]^+$	25
IQ	199 $[M+H]^+$	184 $[M+H-CH_3]^{+\bullet}$	30	157 $[M+H-CH_3-HCN]^{+\bullet}$	35
MeIQ	213 $[M+H]^+$	198 $[M+H-CH_3]^{+\bullet}$	25	197 $[M+H-CH_3-H]^+$	30
MeIQx	214 $[M+H]^+$	199 $[M+H-CH_3]^{+\bullet}$	30	172 $[M+H-CH_3-HCN]^{+\bullet}$	30
4,8-DiMeIQx	228 $[M+H]^+$	213 $[M+H-CH_3]^{+\bullet}$	30	187 $[M+H-C_2NH_3]^+$	25
7,8-DiMeIQx	228 $[M+H]^+$	172 $[M+H-CH_3-C_2NH_3]^{+\bullet}$	35	213 $[M+H-NH_3]^{+\bullet}$	25
4,7,8-TriMeIQx (IS)	242 $[M+H]^+$	227 $[M+H-CH_3]^{+\bullet}$	25	201 $[M+H-C_2NH_3]^+$	30
Glu-P-1	199 $[M+H]^+$	172 $[M+H-HCN]^+$	25	184 $[M+H-CH_3]^{+\bullet}$	25
Glu-P-2	185 $[M+H]^+$	158 $[M+H-HCN]^+$	25	131 $[M+H-HCN-HCN]^+$	30
AαC	184 $[M+H]^+$	167 $[M+H-NH_3]^+$	25	140 $[M+H-NH_3-HCN]^+$	30
MeAαC	198 $[M+H]^+$	181 $[M+H-NH_3]^+$	25	154 $[M+H-NH_3-HCN]^+$	30
Trp-P-1	212 $[M+H]^+$	195 $[M+H-NH_3]^+$	25	168 $[M+H-NH_3-HCN]^+$	30
Trp-P-2	198 $[M+H]^+$	154 $[M+H-NH_3-HCN]^+$	30	181 $[M+H-NH_3]^+$	25

[a] System dwell time was 0.025 s in all studied compounds; IS, internal standard.

Investigation into Cancer and Nutrition (EPIC) defines frying cooking method as cooking of food in either fat or oil. In this study, to prevent the meat sticking to the pan, 5 mL of olive oil was added to the pan at the beginning of the cooking process. The cooking started when the temperature in the centre of the pan with a layer of oil was between 215 °C and 230 °C. The total cooking time was 8 min: the meats were moved around the with oil for 4 min, following which they were flipped and moved around the pan for 4 min more. Subsequently, the cooked meat samples were cleaned and all pan residues, including retained oil, were removed. Cooking weight loss was measured by weighing the meat before and after cooking. Every meat fillet was marinated and cooked independently in duplicate. Control samples were also prepared in duplicate. The meat crusts from the cooked meet were separated, pooled, ground and refrigerated until characterisation. Meat samples were blended using a stardust coffee grinder, CML-1000MKII (Osaka, Japan), and a Microtron® MB800, Kinematica AG (Littau, Switzerland).

2.3. HCAs extraction from meat samples and quantification

The cartridges used for the extraction of HCAs were octadecylsilane (C_{18}, 100 mg) and Bond Elut propylsulfonyl silica (PRS, 500 mg). These solid phase extraction (SPE) cartridges, connecters and stopcocks were obtained from Varian (Harbor City, USA). Extraction columns (Extrelut NT20) were purchased from Merck (Darmstad, Germany). Hydromatrix bulk material (diatomaceous earth) was purchased from Agilent Technologies (Santa Clara, California, USA). The SPE was carried out with Visiprep™ and Visidry™ vacuum manifolds, from Supelco (Gland, Switzerland). They were used for the purification of HCAs, and drying the elution solvent through evaporation, respectively.

The refrigerated ground meat crusts were allowed to equilibrate at room temperature (25 °C) for >30 min. Sodium hydroxide solution (50 mL, 1M) was added to the ground meat crusts (20 g) followed by homogenization using ultra-turrax T25 digital homogenizer (from IKA®-WERKE GmbH, Staufen, Germany). Homogenized meat samples (3 g) were carefully mixed with hydromatrix bulk material (14 g, diatomaceous earth) and moved to an empty column (60 mL) connected to PRS cartridge (500 mg).

The PRS cartridge was previously preconditioned using HCl 0.1 M (5 mL), water (10 mL) and methanol (5 mL). Ethyl acetate (75 mL) was used to extract the HCAs from the homogenized samples dispersed in diatomaceous earth and these were eluted to the PRS cartridge. After the elution, the PRS cartridge was dried under vacuum and washed sequentially using MilliQ water and methanol (4:6, v/v, 15 mL), and Milli Q water (5 mL). The PRS cartridge was then coupled to a C_{18} cartridge (100 mg) which had been preconditioned using methanol (5 mL) and Milli Q water (5 mL). The HCAs were eluted from PRS cartridge to C_{18} cartridge using ammonium acetate (0.5 M, pH 8.5, 20 mL). As a final step, the C_{18} cartridge was washed using Milli Q water (5 mL) followed by drying under low vacuum. The HCAs elution from C_{18} cartridge to a microcentrifuge tube was performed using a methanol and ammonia solution (9:1, v/v, 800 μL). The sample solvent was vaporized mildly using nitrogen. The dried sample extract was reconstituted in methanol containing internal standard (4,7,8-TriMeIQx, 0.5 μg/g, 100 μL). After the reconstitution, the samples were filtered (syringe filter PTFE, 0.22 μm) and analysed by UPLC tandem mass spectrometry (UPLC–MS/MS).

