

Effect of seasoning on formed imidazoquinoline compound concentrate during beef meat cooking methods at high temperatures

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Abstract: This study performed to examine the possibility of inhibiting 2-amino-3-methyl imidazoquinoline (IQ) which formed during cooking methods at high temperature by feeding of rats on experimental diets. The 36 adult male rats (123±2g) were randomly divided into 6 groups; (G1) negative control group that fed on basal diet, (G2) positive control that treated with IQ at concentrate 10ppm_{IQ}/100g_{b.w} for 10 days un-sequence (day after day), (G3) rats fed on boiled beef meat, (G4) rats fed on fried beef meat, (G5) rats fed on grilled beef meat and (G6) rats fed on pre-seasoning grilled meat with 1% ginger. The duration of the experimental period was 16 weeks. Estimation of IQ in different cooked beef meat, serum and urinary excretion were performed. The results obtained a significant differences among them where contained grilled beef meat without seasoning on the highest values of IQ were 14.368 ng/g (ppb), 7.23 ng/ml and 20.19 ng/ml in food, blood serum and urine of rats fed on it, respectively. But seasoning beef meat with 1% ginger over time before grilling that led to decrease IQ concentrate in food, serum and urine to 3.367ng/g, 0.49 ng/ml and 1.74 ng/ml, respectively with decreasing percent reached to 76.56, 93.17 and 91.37%, respectively comparing with un-seasoning grilled meat. Whereas, IQ concentrate in pan fried meat was 8.806 ppb while were 3.02 and 10.45ppb in serum and urine of rats fed on it that equal approximately control (+) group which their rats treated with IQ compared with boiled beef was un detected. Although IQ content in grilled meat higher than pan fried meat because IQ content increase with increasing heat temperature and concentrate internal beef tissues than surface but its effect on biological properties in fried meat was stronger than grilled meat where IQ soluble in the fat. So, its absorption and metabolism in the body is faster and harmless. Also, Feed Coefficient Efficiency (F.C.E) was the best for rats group fed on pre-seasoning grilled beef compared with un-seasoning while rats group fed on fried beef and control (+) were the worst. **Conclusion:** Seasoning of beef meat with herbs before cooking able to reduce the harm effect of IQ that formed at high temperature.

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Key words: Imidazoquinoline (IQ) concentrate - different cooked beef meat - blood serum - urinary excretion - rats - F.C.E.

1-Introduction:

Philippe *et al.* (2000) observed that the amount of heterocyclic aromatic amines (HAAs) formed was highly dependent upon the type of meat and method of preparation. The detection and quantification limits of these HAAs approached 0.015 and 0.045 µg/kg (ppb) respectively, with only 4 g of meat. When cooking meat at high temperatures, as grilling or frying at temperatures over 150°C, more than 25 HCAs have been isolated and identified. The major classes of heterocyclic amines include amino-imidazo-quinolines, or amino-imidazo-quinoxalines (called IQ-type compounds). The highest consumption of processed or well-cooked meat had an increased risk of rectal cancer (Kizil, *et al.*, 2011 and Viegas *et al.*, 2012). Cooking meat at a high temperature forms carcinogenic heterocyclic amines and polycyclic aromatic hydrocarbons that carcinogenic (Santarelli *et al.*, 2008).

HAA are carcinogenic compounds found in the crust of fried meat. Inhibiting HAAs formation in fried beef patties by using marinades with different concentrations of hibiscus extract. The concentration of MeIQx was reduced by about 50% and 40% by applying marinades containing the highest amount of extract compared to sunflower oil and control marinade, respectively (Gibis and Weiss, 2010). Also, Gibis and Weiss, (2012) resulted that the content of MeIQx and PhIP were significantly reduced 57% and 90%, respectively, after use of marinades containing the highest extract concentration of grape seed. Whereas, garlic at 20 gm/100 gm marinade reduced carcinogen production by about 70%. The author determined that "the optimum amounts of onion, garlic, and lemon juice are 31.2%, 28.6%, and 14.6% in marinade respectively (Gibis, 2007). It was found that a "mixture of brown sugar, olive oil, cider vinegar, garlic, mustard, lemon juice and salt" reduced the total detectable heterocyclic amines from 56 to 1.7 ng/g,

