



Cooking methods and the formation of PhIP (2-Amino, 1-methyl, 6-phenylimidazo[4,5-*b*] pyridine) in the crust of the habitually consumed meat in Argentina



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ABSTRACT

Content of carcinogenic molecules like, 2-Amino, 1-methyl, 6-phenylimidazo[4,5-*b*] pyridine in meals is one of the main mutagenic substances formed during meat cooking, and it can be used as a dietary exposure marker. Our objective was to estimate the amount of PhIP consumed from habitual Argentinean diet, rich in red meats, comparing different cooking procedures and meat type.

Samples ($n = 240$) of lean and fatty beef, chicken, pork, and fish were cooked using different methods: griddle, grill, sauté pan, and oven. Samples were: Overcooked, or cooked with a microbiologically suitable or “healthy technique” (HT). The PhIP was determined by HPLC-MS. Meats cooked using HT formed little crust amounts and PhIP was below the detection levels. In overcooked meats, large amounts of crust were formed in lean meats, fatty beef, fatty chicken and baked pork. PhIP was measured in lean meats sautéed or cooked on a griddle, a method reaching temperatures until 250 °C. It was estimated that Argentine people eats about 12,268.0 ng/day of PhIP being these values above those tolerated limits for total dietary heterocyclic amines in some developed countries. Hence, cooking small meat portions, at medium temperature, avoiding prolonged cooking and preferring baked lean meats could be recommended as a healthier habit.

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1. Introduction

Many dietary factors can influence the development of cancer either as initiators (starting the process which results in the disease) or as promoters (modulating the already started process) (WCRF/AICR, 2007). The toxins in food can derive from industrial and environmental sources, or can be produced when food is heated. Cooking procedures may modify food composition itself or their chemical contaminants concentration (Domingo, 2011). Foods cooked at high temperatures could contain carcinogenic substances, including heterocyclic amines, which act as initiators

(Cross and Sinha, 2004; Norat et al., 2005; Toribio et al., 2000). Various studies suggest the presence of these substances in the outer cooked surface of meat (termed “crust” for the purpose of this research) when cooked by direct heat (conduction) such as grilling, baking or sautéing (Dai et al., 2002). However, few studies investigated the cooking techniques and the conditions in which these compounds are formed in habitually used cooking methods (Toribio et al., 2000; Kazerouni et al., 2001).

HAAs formation depends on temperature, time of cooking, cooking method and total fat (Toribio et al., 2000; Galceran et al., 1996). Their presence in meat increase with temperatures above 150 °C (Knize et al., 1994; Skog et al., 1995; Joshi et al., 2012; Gu et al., 2012; Gibis et al., 2015). The longer the cooking time, the higher the content of HAAs. The cooking method is also important. Not only because of temperature but

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also due to heat transmission mechanisms are different. Procedures reaching temperatures near 100 °C like boiling, steaming, or poaching, do not generate detectable mutagenic compounds (Galceran et al., 1996). Neither microwave or indirect convection methods like ovening generate HAA or their quantities are near null (Toribio et al., 2000). On the contrary some studies showed that methods where heat source has a direct contact with food like frying or barbecuing would produce higher mutagens levels (Galceran et al., 1996; Sinha et al., 1998a,b). Also, fat amount plays a relevant role because it would increase the efficiency of heat transmission increasing, in turn, mutagenic compounds amount (Johansson et al., 1993; Toribio et al., 2000; Rohrmann et al., 2002). The different composition in amino acids, creatine and other proteins of different varieties of meats, results in different amount of HAAs according to meat type, even when processed in a similar way (Toribio et al., 2000; WCRF/AICR, 2007). A recent study postulates that not only HAAs but also other environmental pollutants may be responsible for meat carcinogenicity (Domingo and Nadal, 2016), and they can impact the exposure, metabolism, and cell proliferation response of heterocyclic amines. Indeed, PhIP is the main HAA formed by high temperatures cooked meats and a potential risk factor derived from diet for different cancer types (Rohrman, 2007).