The quantification of HCAs in meat samples was carried out by standard additions method, which consisted of adding a mixture of HCAs at three levels of concentration (50%, 100% and 200%) with respect to the estimated initial level of HCAs in the sample. A duplicate of the sample was processed and analysed without having been spiked. Specifically, The samples were spiked with DMIP, PhIP, IQ, MeIQ, Glu-P-1, Glu-P-2, AαC, MeAαC, Trp-P-1 and Trp-P-2 at final concentration levels of 0, 10, 50, and 150 ng HCAs/meat g and for harman, norharman, MeIQx, 4,8-DiMeIQx and 7,8-DiMeIQx were 0, 5, 10 and 30 ng HCAs/g meat. The standard addition quantification of every type cooked meat was carried out in triplicate. Recovery rates were estimated from the slope of the linear regression between the added and recovered HCAs amounts in the meat samples.

2.4. Instrumentation

2.4.1. HCAs separation

The optimal HCAs separation was performed using an UPLC (Acquity®, Waters, Milford, USA). The analytical column used was an ethylene bridged hybrid (BEH C_{18}) with (50 mm × 2.1 mm i.d. and 1.7 μm particle size, Acquity® from Waters (Milford, USA). The mobile phase used was acetonitrile (A) and buffer solution (30 mM formic acid/ammonium formate, pH 4.7, B) at 500 μL/min. The elution programme was: 5% A in B; 0–0.1 min; 5–30% A in B, 0.1–1.5 min; 30–60% A in B, 1.5–1.8 min; 60% A in B, 1.8–2.5 min. As precaution, the column was washed for 2 min with methanol:water (50:50) every twenty sample injections. The injection volume was 5 μL. This analytical method was adopted from a previously developed method (Barcelo-Barrachina et al., 2006), with minor changes.

Table 2
Cooking conditions of the meat samples processed with the marinades assayed.

Sample[a], marinating time (h)	Sample code	Raw meat (g)	Raw meat thickness (cm)	Fruits extract (mL)	Cooking temperature (°C)	Cooking time (4 min/side)	Fried meat (g)	Weight loss (%)
Camel[a] (control sample)	CACS	200.80	1.2	50	215–230	8.10	109.36	45.54
Camel with blueberry, (1 h)	CABL-1 h	200.74	1.1	50	215–230	8.00	125.65	37.41
Camel with raspberry, (1 h)	CARA-1 h	200.36	1.1	50	215–230	8.15	129.23	35.50
Camel with strawberry, (1 h)	SACT-1 h	200.25	1.3	50	215–230	8.15	123.65	38.25
Camel with blueberry, (6 h)	CABL-6 h	200.20	1.1	50	215–230	8.05	139.14	30.50
Camel with raspberry, (6 h)	CARA-6 h	200.35	1.1	50	215–230	8.10	142.20	29.02
Camel with strawberry, (6 h)	CAST-6 h	200.45	1.3	50	215–230	8.15	138.45	30.93
Camel with blueberry, (12 h)	CABL-12 h	200.20	1.1	50	215–230	8.00	148.89	25.63
Camel with raspberry, (12 h)	CARA-12 h	200.12	1.1	50	215–230	8.13	152.20	23.95
Camel with strawberry, (12 h)	CAST-12 h	200.32	1.3	50	215–230	8.15	147.42	26.41
Camel with blueberry, (24 h)	CABL-24 h	200.15	1.1	50	215–230	8.10	160.60	19.76
Camel with raspberry, (24 h)	CARA-24 h	200.35	1.1	50	215–230	8.10	168.23	16.03
Camel with strawberry, (24 h)	CAST-24 h	200.42	1.3	50	215–230	8.15	164.42	17.96
Beef[b] (control sample)	BECS	200.52	1.2	50	215–230	8.15	108.11	46.09
Beef with blueberry, (1 h)	BEBL-1 h	200.13	1.1	50	215–230	8.20	123.32	38.38
Beef with raspberry, (1 h)	BERA-1 h	200.42	1.3	50	215–230	8.10	125.20	37.53
Beef with strawberry, (1 h)	BEST-1 h	200.35	1.2	50	215–230	8.00	128.12	36.05
Beef with blueberry, (6 h)	BEBL-6 h	200.46	1.3	50	215–230	8.15	140.32	30.00
Beef with raspberry, (6 h)	BERA-6 h	200.42	1.2	50	215–230	8.00	135.45	32.42
Beef with strawberry, (6 h)	BEST-6 h	200.56	1.1	50	215–230	8.20	138.65	30.87
Beef with blueberry, (12 h)	BEBL-12 h	200.32	1.3	50	215–230	8.15	147.95	26.14
Beef with raspberry, (12 h)	BERA-12 h	200.85	1.3	50	215–230	8.10	152.10	24.27
Beef with strawberry, (12 h)	BEST-12 h	200.45	1.1	50	215–230	8.10	149.65	25.34
Beef with blueberry, (24 h)	BEBL-24 h	200.60	1.1	50	215–230	8.00	162.32	19.08
Beef with raspberry, (24 h)	BERA-24 h	200.78	1.2	50	215–230	8.10	161.18	19.72
Beef with strawberry, (24 h)	BEST-24 h	200.95	1.1	50	215–230	8.00	163.35	18.71
Chicken[c] (control sample)	CHCS	200.86	1.2	50	215–230	8.20	104.25	48.10
Chicken with blueberry, (1 h)	CHBL-1 h	200.65	1.1	50	215–230	8.10	117.52	41.43
Chicken with raspberry, (1 h)	CHRA-1 h	200.12	1.1	50	215–230	8.15	118.98	40.55
Chicken with strawberry, (1 h)	CHST-1 h	200.30	1.2	50	215–230	8.00	120.54	39.82
Chicken with blueberry, (6 h)	CHBL-6 h	200.25	1.3	50	215–230	8.00	141.20	29.49
Chicken with raspberry, (6 h)	CHRA-6 h	200.50	1.3	50	215–230	8.10	143.65	28.35
Chicken with strawberry, (6 h)	CHST-6 h	210.87	1.1	50	215–230	8.20	152.65	27.61
Chicken with blueberry, (12 h)	CHBL-12 h	200.45	1.2	50	215–230	8.10	149.21	25.56
Chicken with raspberry, (12 h)	CHRA-12 h	200.65	1.1	50	215–230	8.00	151.10	24.69
Chicken with strawberry, (12 h)	CHST-12 h	200.25	1.3	50	215–230	8.30	150.65	24.77
Chicken with blueberry, (24 h)	CHBL-24 h	200.50	1.2	50	215–230	8.20	162.36	19.02
		200.30	1.2	50	215–230	8.00	158.96	20.64