from 158 to 10 ng/g and from 330 to 44 ng/g for grilling times of 20, 30 and 40 min, respectively. Also, green tea extracts lower HCAs (Arimato-Kobayashi *et al.*, 2003). While, the results revealed no significant differences between control samples and green tea marinated samples on the formation of heterocyclic aromatic amines (HAAs) in pan-fried beef cooked at 180–200°C for 4 min each side (Quelhas *et al.*, 2010). To be useful cooking meat with foodstuffs containing phenolic antioxidants or with tomatoes, carrots or other vegetables rich of carotenoids and antioxidant vitamins Alaejos *et al.* (2007).

Alaejos, and Afonso, (2011) describes the factors that affect the formation of HAAs in foods, such as the cooking method, including temperature and time of cooking. The three high-temperature cooking methods (pan-frying, oven broiling and grilling/barbecuing) seem to produce the highest HAAs concentrations. Whereas, boiling, oven roasting, deep-frying, and charcoal grilling are generally “milder”, and much lower amounts of HAAs are formed. Investigations of several cooking methods have shown that contact frying, deep fat frying, and broiling all cause much higher levels of mutagenic activity than other cooking methods such as roasting, stewing, and microwave cooking (Hargraves and Pariza, 1984). Also, removing HCAs from beef patties by microwave pretreatment before frying was shown by (Felton *et al.*, 1994).

Smith *et al.* (2008) revealed the higher amount of HCAs present in the external surface of steaks. The internal part of the meat contained 2.27 ng/g MeIQ and 2.14 ng/g MeIQx, while the external part in the control steaks contained 12.78 ng/g MeIQ and 24.23 ng/g MeIQx. The formation of HAAs in the crust is the result of steady transportation of water and dissolved compounds such as amino acids and creatinine to the surface by capillary flow to the evaporation zone. Thus, the precursors of HAAs are concentrated on or near the surface of the meat where the temperature is highest. Ni *et al.* (2008) resulted that the concentrations of heterocyclic aromatic amines (HAAs) ranged from <0.03 to 3.5 µg/kg. PhIP was the most abundant HAA formed in very well done barbecued chicken (up to 305 µg/kg), broiled bacon (16 µg/kg), and pan-fried bacon (4.9 µg/kg). 7-MeIQx was the most abundant HAA formed in very well done pan-fried beef and steak, and in beef at concentrations up to 30 µg/kg.

HAAs detected in amounts up to 480 ng/g whereas, the other HAs are generally lower, ranging from not detectable to 15 ng/g for IQ, MeIQ and 4,8-DiMeIQx and to 50 ng/g for MeIQx. (Fay *et al.*, 1997). The results showed that the total HCAs in the smoke condensate were 3 ng/g from bacon and 0.37 ng/g from hamburgers, compared with 163 ng/g in cooked bacon and 110 ng/g in cooked beef. researchers found that

beef cooked at 198 °C and 277°C contained 10.5 and 110 ng/g of HCAs, respectively (Environmental Health Perspectives, 1996).

The spices contain antioxidants that can reduce the formation of HAAs during heating, the phenolic compounds isolated from common spice plants show strong inhibitory effects. Also, antimutagenic and anticarcinogenic effects were detected (Milic and Milic, 1998). The spices used were rosemary, sage, thyme, and garlic, the addition of dried spices or brine to the surface of the meat prior to heating leads to a significant reduction in the HAAs content (Murkovic *et al.*, 1998).

Kassie *et al.* (2003) resulted that the amounts of red cabbage juice which caused protective effects were quite small (0.6–0.7 ml/day/rat, body weight 250g) and correspond to consumption of 180–210 ml juice/day/person.

World Cancer Research Fund /UK, (WCRF/UK, 2009) recommended for cancer Prevention, Limit consumption of red meats (such as beef, pork and lamb) and avoid processed meats and eat more of a variety of vegetables, fruits, whole grains, and pulses such as beans. Adding both starch and glucose to the beef patties inhibited mutagenic activity by up to 54% (Skog *et al.*, 1992). Starch from potatoes inhibited the formation of HAAs (Persson *et al.*, 2004). Avoiding direct an open flame or a hot metal surface at high temperatures and long time and using a microwave oven to cook meat reduce HCA formation by reducing the time (Knize, and Felton, 2005).

