Dosage of Benzo(A)Pyrene in Skewers and Grilled Chicken, Sold around 67ha and ankatso, Antananarivo, Madagascar

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ABSTRACT

A study about sales and consumption of beef skewers and grilled chicken around 67ha and Ankatso showed that this small business is mainly practiced by men (57.74% men and 42.25% women). The most concerned consumer are students, 88.46% of the consumers in Ankatso and 47.88% of the consumers for 67ha. The preparation of the meet for the grilled chicken generates potentially toxic molecules like Benzo(a)pyrene that can be carcinogenic. The grilling time is one of the factors that cause this risk. Skewers and grilled chicken cooked for 10 to 20 minutes on charcoal have an amount of high benzo(a)pyrene (0.66µg/kg and 4,09 µg/kg) compared to cooked on intense fire for less than 10 minutes (0.25µg/kg and 1.34µg/kg). Grilled chicken contains a high quantity of Benzo(a)pyrene more than recommended standard (2µg/kg).

Keywords: skewers, benzo(a)pyrene, carcinogenic, grilled chicken

I. Introduction

Selling street food increased 60 years ago in the whole African countries including Madagascar. The urban population growth and the economic problems enable the development of open food market [5, 19]. Food sold in the urban commune of Antananarivo is composed of ingredients rich in carbohydrate (cereals and tubers), fat (fries and meat) and protein (sausage, skewers and barbecue) [6]. Beef skewers and grilled chicken are among the most consumed street food in Antananarivo [6,7]. Barbecuing have been used for centuries in many countries to get the food taste and characteristic appearance [4]. Recently, nutritional messages alarm the cooking of the grilled meat. Carelessness during the grilling generates toxic molecules like polycyclic aromatic hydrocarbon (PAH) particularly the Benzo(a)pyrene (BaP); it is carcinogenic, mutagenic and teratogen [14,15,16]. BaP is used to indicate the contamination of the foods by the PAH. This study will focus on the dosage of BaP during the cooking of the sold beef skewers and grilled chicken around 67ha and Ankatso in Antananrivo city. These places are chosen because of the quantity of seller and consumers there. Besides, those types of food are mostly appreciated by students who will lately lead the nation and it is important to know about the quality of the food they eat.

II. Materials and methods

2.1 Materials

- **Biological materials**

It constists by different grilled meat samples: beef skewers and grilled chicken sold around 67ha and Ankatso.

- **Reagent**

A standard solution containing 1g/l of BaP, acetonitrile, acetone, methanol, N-hexane, toluene, dichloromethane and deionized water were used for the dosage of BaP.

- **Materials and laboratory devices**

The dosage of BaP was done by high performance liquid chromatography (HPLC)/ UV Visible Spectrophotometer, with C18column.

1.2 Methods

1.2.1 Sampling method

For this study, two samples for each food were taken : the first one is braised and the other one is grilled with hot fire. Then the samples were covered with aluminum foil to protect them from dirtiness before keeping them in an amber glass jar. The samples were kept in 4°C cold and transferred to the laboratory. This precaution is necessary because of the photosensitivity of some HAP [17].

2.2.2 Survey

A survey was done according to the availability of the sellers; it was based on survey. Information about the characteristics of the sellers, the grilling practice (including the kind of fire, the cooking time and the grilling kind) were observed during the survey.

2.2.3 Dosage method of BaP

Extraction

The realization of the extraction and the analysis of the BaP followed the standard ISO 15753-2004 [8]. We have tested 2.5g sample (beef skewers/grilled chicken) in a centrifuge tube, then, 10 ml of combined acetonitrile/acetone (v/v: 60/40) were added. The solution has been homogenized in vortex for 30 s and in ultrasound for 5 min before being centrifuged for 5 min at 4 000 t/min. The supernatant has been transferred in a crucible conic tube and the solvent was evaporated with a rotavapor at 35°C. The extraction has been repeated twice with 10 ml of combined acetonitrile/acetone (v/v: 60/40). The extract has been purified on grafted cartridge phase of C18. So, 2 ml of combined acetonitrile/acetone are introduced in a conic tube containing the extract and shake with vortex for 15s then centrifuged for 30 s. The supernatan was transferred in prior conditioned C18 cartridge with 12 ml of methanol and 12 ml of acetonitrile. The elution was done with 5 ml of combined acetonitrile/acetone on atmospheric pressure. The eluent was concentrated with a rotavapor at 35°C. The purified extract as been taken in 1 ml of hexane. The tube was then closed and conserved at -4°C before the analysis.