(*continued on next page*)

Table 2 (*continued*)

Sample[a], marinating time (h)	Sample code	Raw meat (g)	Raw meat thickness (cm)	Fruits extract (mL)	Cooking temperature (°C)	Cooking time (4 min/side)	Fried meat (g)	Weight loss (%)
Chicken with raspberry, (24 h)	CHRA-24 h							
Chicken with strawberry, (24 h)	CHST-24 h	200.15	1.1	50	215–230	8.10	155.52	22.30

a,b,c meat cooked without fruits extract (control samples).
[a] Marinating temperature (4 °C).

2.4.2. HCAs determination

The HCAs were detected with a triple quadrupole mass analyser model Quattro Premier Micromass (Milford, USA) equipped with electrospray (ESI) working in positive mode. The quantification was carried out in multiple reaction monitoring (MRM) mode. The protonated HCA molecular ions $[M+H]^+$ were the precursor ions that were fragmented to product ions that were used for the quantification and confirmation of the analytes (see Table 1). The working conditions of the ESI source were: 100 °C source temperature; 350 °C desolvation temperature; 3.6 KV capillary voltage; 38 V cone voltage; 700 L/h desolvation gas; 70 L/h cone gas. High purity of nitrogen gas was used, produced from Peak Scientific nitrogen generator (NM30LA, Inchinnan, United Kingdom) for the for the cone gas. High purity argon for the collision gas was from Speciality Gas Centre, (Jeddah, Saudi Arabia). The software used for the analysis was Waters MassLynx V4.1 (Milford, USA).

2.4.3. Statistical analysis

The comparison of the concentration of HCAs with marinating time and berry extrats was carried out with 2-way ANOVA with replicates and student-t test comparing means using Microsoft™ Excel 2019.

3. Results and discussion

Marinating meat prior cooking has shown to be among the most effective ways to reduce the overall formation of HCAs (Busquets, Puignou, Galceran, & Skog, 2006; Manful et al., 2020). This is due to both physical and chemical effects of marinades on the Maillard reaction leading to the formation of HCAs. This study explores whether HCAs levels in commonly consumed meat can be reduced effectively with fruit extracts. The fruit extracts tested here have potential to affect the formation of HCAs and they can be used in recipes that consumers may accept.

The composition of the marinades was chosen on the basis of well accepted health benefits of the studied berry marinades (Gowd, Bao, & Chen, 2019;Zhou, Xie, Yang, & Liu, 2020).

The cooking was carried out with full control of temperature. An example of the temperature profile is given in Fig. 1. During the cooking processes, the temperature measured at 2 mm below the meat surface (probe 1 and 4) did not go over 120 °C. The cooking conditions of every experiment are summarised in Table 2. Under these conditions, the meat weight loss was affected by the duration of the marinade, as displayed in Fig. 2. Control samples experienced the same cooking weight loss (46–48%) regardless the meat type. The minimum cooking weight loss, 18–22%, was achieved with the longest marination time (>6h). When comparing cooking weight loss in the present study with an earlier study using wine marinades (Busquets et al., 2006), cooking weigh loss was lower with the berry marinades. This can be important when comparing the effectivity of different marinades because a reduction of cooking loss, through the addition of ingredients with water holding capacity, was responsible for a significant reduction on the formation of PhIP and quinoxalines in burgers (Persson et al., 2003). Hence, the reduction of cooking loss, and its consequent effect on the transport of HCA precursors within the meat, could play a role on decreasing the formation of HCAs in the current study, besides chemical effects by the marinade components.