The objective was to examine the possibility of inhibiting IQ as HAA that formed in fried and grilled beef meat by seasoning beef with 1% ginger before cooking.

2-Materials and Methods of IQ:

2-1-Materials:

1- Beef meat: Imported frozen beef meat from boneless round (Brazilian product) was purchased from private shop in the local market at Giza, Egypt.

2- The ginger herb was obtained from local market at Giza, Egypt, was powdered in a laboratory mill and 1% of powdered ginger was mixed well with beef meat.

3- 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) standard was obtained from Sigma- Aldrich, St. Louis, USA.

2-2-Methods:

1-Technological methods:

Cooking methods of beef meat:

Imported frozen beef meat were cut to slices and divided into four parts cooked by three methods; The first: beef slices boiled in boiling water at 100°C for hour, the second: beef slices fried in pan vegetable oil at 180°C for 5-7 minutes for each side, the third: beef

slices grilled on the open direct flame at more than 200C° for 5-7 minutes for each side, the forth: beef slices were marinated by 1% ginger well over night in refrigerator before grilled. All samples after cooking methods cooled at room temperature for an hour, were minced in a grinder, packed in polyethylene pages and preserved in deep freezer at -18C° until tested.

2-Analytical methods:

Gross chemical composition of cooked beef meat for preparing different diets were evaluated according to **A.O.A.C. (2005)**.

Determination of IQ):

Determination of IQ by using HPLC apparatus in foods (cooked beef meat), blood serum and urine excretion samples according to methods described by **Knize et al. (1995)**, **Robert et al., (1993)** and **Reistad et al. (1997)** respectively.

3-Biological methods:

Animals and experimental design:- (IQ)

Thirty six adult male Sprague-Dawley albino rats average weight (123±2 g) were purchased from the Laboratory Animal Department, Research Institute of Ophthalmology, Giza, Egypt. The animals were housed in plastic cages under normal health laboratory condition at 21°C ± 2°C with timed lighting 12h and relative air humidity of 40 - 60% and fed on basal diet for one week as an adaptation period. After the adaptation period (1 week), the rats were randomly divided into 6 groups (6 rats for each group). The composition of the experimental diet for each group are shown in table (1) according to the method described by **A.O.A.C. (2005)**.

The data explain cooked beef meat weight equal 15% protein, supplemented with 4% salt mixtures and 1% vitamin mixtures and completed to 100% with sucrose and starch where the last 2-fold the first.

Table (1): Composition of the experimental diets (%):

Ingredients	Control (-)	Control (+)	Boiled meat	Fried meat	Grilled meat	Pre-seasoning grilled meat
Casein as protein	17	17	--	--		--
Boiled meat	--	--	62.5	--		--
Fried meat	--	--	--	60		--
Grilled meat	--	--	--	--	50	--
Pre-seasoning grilled meat	--	--	--	--	--	50
Fat (corn oil)	10	10	5.7	3.7	6.8	6.8
Cellulose	5	5	5	5	5	5
Vitamins mix.	1	1	1	1	1	1
Salt mix.	4	4	4	4	4	4
Sucrose	21	21	7.3	8.8	11.1	11.1
Starch	42	42	14.5	17.5	22.1	21.1
Ginger	--	--	--	--	--	1

*(AOAC,2005)

The blood samples were collected each 15 days throughout and at the end of experimental period. The blood samples were collected from eye plexuses into both heparinized tubes and into a dry clean centrifuge glass tube without any coagulation to prepare serum. Blood samples were left for 15 minutes at room temperature, then the tubes were centrifuged for 15 min at 3000 rpm and the clean supernatant serum was kept frozen at -20 °C until the time of analysis. At end of the experimental period rats were weighed and euthanized under deep anesthesia using ether, their organs were

separated and collection of tissue specimen were performed for further histological examination.