Analysis on HPLC/UV

The analyzed extract by HPLC was equipped with fluorescence detector UV-visible due to their universal character, the relational transparency in many solvents and the simplicity of the method [13]. The C18 column (15 m*4.6 mm*5 µm), the combination solvents acetonitrile/acetone (60%/40%) and acetonitrile/water (50/50) were used as mobile phase of 0.6 ml/min flow. An extract of 20 µl was injected in the column. The product signal during the composed detection cell passage was resulted from a chromatogram peak whose surface would be proportional to the concentration. The quantification of BaP was done from the calibration curve.

2.2.4 Statistical analysis

The statistical processing was achieved with the XLSTAT 2014 software and the significancethreshold is hold at 0.05. The result of the BaP dosage is analyzed with the test Khi2 to justifythe hypotheses on the principal causes of the toxic product in the beef skewers and the grilledchicken.

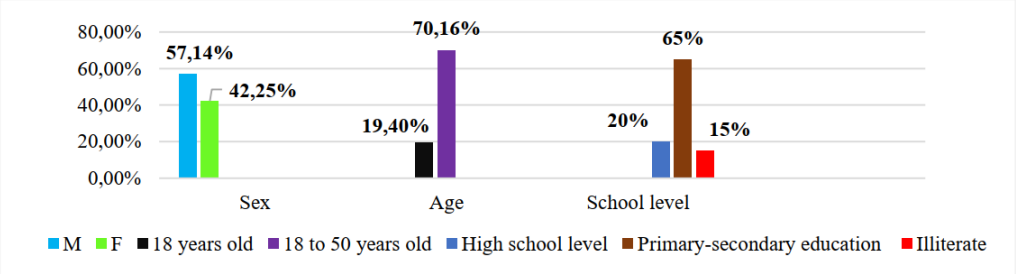
III. Results

3.1. Surveys results

3.1.1. Sellers

The age, the sex and the education level of the sellers in the two areas of study arepresented in this figure 1.

Figure 1 : Sellers profile



The survey shows that 19.40% of the sellers are younger than 18 years, 70.16% are included in the age of 18 to 50 and 10.44% are aged more than 50. The majority of the sellers are men 57.74% and 42.25% women. The sellers are less educated, only 20% of them achieved high school education, 65% have finished primary-secondary education and 15% are illiterate. Personals who does this business is varied: those who cut the meat into small pieces, those who cook, those who serve and those who manage. The main used fuel for grilling is charcoal. If the meat is braised, the cooking time is 10 to 25min. But if the meat is grilled with hot fire, the duration is only 10 min. This following table illustrates the cooking duration depending on the fire intensity for the barbecue.

Table1: Cooking timing and percentage of the skewers according to the kind of used fire

	Braise	*Hot fire grill*
Cooking timing	*10 min to 25 min*	*About 10 min*
Pourcentages of the skewers according to the kind of fire	*57,64% More carbonized meat*	*40,84% Less carbonized meat*

The cooking duration on the barbecue also depends on the customers' needs. Someconsumers prefer to eat the rare meat and some prefer it well-cooked.

The meat is well-cooked and more carbonized if it is braised and it is rare or "masak'afo" and less carbonized if the fire is intense. As we can notice in the table 1, 57.64% of the grilling is practiced with braised heat and 40.84% is practiced with intense fire.

3.1.2 Consumers

Three hundred consumers were surveyed around 67 ha and Ankatso including 68.64% men and 31.35% women. Their ages vary between 16 and 54, with 29.60% married and 70.33% single. The figure 2 shows the profile of consumers.