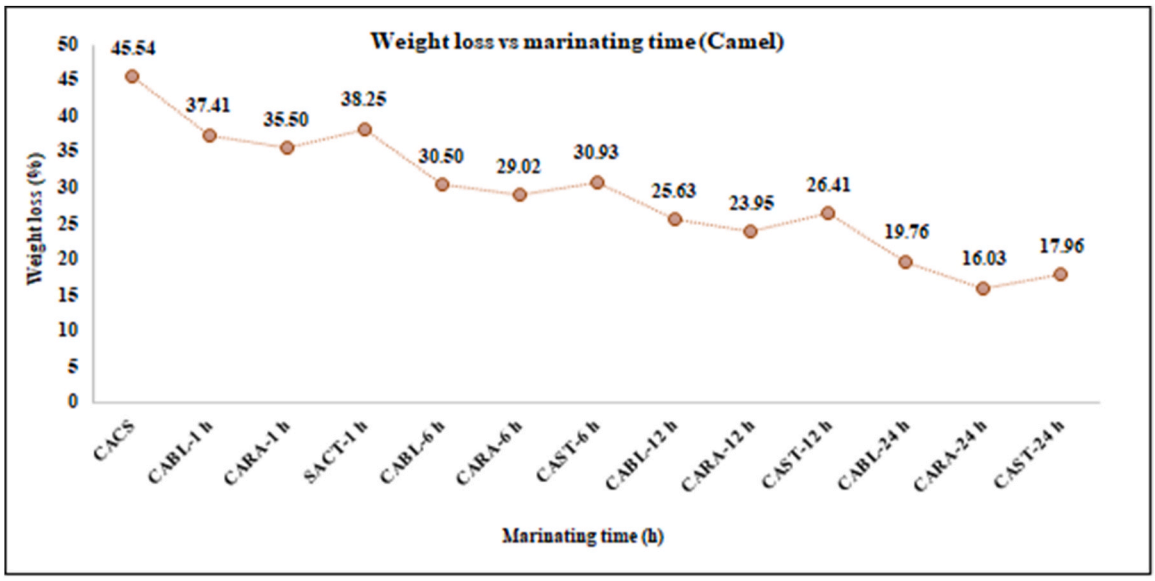
The concentrations of HCAs in unmarinated and marinated samples are reported in Table 3. Among unmarinated samples (control samples), chicken, with 41 ng mutagenic HCAs/g, was the most contaminated food, as compared to unmarinated camel (18 ng mutagenic HCAs/g) and beef (12 ng mutagenic HCAs/g). Although there are numerous examples of HCA levels in cooked chicken and beef samples reported in the literature, it is interesting to know how the total levels of mutagenic HCA in unmarinated camel relate to unmarinated chicken and beef cooked under the same conditions. The different concentration if HCAs can be due to different levels of HCA precursors in the raw meat. For instance, Gibis and Weiss (2015) confirmed that the ratio of creatin(in)e to glucose was correlated with PhIP, MeIQx and harman levels in different types of cooked meat. The greater concentration of PhIP in chicken, an item with low glucose concentration, was attributed to the presence of certain free amino acids and creatinine (Gibis & Weiss, 2015).

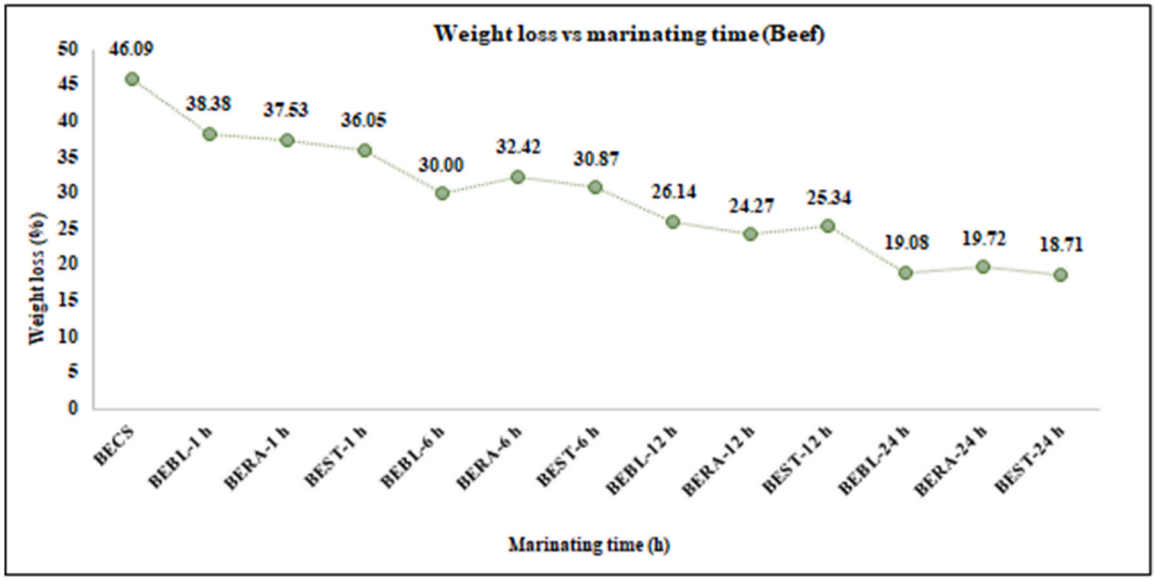
In this study, even α-carbolines and ɣ-carbolines, which are traditionally reported to form at 300 °C, were identified in chicken cooked under temperatures below 120 °C (Table 3). This suggests that the definition of thermal amines needs to be revised. The very sensitive analysis carried out (limits of detection and recoveries in the analysis reported in Supporting Information Table S1) also made possible the quantification of the Glu-P-1 and Glu-P-2 in fried chicken (only). These pyridoimidazoles have been seldomly reported in the literature.