Mean body weight changes (g), body weight gain (g), body weight gain (%), total food intake of rats fed on the experimental diet for 16 weeks and Feed Conversion Efficiency were calculated as follow;

F.C.E = Feed Conversion Efficiency = Body weight gain (g) / Total food intake (g)

3-Results and Discussion:

Gross chemical composition of cooked beef meat:

Gross chemical composition of different cooked beef meat samples are shown in table (2).

Table (2): Chemical composition of different cooked beef meat.

Items	Moisture	Protein	Fat	Ash
Boiled meat	66.5	24	6.9	2.6
Fried meat	62.0	25	10.5	2.5
Grilled meat	59.7	30	6.4	3.9

The obtained results indicated that the moisture content of grilled beef was lower than boiled and fried beef reached to 59.7, 66.5 and 62.0 g/100g sample, respectively. These results might be attributed to the loss of water holding capacity (W.H.C) which result about occurring denaturation of protein during cooking methods.

These results are in agreement with those obtained by **Lawrie, (1968)** who cited that the loss of water-holding capacity in grilled meat due to change in myofibril proteins of muscle, moreover, conversion of collagen to gelatin causing shrinking and toughness.

The previous data indicated that both protein and ash content were higher in grilled beef than boiled and fried beef meat reached to (30 and 3.9), (24 and 2.6) and (25 and 2.5) g/100g sample, respectively. It explained that the increasing of protein and ash content in grilled beef led to loss of some contents such as moisture and fat during high heat temperatures.

Fat content have a high importance in this study due to its feasibility to soluble of 2- amino-3-methyl imidazoquinoline (IQ) content.

Fat content in fried beef was higher than other cooked that led to absorption some of frying oil, moreover, melting of some beef fat by cooking heat temperature and drip way in soup in case of boiling or burning on the surface in case of grilling.

The above mentioned data are in the line with **Health Advisories, (1999)** that stated that the reduction in fat content of boiled food is due to melting of some fat and drip way in soup.

Concentration of 2-amino-3-methyl imidazoquinoline (IQ) in different cooked beef meat:

2-amino-3-methyl imidazoquinoline (IQ) concentrate (ppb) in different cooked beef meat are shown in figure (1).

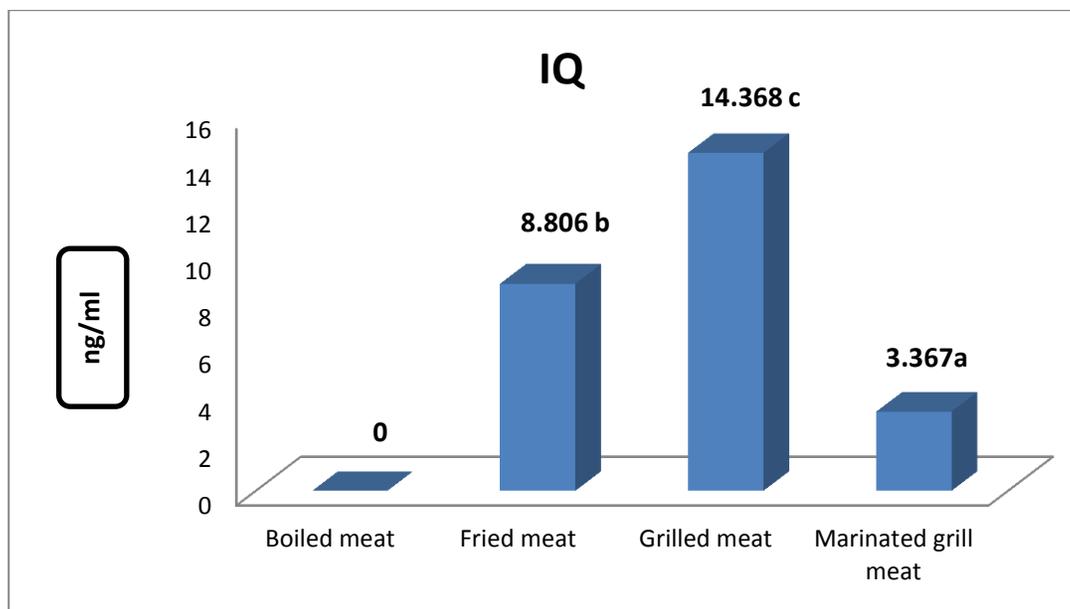


Fig (1): 2- amino-3-methyl imidazoquinoline (IQ) concentrate in boiled, fried, grilled and pre-marinated grilled beef meat.