Many epidemiological studies have associated unbalanced meat consumption with cancer. Some of them have suggested that higher consumption of mutagens from meats cooked at higher temperature and longer exposition may be associated with higher risk of colon adenoma perhaps due to HAAs contents, such as PhIP (Sinha et al., 2001; Wu et al., 2006; Li et al., 2007; Rohrmann et al., 2015). In vitro, ex vivo and in vivo studies have confirmed their carcinogenic effects (Jamin et al., 2013; Rohrmann et al., 2015). Carcinogenic potentials of PhIP derives from its ability to build DNA adducts, or genetic differences in the ability to transform HAAs and repair DNA as well (Felton et al., 1986; Fede et al., 2009; Joshi et al., 2012). All of these findings led the International Agency for Research on Cancer (IARC) to include processed red meat as potentially carcinogenic (IARC, 2015).

The nutritional profile of the Argentinean population is characterized by the high consumption of meats (Navarro et al., 2003, 2004). Meats possess an important nutritional value; however, the characteristics that make them so highly recommended are not always accompanied by benefits. The habitual cooking methods in Argentina population (as in South America Cone too) often cause a formation of a crust (Navarro et al., 2003, 2004). Of the most used methods of cooking, such as the traditional Argentinean “asado” on the grill (much like a barbecue, hereon “barbecued”), the meat is cooked over charcoal or wood. Another common cooking method is on the griddle, which consists of placing a flat iron pan on the stovetop and cooking the meat at higher temperatures.

Argentina has also a high incidence of colon cancer (Díaz et al., 2009, 2010), mostly perhaps related to dietary habits. Some authors have suggested that the traditional cooking methods could be responsible for the tumorigenic processes due to the formation of heterocyclic compounds on the surface of the meat (Dai et al., 2002; Faramawi et al., 2007; Knize et al., 1999, 2002; Zheng and Lee, 2009). Taking into account nutritional standards of the Argentinean population and the association between the consumption of meats cooked at high temperatures and different types of cancer (Zheng and Lee, 2009), our main goal was to estimate the carnit PhIP amount consumed from the habitual Argentinean diet, considering traditional cooking methods, the time, temperature, and doneness of habitually consumed meats.

2. Materials and methods

2.1. Food samples meat

Meat was bought in the central market, where it arrives from different points of the province territory and it is the main selling point in the city. Of all 240, 224 meat sample units 100 g each (raw?), of 13 cm long x 10 cm wide, 0.5 cm thickness approximately, were distributed in 8 groups: lean bovine (sirloin tip-LB), fatty bovine (chuck-roast-FB), lean and fatty chicken (breast-LC; meat with skin-FC), lean and fatty fish (hake-LF, white salmon-FF), and lean and fatty pork (ribs-LP, and FP). These samples were half cooked using a 1 min preheated griddle and the other half were sautéed in a 30 s preheated pan (Only the two methods forming a crust in all meat types according to a pilot study), until reaching 70 °C in the center of the meat and flipped every minute (Healthy cooking technique – HT). For sauté, 10 cc of sunflower oil was added previous to preheating. The remaining 16 sample units (one per meat group and method) were overcooked as positive controls (OC).

2.2. Cooking methods

In accordance with the cooking practices in Argentina, the cooking methods are defined as follows: griddle, grill, sautéed, and oven. Temperatures and meat-crust formation were assessed for all usual cooking methods. Previous to the cooking, all utensils were pre-heated: 1 min the griddle, 30 s the for sauté pan, 5 min the oven and grill for barbecuing. In the four methods temperature was measured during 10 s after cooking the meats. While cooking, meats were flipped every minute until 70 °C were reached in the middle of the portions, and temperature of the meat surface, and of the cooking utensils were also registered. The fire was graduated in a middle point. In the grill, the floor of the roaster was covered with coals in the area where the samples were cooked. A mixture of charcoal from different types of wood (mainly *Aspidosperma quebracho* white) was used. The loss of weight as a result of cooking was also evaluated. All cooked samples were stored at –80 °C until analysis.

2.3. Materials

Balance Arte Ariete, digital thermometer Stainless Steel Probe, Luftgarmany –50 °C–150 °C, exactness ± 1 °C/ ± 2 °F. TES-1322A Infrared/Type – K Thermometer, –20 °C–500 °C. Resolution: 0, 1 °C. Methanol (HPLC grade) and formic acid (puriss. p.a. for mass spectroscopy) were obtained from J. T. Baker (Edo. de México, Mexico) and Fluka (Steinheim, Germany), respectively. PhIP standard was obtained from Toronto Research Inc. (Canada).

2.4. Sensory evaluation

To determine the degree of cooking and the presence of toasted crust a sensory evaluation of the meat samples was conducted with a group of trained judges. A description of the observed characteristics was performed for each meat cut and cooking method. Appearance attributes evaluated were: percentage distribution and color of toasted crust and the color of the inner flesh.