The probable mutagens IQ and MeIQ (IARC, 1993) have been detected in the study chicken samples only. These 2 quinolines were also detected, at a level with the same order of magnitude, in the chicken sample (namely Shawaya) from a traditional dish prepared at a Saudi restaurant (Alsohaimi et al., 2019). IQ and MeIQ do not form in chicken exclusively as they have been detected in other matrices (e.g. fish, beef, pork and goose) (Busquets, 2012; Barzegar et al., 2019). Given the high toxicity of the quinolines detected in cooked meat, which have been linked to causing tumours in animal studies (Sugimura, Wakabayashi, Nakagama, & Nagao, 2004), the consumption of fried chicken should be questioned at least for people who are at greater risk of developing cancer until more is known about the link between cooked meat and different types of cancer.

The quantification of HCAs in the 3 types of meat, with individual blueberry, raspberry and strawberry marinades, which are rich in antioxidants, under conditions resembling marinating in Saudi Arabian recipes, informs about the change of HCA contamination in these meats caused by the berry marinades (Fig. 3, Table 3). The marinades were selected because that approach can be easily adopted by the public. The 3 marinades affected the formation of HCAs in the 3 types of meat with a similar trend: there was a strong reduction in the formation of the pyridines DMIP and PhIP; and the β-carbolines harman and norharman with increased marinating time. Harman and norharman were not enhanced by the marinade in this study as opposed to when common cooking recipes that included multiple ingredients were used (Khan, Busquets, Naushad, & Puignou, 2019). Although harman levels can be correlated with glucose (Gibis & Weiss, 2015), they were not increased with the application of fruit juices in this work. However, it is possible that the enhancing effect of glucose on harman could be masked by the reaction caused by other mechanisms.

Marinating for 12 and 24h was found to cause a significantly greater





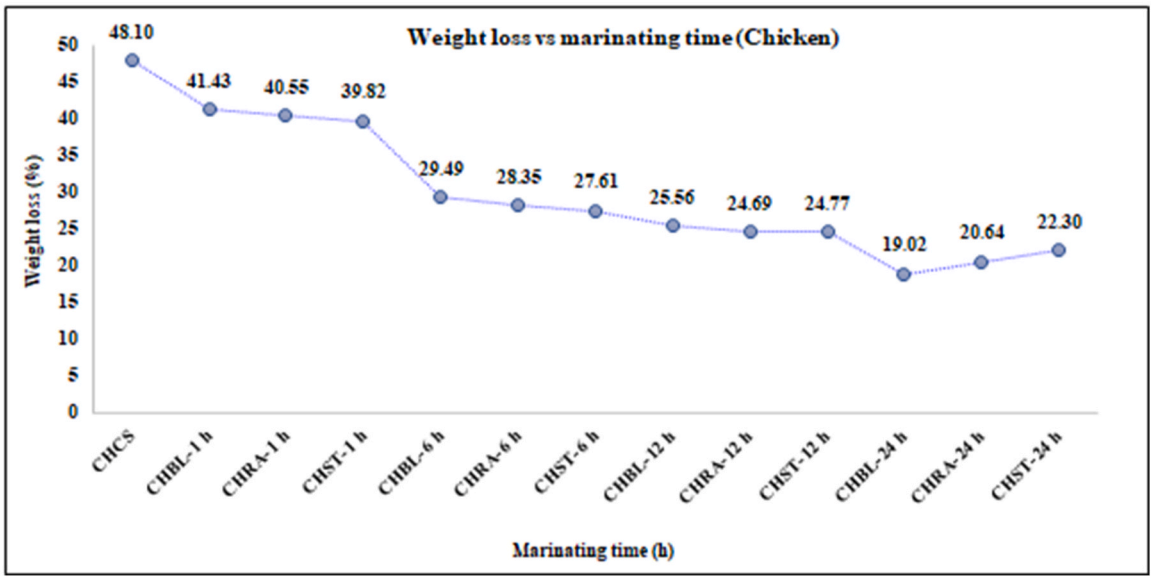


Fig. 2. Meat weight loss vs. marinating time under the study conditions (n = 2).

Table 3
HCAs identified in thermally processed camel, beef and chicken meat samples marinated with highly antioxidant fruits. The acronyms CA (camel), BE (beef), CH (chicken), BL (blueberry), RA (raspberry), ST (strawberry) are used