The results obtained a significant differences among them where contained grilled beef meat on the highest value was 14.368 ng/g (ppb) but when marinated with 1% ginger over time before grilling that led to decrease IQ concentrate to 3.367ng/g with decreasing percent reached to 76.6 % comparing with un-marinated grilled meat followed it fried meat was 8.81ng/g and the last, boiled beef meat not be detected any concentrate.

The previous results were in agreement with **Yao et al.(2013)** who determined the content of HAAs in sauce beef, Nine kinds of HAAs were found in the

samples and the total amounts of HAAs ranged from 4.33 to 27.15 ng/g. High concentration of total HAAs (up to 50.86 ng/g) was detected in very well-done Brazilian barbecue beef, but it dropped to 1.25 ng/g when a marinade was used (**Oz et al.,2007** and **Iwasaki et al.,2010**). The total HCA content in well-done meat was 3.5 times higher than that of medium-rare meat. Fried pork (13.91 ng/g) had higher levels of total HCAs than fried beef (8.92 ng/g) and fried chicken (7.00 ng/g) (**Puangsoombat et al., 2012**). the levels of other HAAs such as MeIQx (2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline) generally range

from not detectable up to 10 ng/g in foods, estimated daily intake of HAs in different studies ranges from 0 to around 15 ng/person/day (Skog, 2002).

Commonly available spice-containing marinades can be effective inhibitors of HCAs formation and provide reduced exposure to some of the carcinogens formed during grilling, these amounts decreased to 87% of MeIQx and 87.3% of PhIP in the Caribbean marinated steak (Baloghet *et al.*, 2000). Also, Murkovic *et al.* (1998) showed that sprinkling dried spices (rosemary, thyme, sage, and garlic) on the surface of steak decreased the amount of HCAs in

meat, reduced the content of the HCAs below 60%. Verdin (2002) studied several spices as antioxidants and possible HCA reducers, the spices used were basil, garlic, ginger, onion, oregano, rosemary, sage, thyme.

Concentration 2-amino-3-methyl imidazoquinoline (IQ) in blood serum and urinary excretion of rats fed on experimental diets:

2-amino-3-methyl imidazoquinoline (IQ) concentrate (ppb) in blood serum and urinary excretion of rats fed on experimental diets are shown in table (3).

Table (3): 2-amino-3-methyl imidazoquinoline (IQ) concentrate (ppb) in blood serum and urinary excretion of rats fed on experimental diets.

Rat groups	IQ (ppb)	
	Serum	Urine
Control (-)	ND	ND
Control (+)	2.677±0.102 ^b	10.269±0.415 ^b
Boiled meat	ND	ND
Fried meat	3.018±0.375 ^b	10.451±0.511 ^c
Grilled meat	7.225±0.268 ^c	20.190±0.173 ^d
Pre-seasoning grilled meat	0.493±0.021 ^a	1.742±0.071 ^a

ND: not detected. **Each value in a row followed by the same letter are not significantly different at ($p \leq 0.05$).

The results obtained a significant differences among rats groups. Both blood serum and urinary excretion contained the highest IQ concentrate for rats group fed on grilled meat diet without marinated were 7.23 and 20.19 ng/ml (ppb), respectively, but when marinated with 1% ginger over time before grilling that led to decrease IQ concentrate to 0.49 and 1.74 ng/ml in serum and urine, respectively with decreasing percent reached to 93 and 91% about un-marinated, respectively. Whereas, pan fried meat rats group recorded values equal approximately the control (+) which their rats intake 2-amino-3-methyl imidazoquinoline (IQ) for 10 days were (3.02 and 2.677) in serum and (10.451 and 10.269) ng/ml in urine, respectively. While, IQ level not detected in both control (-) group which contain basal diet only without any additives and rats group which their diet eaten boiled beef meat.