The degree of doneness for the different types of foods was primarily defined based on the maximum internal temperature, as follow a) Middle raw or undercooked roast: a temperature between 50 °C and 60 °C: bright red center and a narrow outer layer taupe. b) Not cooked or “half done” pink center with a temperature between 60 °C and 70 °C, and c) cooked: Temperature above 70° C, gray uniform inside.

Color measurement was conducted using scale samples of Argentina paints catalogs. Three reference colors (No. 8570 to 10%, 50% No. 8584 and No. 8654, 90%) were used.

2.5. Samples preparations

Cooked samples were cut into small pieces and processed until a homogeneous mass. Then, 9 g from each one were homogenized with 9 g of quartz and conserved at -20°C according to Galceran et al. (1996), based on Gross and Fay (1995) method. Two grams of the homogenized samples were agitated during 15 min with 12 mL of 1 M NaOH to separate quartz. The supernatant was centrifuged for 15 min, at 2500 rpm. The supernatant underwent the clean up, solid phase extraction as detailed in Galceran et al. (1996).

2.6. Identification and quantification of PhIP

2.6.1. HPLC-DAD-ESI-MS/MS analysis

PhIP levels were analyzed in meat samples by HPLC-ESI-MS/MS method, using an Agilent Technologies 1200 Series system equipped with gradient pump (Agilent G1312B SL Binary), solvent degasser (Agilent G1379 B) and auto sampler (Agilent G1367 D SL + WP). The HPLC system was connected to a QTOF mass spectrometer (microQTOF-QII Series, Bruker Daltonics) equipped with electrospray ionization (ESI) interface.

The chromatographic separation was achieved on an Agilent ZORBAX Eclipse XDB C18 (50 mm \times 3 mm i.e.; 1.8 μm) reversed-phase column. The column temperature was thermostated at 30°C using a column heater module (Agilent G1316 B). The mobile phase consisted of 0.5% formic acid in ultrapure water (v/v, solvent A) and 0.5% acid formic in methanol (v/v, solvent B), starting with 5% B and changing to 40% B during 5 min, held 4 min, followed by a second ramp to 5% B in 1 min, remaining at this last condition for 4 min before next run. The flow rate was 0.3 mL/min, injecting 10 μL on column. Mass spectra were recorded in positive ion mode between m/z 100 and 400. The working conditions for the ionization source were as follows: capillary voltage, 4500 V; nebulizer gas pressure, 4.0 bar; drying gas flow, 8.0 L/min and drying gas temperature, 200°C . Nitrogen was used as nebulizer gas. Identification was based retention time and mass spectra, as compared with commercial standard. All extracts and standard solutions were filtered (0.45 μm) and injected into the HPLC-ESI-MS system.

Quantification was based on external calibration curve from PhIP commercial standard, using the mass peak area obtained from the extracted ion chromatograms (m/z 225.11), at concentrations between 0.02 and 6.25 $\mu\text{g mL}^{-1}$. The Compass Version 3.1 software and Data Analysis Version 4.0 software were used for data acquisition and processing, respectively. Limit of detection (LOD) and limit of quantification (LOQ) was 0.17 ng/g and 0.51 ng/g, respectively.

2.7. Estimation of PhIP consumption in Argentinean population

This work has been undertaken in the frame of an Epidemiological Study of food patterns being conducted in Córdoba, Argentina (Pou et al., 2014). Briefly, after accepting to participate a sample of ($n = 1125$) healthy subjects have been enrolled and asked about their habitual food consumptions and other bio-socio-cultural characteristics using a validated food frequency questionnaire (Navarro et al., 2001) and with the aid of a validated Athlas (Navarro et al., 2007) After including these data into a nutritional software (Peyrano et al., 1998), the average grams a day of each food and each cooking method were obtained.

By simply multiplying average grams a day of consumed meats with PhIP content of each meat type and cooking methods, it was

calculated the average consumption of PhIP for the selected population.

2.8. Statistical analysis

Mean values for PhIP (ng/g) were calculated based on triplicate samples for each single cooked food and method.

To assess the mean difference among temperatures we performed a t-test with significance level of 95%. The results are shown in Tables 1 and 2.