Sample code	DMIP (ng/g) ± sd	PhIP (ng/g) ± sd	Harman (ng/g) ± sd	Norharman (ng/g) ± sd	IQ (ng/g) ± sd	MeIQ (ng/g) ± sd	MeIQx (ng/g) ± sd	4,8-DiMeIQx (ng/g) ± sd	7,8- DiMeIQx (ng/g) ± sd	Glu-P-1 (ng/g) ± sd	Glu-P-2 (ng/g) ± sd	AαC (ng/g) ± sd	MeAαC (ng/g) ± sd	Trp-P-1 (ng/g) ± sd	Trp-P-2 (ng/g) ± sd
CACS[a,]	4.23 ± 0.31	8.65 ± 0.53	2.36 ± 0.16	5.45 ± 0.34	Nd	Nd	2.85 ± 0.15	1.82 ± 0.10	0.84 ± 0.03	nd	nd	nd	nd	0.03 ± 0.002	0.03 ± 0.002
CABL-1 h	1.82 ± 0.08	4.36 ± 0.04	1.82 ± 0.07	4.68 ± 0.26	Nd	Nd	3.62 ± 0.12	2.54 ± 0.13	1.06 ± 0.02	nd	nd	nd	nd	0.01 ± 0.001	0.01 ± 0.001
CARA-1 h	2.68 ± 0.12	5.45 ± 0.06	1.94 ± 0.09	4.82 ± 0.28	Nd	Nd	3.38 ± 0.14	2.63 ± 0.14	1.21 ± 0.04	nd	nd	nd	nd	0.01 ± 0.001	0.01 ± 0.001
CAST-1 h	2.42 ± 0.11	4.67 ± 0.04	1.87 ± 0.08	4.72 ± 0.26	Nd	Nd	3.74 ± 0.12	2.86 ± 0.13	1.06 ± 0.05	nd	nd	nd	nd	nd	nd
CABL-6 h	1.62 ± 0.05	4.03 ± 0.04	1.62 ± 0.05	4.12 ± 0.21	Nd	Nd	1.52 ± 0.12	1.26 ± 0.10	0.01 ± 0.001	nd	nd	nd	nd	nd	nd
CARA-6 h	2.41 ± 0.12	4.85 ± 0.05	1.74 ± 0.06	4.19 ± 0.23	Nd	Nd	1.75 ± 0.13	1.45 ± 0.11	0.01 ± 0.001	nd	nd	nd	nd	nd	nd
CAST-6 h	2.12 ± 0.10	4.21 ± 0.03	1.68 ± 0.06	4.15 ± 0.24	Nd	Nd	1.63 ± 0.12	1.34 ± 0.11	0.01 ± 0.001	nd	nd	nd	nd	nd	nd
CABL-12 h	1.26 ± 0.03	2.35 ± 0.02	1.48 ± 0.04	3.85 ± 0.18	Nd	Nd	0.87 ± 0.05	0.56 ± 0.03	nd	nd	nd	nd	nd	nd	nd
CARA-12 h	1.42 ± 0.05	2.67 ± 0.02	1.53 ± 0.05	3.89 ± 0.20	Nd	Nd	0.99 ± 0.08	0.69 ± 0.04	nd	nd	nd	nd	nd	nd	nd
CAST-12 h	1.38 ± 0.04	2.53 ± 0.02	1.61 ± 0.04	3.87 ± 0.20	Nd	Nd	0.92 ± 0.08	0.63 ± 0.04	nd	nd	nd	nd	nd	nd	nd
CABL-24 h	nd	0.66 ± 0.04	1.36 ± 0.03	3.21 ± 0.16	Nd	Nd	Nd	nq	nd	nd	nd	nd	nd	nd	nd
CARA-24 h	nd	0.74 ± 0.04	1.38 ± 0.03	3.26 ± 0.13	Nd	Nd	Nd	nq	nd	nd	nd	nd	nd	nd	nd
CAST-24 h	nd	0.68 ± 0.04	1.42 ± 0.02	3.24 ± 0.17	Nd	Nd	Nd	nq	nd	nd	nd	nd	nd	nd	nd
BECS, [b]	2.34 ± 0.13	4.72 ± 0.34	2.25 ± 0.11	3.61 ± 0.18	Nd	Nd	2.13 ± 0.13	1.74 ± 0.12	0.67 ± 0.05	nd	nd	nd	nd	nd	nd
BEBL-1 h	1.95 ± 0.10	3.35 ± 0.23	1.95 ± 0.10	2.61 ± 0.11	Nd	Nd	3.46 ± 0.17	2.12 ± 0.11	0.78 ± 0.04	nd	nd	nd	nd	nd	nd
BERA-1 h	2.23 ± 0.12	3.65 ± 0.43	1.98 ± 0.10	2.68 ± 0.12	Nd	Nd	3.40 ± 0.16	2.33 ± 0.12	0.82 ± 0.03	nd	nd	nd	nd	nd	nd
BEST-1 h	2.05 ± 0.12	3.21 ± 0.53	2.03 ± 0.13	2.85 ± 0.12	Nd	Nd	3.43 ± 0.16	2.02 ± 0.11	0.46 ± 0.06	nd	nd	nd	nd	nd	nd
BEBL-6 h	1.82 ± 0.10	3.25 ± 0.63	1.86 ± 0.12	2.54 ± 0.10	Nd	Nd	0.17 ± 0.01	0.01 ± 0.001	nq	nd	nd	nd	nd	nd	nd
BERA-6 h	2.01 ± 0.12	3.32 ± 0.43	1.85 ± 0.12	2.61 ± 0.13	Nd	Nd	0.19 ± 0.01	0.01 ± 0.001	nq	nd	nd	nd	nd	nd	nd
BEST-6 h	1.98 ± 0.12	2.86 ± 0.02	1.76 ± 0.10	2.65 ± 0.13	Nd	Nd	0.28 ± 0.02	0.01 ± 0.001	nq	nd	nd	nd	nd	nd	nd
BEBL-12 h	0.54 ± 0.02	0.72 ± 0.04	1.32 ± 0.08	1.48 ± 0.06	Nd	Nd	Nq	nd	nd	nd	nd	nd	nd	nd	nd
BERA-12 h	0.46 ± 0.01	0.64 ± 0.03	1.45 ± 0.08	1.62 ± 0.07	Nd	Nd	Nq	nd	nd	nd	nd	nd	nd	nd	nd
BEST-12 h	0.53 ± 0.01	0.68 ± 0.04	1.38 ± 0.07	1.56 ± 0.06	Nd	Nd	Nq	nq	nd	nd	nd	nd	nd	nd	nd
BEBL-24 h	nd	0.01 ± 0.001	1.23 ± 0.05	1.42 ± 0.04	Nd	Nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd
BERA-24 h	nd	0.02 ± 0.002	1.35 ± 0.05	1.56 ± 0.03	Nd	Nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd
	nd		1.28 ± 0.04	1.53 ± 0.03	Nd	Nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd

(*continued on next page*)

Table 3 (*continued*)