The previous data agree with Murray *et al.* (1989) who observed that meal of pan-fried meat have approximately 12 µg of HAAs, which a small fraction (<5%) is excreted un metabolized in urine. Also, a quantity equivalent up to several percent of the ingested dose of 8-MeIQx and PhIP are excreted in urine as the un metabolized compounds (Stillwell *et al.*, 1999). Also, the major metabolite of 8-MeIQx formed in humans, and it accounts for more than 50% of the dose eliminated in urine (Turesky *et al.*, 2002). Also, Pavanello *et al.* (2002) indicated that pan fried hamburger (rich in HHAs) and consequently their potential genotoxic risk. HAAs are present at the ppb level depending on temperature and type of cooking. HCAs have been found in the urine of healthy people who consume ordinary diets (Nagao *et al.*, 1996).

Table (4): Mean body weight changes (g), body weight gain (g), body weight gain percent (%), Total food intake and Feed Conversion Efficiency of rats fed on the experimental diet for 16 weeks :

Rat groups	Mean body weight changes (g)					Body weight gain (g)	Body weight gain percent (%)	Total food intake (g/rat/16ws)	**F.C.E
	Initial body weight	After 4 weeks	After 8 weeks	After 12 weeks	Final body Weight				
Control (-)	122.3±3.1 ^a	142.6±6.1 ^a	173.3±7.2 ^{ab}	188.6±5.2 ^b	201.6±3.1 ^b	79.3±3.3 ^b	64.8	761.8±4.7 ^b	0.104±0.11 ^b
Control (+)	123.1±2.7 ^a	154.6±4.2 ^b	163.0±6.4 ^a	175.0±4.6 ^a	172.0±2.8 ^a	48.9±7.1 ^a	39.7	672.0±3.1 ^a	0.073±0.07 ^a
Boiled meat	125.0±2.1 ^a	158.0±4.3 ^b	188.0±8.2 ^{bc}	208.0±6.3 ^{bc}	235.6±4.3 ^c	110.6±5.2 ^c	88.5	687.5±3.3 ^a	0.161±0.14 ^{bc}
Fried meat	121.6±2.9 ^a	152.0±4.4 ^b	194.0±7.9 ^{bc}	255.0±7.1 ^c	233.0±4.1 ^c	111.4±5.3 ^c	91.6	872.3±5.2 ^c	0.128±0.12 ^{bc}
Grilled meat	121.0±3.3 ^a	146.0±3.1 ^{ab}	180.0±7.3 ^b	235.7±6.9 ^c	219.7±3.0 ^{bc}	98.0±4.7 ^{bc}	80.99	735.1±4.2 ^{ab}	0.133±0.12 ^{bc}
Pre-seasoning grilled meat	125.6±3.1 ^a	148.3±2.9 ^{ab}	173.0±6.9 ^{ab}	204.0±6.5 ^{bc}	248.8±4.3 ^c	123.2±6.6 ^c	98.6	647.4±2.9 ^a	0.190±0.71 ^c

* Each value in a column followed by the same letter are not significantly different at ($p \geq 0.05$).

**F.C.E = Feed Conversion Efficiency

The previous results obtained a significant differences among rat groups. Although pre-seasoning grilled meat rats group consumed the lowest food was 647.4 but have the highest body weight gain was 123.2g compared with control (+) rats group that have the lowest body weight gain. For this reason, feed conversion efficiency (F.C.E.) for rats group fed on pre-seasoning grilled beef meat recorded the highest value was 0.190 comparing with control (+) rats group was 0.073. It could be noticed that, seasoning beef meat with 1% ginger over night in the refrigerator before cooking improvement F.C.E compared with un-seasoning meat may be led to bind formed IQ during cooking at high temperature by total phenols and flavonoids exist in ginger herb.

When comparing cooking methods effect without seasoning on F.C.E, it could be observed a significant differences among rat groups where contained the fried meat rats group the lowest value was 0.128 although eating them the highest quantity was 872.3g that maybe led to soluble of IQ in fatso effect it faster and harmless.

These data were in agreement with **Eshak and Farag, (2011)** who resulted that ginger have the highest content for total phenols and flavonoids when used for marinating different animal foods compared with other herbs like; rosemary, thyme and green basil.

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