To evaluate whether a) the temperature of the crust formation and the temperature reached by each method depends on the type of meat cut and cooking method and b) the average weight reduction considering the type of meat and the cooking method, it was performed a Generalized linear model (GLM). GLM models are a generalization of the linear model which specifies the relationship between a dependent variable and a set of predictor variables (covariates) and allows the response variable to have other than a normal distribution (McCullagh, 1984; Madsen and Thyregod, 2010). In the present study, a multivariate gamma regression model with reciprocal link function was used (McCullagh and Nelder, 1989). In both cases, a) and b), the model considered the temperature variable as the response variable and meat type and cooking method in the linear predictor as factor covariates. A significant effect was considered when the p value for any of the treatments did not exceed 0.05.

2.9. Ethical aspects

All the protocols were approved by the local ethical committees and register in the Provincial Register for Health Research (RePIS). HAAs determination protocol RePIS N° 104/11 Feeding habits protocol RePIS N° 345/09.

3. Results

3.1. Formation of the crust

Cooking in the oven only resulted in the formation of crust in fatty meat. On the contrary, the griddle formed a crust in nearly all of the samples of meat. In the sauté pan 75% of the meats formed a crust. On the grill the lean cuts of beef, chicken, pork and fish did not form a crust. However, the fatty cuts of beef and chicken formed a crust in 100% of the samples. The time in which the samples of meat were fully cooked, when they reached 70°C in the middle, was between 9 and 11 min in the oven and grill and 7 and 8 min on the griddle and in the sauté pan. The meats cooked by radiation (oven and grill) had a longer final cook time (between 9 and 11 min) compared to the cooking methods by conduction (griddle and sauté

Table 1

Temperature^a of the crust according to the different types of meat.

Type of meat ^d	Temperature of the crust \pm SEM ^e
Lean beef (LB)	97 \pm 15.1
Fatty beef (FB) ^b	89 \pm 17.1
Lean chicken (LC)	101 \pm 11.2
Fatty chicken (FC) ^b	116 \pm 23.5
Lean pork (LP) ^c	98 \pm 29.6
Fatty pork (FP) ^c	115 \pm 22.8
Lean fish (LF) ^c	91 \pm 4.5
Fatty fish (FF) ^c	100 \pm 10.5

^a In $^{\circ}\text{C}$.

^b Significantly different ($p < 0.05$).

^c $p = 0.01$ FP respect all the others.

^d $n = 7$ in each group.

^e SEM: Standard error of the mean.

Table 2
Temperature^a of the different traditional cook methods and types meat with crust.

^b Method	Chicken		Fish		Beef		Pork	
	Lean	Fatty	Lean	Fatty	Lean	Fatty	Lean	Fatty
Griddle	259 ± 38.9	275 ± 16.4	–	–	204 ± 34.9	173 ± 36.1	158 ± 19.6	208 ± 36.1
Grill	–	263 ± 69.0	–	–	–	203 ± 49.6	–	–
Sauté Pan	218 ± 17.0	219 ± 29.3	134 ± 15.6	139 ± 22.3	209 ± 7.1	221 ± 11.1	146 ± 15.8	192 ± 12.5
Oven	–	–	–	–	–	–	–	117 ± 6.6

^a In °C mean ± standard deviation.^b n = 7 in each group.

pan), in which the times were between 7 and 8 min.

The highest median temperature of the crust according to the different cuts of meat was found in the fatty chicken (Table 1). The crust of fatty beef and fatty chicken reached the highest temperatures in relation to their lean pairs. A significant difference exists between the temperatures of the crust of the fatty pork and the rest of the meats and between the lean pork and lean fish ($p = 0.01$). On the other hand, temperatures reached by using griddle, sauté pan and oven were significantly different ($p = 0.000$).

3.2. Temperature of the cooking method

It was observed that the griddle reached the highest temperature in relation to the other cooking methods for all types and cuts of meat analysed (Table 2).

The mean temperature for the cooking methods in lean and fatty beef and chicken was significantly less than 250 °C ($p = 0.02$), when the centre of the sample reached 70 °C (optimal cooked temperature for microbiological concerns). Also a significant difference was observed in the temperature of the cooking methods between the different lean and fatty fish and pork.

3.3. Toasted crust temperature

About the crust temperature, a significant difference exists among lean pork and lean fish ($p = 0.03$). Temperature for crust formation is higher for fatty chicken and pork in relation to the other cuts of meat that underwent the same cooking methods, being pork crust formed at higher temperatures ($p < 0.05$) (Table 3).