Sample code	DMIP (ng/g) ± sd	PhIP (ng/g) ± sd	Harman (ng/g) ± sd	Norharman (ng/g) ± sd	IQ (ng/g) ± sd	MeIQ (ng/g) ± sd	MeIQx (ng/g) ± sd	4,8-DiMeIQx (ng/g) ± sd	7,8- DiMeIQx (ng/g) ± sd	Glu-P-1 (ng/g) ± sd	Glu-P-2 (ng/g) ± sd	AαC (ng/g) ± sd	MeAαC (ng/g) ± sd	Trp-P-1 (ng/g) ± sd	Trp-P-2 (ng/g) ± sd
BEST-24 h		0.01 ± 0.001													
CHCS[c]	8.82 ± 0.51	24.95 ± 2.44	5.87 ± 0.55	14.62 ± 1.82	0.05 ± 0.003	0.23 ± 0.03	3.55 ± 0.18	2.84 ± 0.14	0.14 ± 0.006	0.04 ± 0.003	0.02 ± 0.001	0.06 ± 0.004	0.14 ± 0.02	0.03 ± 0.002	0.03 ± 0.002
CHBL-1 h	6.23 ± 0.35	20.55 ± 2.20	4.14 ± 0.46	12.61 ± 1.64	0.03 ± 0.002	0.17 ± 0.02	4.87 ± 0.16	3.95 ± 0.12	0.29 ± 0.06	0.03 ± 0.002	nq	0.04 ± 0.003	0.08 ± 0.005	0.01 ± 0.001	0.01 ± 0.001
CHRA-1 h	7.21 ± 0.45	21.12 ± 2.20	5.22 ± 0.54	12.68 ± 1.58	0.04 ± 0.003	0.19 ± 0.02	4.03 ± 0.13	3.77 ± 0.15	0.22 ± 0.08	0.02 ± 0.001	nq	0.03 ± 0.002	0.03 ± 0.002	0.02 ± 0.001	0.02 ± 0.002
CHST-1 h	6.72 ± 0.40	20.87 ± 2.10	4.35 ± 0.32	12.85 ± 1.87	0.03 ± 0.002	0.18 ± 0.02	4.94 ± 0.12	3.82 ± 0.16	0.26 ± 0.05	0.02 ± 0.001	nq	0.03 ± 0.002	0.02 ± 0.001	0.01 ± 0.001	0.01 ± 0.001
CHBL-6 h	5.21 ± 0.36	18.65 ± 2.00	3.86 ± 0.25	12.54 ± 1.71	0.02 ± 0.001	0.13 ± 0.01	2.17 ± 0.12	1.78 ± 0.14	0.66 ± 0.05	nq	nd	nq	0.06 ± 0.004	nd	nd
CHRA-6 h	5.65 ± 0.41	19.44 ± 2.20	3.85 ± 0.26	12.52 ± 1.67	0.03 ± 0.002	0.16 ± 0.02	2.19 ± 0.12	1.84 ± 0.14	0.71 ± 0.06	nq	nd	0.02 ± 0.001	0.04 ± 0.003	nd	nd
CHST-6 h	5.33 ± 0.40	18.13 ± 2.22	3.76 ± 0.16	12.54 ± 1.89	0.02 ± 0.001	0.14 ± 0.01	2.28 ± 0.14	1.82 ± 0.13	0.69 ± 0.07	nq	nd	0.02 ± 0.001	0.03 ± 0.003	nd	nd
CHBL-12 h	1.65 ± 0.10	6.69 ± 0.32	2.46 ± 0.14	10.42 ± 1.30	nd	Nd	1.18 ± 0.05	0.56 ± 0.04	nq	nd	nd	nd	0.01 ± 0.001	nd	nd
CHRA-12 h	2.24 ± 0.02	7.84 ± 0.34	2.58 ± 0.32	10.35 ± 1.25	nd	Nd	1.42 ± 0.06	0.84 ± 0.06	nq	nd	nd	nd	nd	nd	nd
CHST-12 h	1.74 ± 0.11	6.36 ± 0.34	2.49 ± 0.32	10.46 ± 1.24	nd	Nd	1.26 ± 0.04	0.71 ± 0.05	nq	nd	nd	nd	nq	nd	nd
CHBL-24 h	0.56 ± 0.03	1.33 ± 0.06	1.95 ± 0.13	7.89 ± 0.51	nd	Nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd
CHRA-24 h	0.71 ± 0.04	2.15 ± 0.12	2.33 ± 0.10	8.72 ± 0.56	nd	Nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd
CHST-24 h	0.68 ± 0.04	1.41 ± 0.05	2.11 ± 0.12	7.64 ± 0.49	nd	Nd	Nd	nd	nd	nd	nd	nd	nd	nd	nd

a,b,cCooked without addition of fruit juice (control samples); sd, standard deviation (n = 3), obtained from addition standard calibration curve; nq: below quantification limit; nd, not detected.

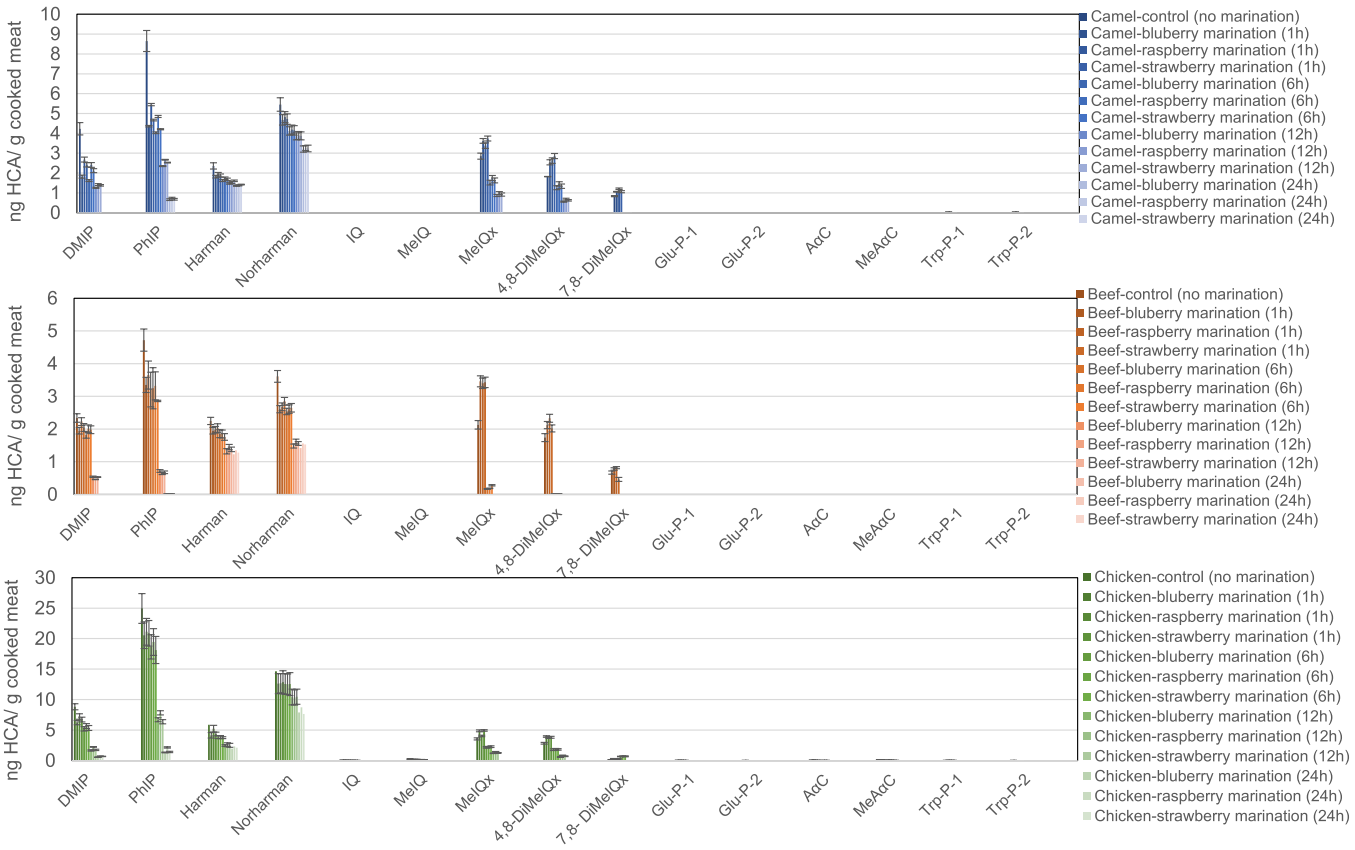


Fig. 3. Variation of HCAs over marinating time in the studied meat samples.

reduction on pyridines and β-carbolines with respect to marinating for less than 6h (P 0.05). The reduction of the pyridine HCAs was 91–100% and β-carbolines decreased by 40–67% with the 24h marinade. Noticeably, with all 3 marinades, the concentration of quinoxalines was enhanced within shorter marinating times (1h) and was reduced after 6h marination time, with a 100% reduction with the 24h marination time. This trend was also observed with MeIQx and 4,8-DiMeIQx when marinating with wines (Busquets et al., 2006). Hence, this research shows that marinades from fruits can promote the formation of quinoxalines, and that long marination time (>6h) is desirable because the enhancement of quinoxalines is mitigated, probably by other chemical reactions such as the capture of free radicals in the meat leading to the formation of quinoxalies. Previous works demonstrated a correlation between the radical scavenging activity of the marinades and the reduction of quinoxalines with time (Busquets et al., 2006; García-Lomillo, Viegas, Gonzalez-SanJose, & Ferreira, 2017). Future sensory analysis and optimisation of the sensory properties of the prepared meat will be important to expand the use of berry extracts for cooking meat.

4. Conclusions

In this study, the effect of marinating with blueberry, raspberry and strawberry on commonly consumed meats has been tested under well-controlled conditions resembling home cooking. Chicken was the most contaminated meat in terms of amounts of pyridines and β-carbolines, with 34 ng/g and 21 ng/g respectively; followed by camel (13 and 8 ng/g) and beef (7 and 6 ng/g). This study has found that marinating meat with fruit juice (blueberry, raspberry and strawberry) can have a positive reduction on the formation of HCAs (pyridines, carbolines and quinoxalines), especially at marinating time of at least 6h, which was characterised by a 40–100% reduction in HCA. In contrast, marinades of just 1h can enhance (even doubling) the formation of quinoxalines, which are potential human carcinogens. The occurrence of HCAs when using 3 independent marinades was not found to be dependent on the type of meat or fruit marinade. Guidelines on recommending of marinating meat should emphasise on the importance of using long marination times.

CRediT authorship contribution statement

Mohammad Rizwan Khan: Conceptualization, Methodology, Investigation, Funding acquisition. **Rosa Busquets:** Data curation, Validation, Writing - original draft, Supervision. **Mohammad Azam:** Formal analysis, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2020.107852.

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