When grilling, lean meats did not form a crust, instead fatty beef and chicken did. Also, a significant difference exists in the temperature reached by methods employing the griddle, sauté pan and oven. The cooking temperature between the different meats and cooking methods was also significantly different (Table 3).

With respect to weight reduction during cooking, lean beef resulted in the largest reduction in relation to the other meats which had a similar weight reduction under different cooking methods. The weight reduction (lean and fatty beef, and chicken) depended on the combination of the method and type of meat used

($p = 0.002$).

3.4. Presence of PhIP

PHIP was measured only in those samples that formed a toasted crust while cooked until 70 °C in the middle (microbiologically safe) in all meat types: griddle and sauté methods, and their overcooked positive controls.

PhIP was detected only in meat LB sample for overcook method or positive control. With an amount of 341 ng/g.

3.5. Estimation of HAAs intake in Argentine population

Different studies conducted in Córdoba, the second city situated in the middle of the country, about Argentinean food patterns, show that the habitual meat intake in this population is in average 320 g/day, from which 150 are of meat with crust (Table 4) (Pou et al., 2014; Román et al., 2014 and yet unpublished data). Considering the amount of PhIP found in meats, and habitual dietary intake of Argentinean population, whose main dietary pattern is characterized by high intakes of meat, starchy vegetables, refined grains and wine, it is estimated that an Argentinean person

Table 4
Average daily consumption^a of red meat and preference browned crust in Córdoba Province, 2014.

Meat	Cases (n: 586)	Controls (n: 1125)
Total meat	340.96 ± 8.38	309.13 ± 5.39
Total meat crusted	158.98 ± 6.29	144.54 ± 3.79
Total meat not-crusted	122.58 ± 5.37	110.87 ± 3.79
Red meats		
Total red meat	219.24 ± 6.85	189.83 ± 4.00
Crusted red meat	106.83 ± 5.12	91.42 ± 2.86
Red meat not-crusted	112.41 ± 5.06	98.40 ± 3.15
Fatty red meats crusted	54.81 ± 3.62	47.11 ± 1.97
Fatty red meats uncrusted	43.30 ± 3.46	34.49 ± 1.75
Lean red meat crusted	34.96 ± 2.04	32.07 ± 1.28
Lean red meat without crust	24.76 ± 1.69	22.43 ± 1.20

^a g/day ± standard error of the mean.**Table 3**
Temperature^a of the crust in distinct types of meat with respect to the different cooking methods.

^a Method	Chicken ^b		Fish		Beef		Pork	
	Lean	Fatty ^d	Lean ^c	Fatty	Lean	Fatty	Lean ^c	Fatty ^d
Griddle	90.5 ± 4.6	109.8 ± 6.8	–	–	83.5 ± 7.4	74 ± 3.8	78 ± 7.7	135 ± 15.3
Grill	–	104.8 ± 9.1	–	–	–	83 ± 6.4	–	–
Sauté Pan	110.6 ± 2.9	131.9 ± 35.7	91 ± 4.5	100 ± 10.5	111 ± 5.3	111.1 ± 5.8	118 ± 20.2	122 ± 8.4
Oven	–	–	–	–	–	–	–	88 ± 7.4

^a In °C, mean ± Standard deviation).^b All meat types significantly different.^c Different for $p < 0.03$.^d Both different from all the other cuts $p < 0.05$.^e n = 5 in each group.

eats around 12268.0 ng/day of PhIP if meats are overcooked. These values only from PhIP are highly above those reported for dietary HAAs as a whole in other countries. Keating et al., (2004), point that the average dietary HAA intake can range from less than 2 to >25 ng/kg per day in the United States.

When subjects from a case control study of diet and cancer in this population are analysed, those having (prostate, colon, breast, and urothelial) cancer consume in general more meat, but their preference for crust is similar (unpublished data).

4. Discussion

This work assessed the time and the temperature of the crust formed on the surface of the meats. We also evaluated the temperatures reached and the presence of PhIP according different traditional cooking methods for cuts of meat habitually consumed and we estimated the average PhIP intake eaten by Argentine population as well. Cooking meats on the griddle was the cooking method that forms crust in almost 90% of the time studied, followed by sautéed, the cooking method most commonly used in preparations for the first part of a stew or casserole (mixed).

Previous studies showed the presence of mutagenic substances of meats which were subject to high cooking temperatures comparable to those used in our research (Toribio et al., 2000; Knize et al., 2002; Le Marchand et al., 2002; Rohrmann et al., 2002, 2007; Anderson et al., 2002). High levels of polycyclic aromatic compounds (PAHs), like benzo[*a*]pyrene, were found in very well done steaks and hamburgers in USA. Total PAHs were higher in smoked meats and Ham in Estonia, and in fried fish in Spain (Domingo, 2011). However, in most of those research the used cooking methods were not fully detailed. Furthermore, we have not found works describing if, at the moment of consumption the meat cooked in these ways is microbiologically appropriate (70 °C), being a crust present or not.

As in other studies (Sinha et al., 1995, 2001; Thomson, 1999; Vikse et al., 1999; Gerhardsson de Verdier et al., 1991; Rahman et al., 2014; Joshi et al., 2012; Binnie et al., 2014) we observed that the type of meat as well as the cooking method were significant factors in the temperature at which the crust forms and that there is not a synergistic effect between one type of meat with one determined method. So, the temperature of the cooking method essentially depends on the type of meat chosen.

In relation to the cooking methods griddle and the sauté pan both are not previously studied regarding the temperature these methods reach nor information about crust formation in different meat cuts are available. Several studies devoted to determine amounts of mutagenic substances produced during meat cooking, focused on the analysis of the concentration of HAAs in portions of very roasted meat, submitted to extreme temperatures. However, suitability of cooking techniques or the portions obtained for baking or normal recommendation were not considered (Knize et al., 1999; Skog et al., 1995; Keating and Bogen, 2004; Pou et al., 2014; Puangsombat et al., 2012; Pham et al., 2014). Thus, in this work, we determined the temperatures and cooking times of each traditional method for habitually consumed meat portions in our population.

As proposed by Layton et al. (1995), the acceptable levels of HAAs consumption in the USA diet range from 0 to 7.1 ng/day per kilogram, and considering an adult of medium shape with a maximum 70 kg should consume around 497 ng daily of amine. Relative to South America' Cone diet, this value is well below regarding that Córdoba's population consumes. This is around 12268.0 ng of PhIP, according to the average daily LB crusted meat consumption recorded from epidemiological studies in our population (36 g/day) (Pou et al., 2014). In 2015, IARC classified

processed meats as carcinogenic to humans with n-nitrous compounds being perhaps the major responsible for this deleterious effect. Other environmental pollutants were not considered even when their content may be modified by cooking procedures (Domingo and Nadal, 2016) These facts commits us to suggest potentials actions emphasizing for adequate nutritional education to improve healthy food selection, minimize carcinogenic compounds formation and therefore avoiding risky exposure to them. These findings also provide a benchmark that could be used as tools to regulate and control the sale and labelled nutritional cooked meat products ready for consumption.

On the other hand, our previous studies about the relationship between colorectal cancer and the consumption of heavily browned meats showed that there was not an association between this pathology and the consumption of oven-cooked meats. Nevertheless, it showed a positive association between the consumption of grilled meats and those cooked on the griddle with the risk of suffering from colorectal cancer. Heavily browned meats is preferred e in our population (Navarro et al., 2003, 2004). However, to our knowledge, there were not studies devoted to establish the temperature reached by different traditional cooking methods and the toasted crust formation in commonly consumed meats, or the presence of carcinogenic compounds. Hence we characterized the traditional cooking methods of barbecued, griddle, oven, and sautéed, used in our region, particularly in terms of iron griddle cooking which is so typical of South Cone and habitually recommended as a "healthy method" in many eating plans because it is used for lean beef. Perhaps our findings of PhIP content in griddle-cooked and mainly overcooked meats can, in part, explain the relationship between red meat consumption heavily cooked and colorectal cancer risk in Argentinean population. These results support further investigations related to the measurement of HAAs metabolites in biological fluids of healthy and diseased volunteers as possible exposure markers and to study population susceptibility to these carcinogenic compounds.

5. Conclusion

Our findings suggest that exposure to HAAs from meat cooked on the griddle can be reduced by moderating meat consumption, cooking meats small portions for shorter times reaching lower surface temperatures, frequent meat flipping and pre-treatment of meat. It is important to improve culinary techniques and minimize the use of the griddle since this is what produces the largest crust formation, and so to avoid carcinogenic compounds formed during meat cooking.

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