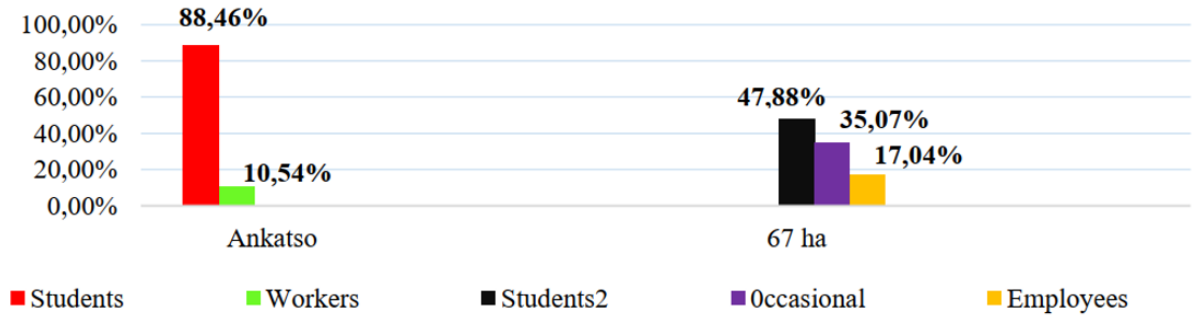


Figure 2 : Consumers profile

In Ankatso, 88.46% of the consumers are students and 10.54% are workers. In 67ha, most of the consumers are students 47.88%, occasional consumers are 35.07% and 17.04% are employees. Generally, a large number of the consumers in the two areas are students. The purchase frequency per week and the demand of the consumers s are illustrated in the figure 3.

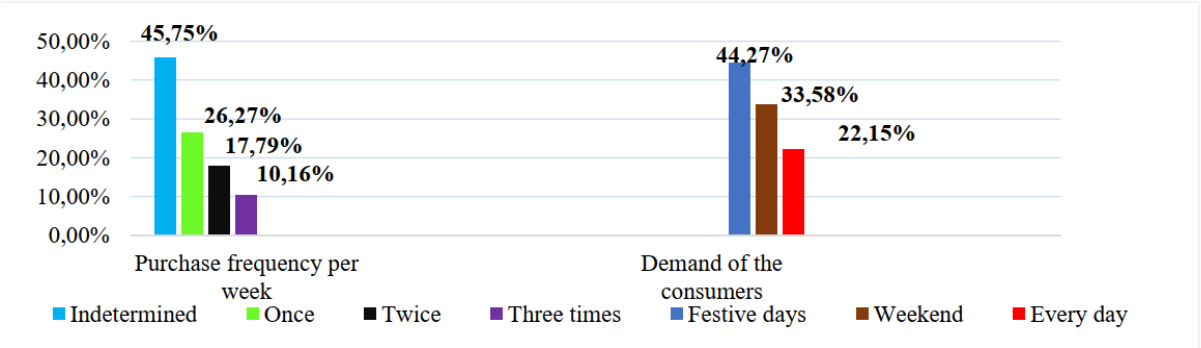


Figure 3 : Weekly supply and demand

The surveyed consumers (10.16%) eat one the products three times a week, 17.79% eat it twice a week and 26.27% once a week whereas 45.75% consume the product occasionally. Like any commercial activity, there is high season for the sale. The skewers and grilled meat are especially consumed on festive days (44.27%) and weekends (33.58%). Every day, the demand of the consumers is at 22.15%.

3.2. Results of the BaP dosage

The results of the BaP in the beef skewers and grilled chicken are shown in the table 2.

Table 2: The content of BaP.

Type of used fire and cooking duration	*BaP in beef skewers*	*BaP in grilled chicken*	*Standard CE N°835/2011*
Braise fire 10 à 25 min	*0,66 µg/kg*	*4,09 µg/kg*	*2 µg/kg*
Intense fire Fewer than 10 min	*0,25 µg/kg*	*1,34 µg/kg*	*2 µg/kg*

The quantity of BaP in beef skewers is less than BaP in grilled chicken. The quantity of formed BaP during the 10 to 25 min cooking with braised heat fire is superior to the 10 min cooking with intense fire. It reaches 0.66 µg/kg for the beef skewers and 4.09 µg/kg for the grilled chicken. With intense fire, this content decreases to 0.25 µg/kg, the grilled chicken cooked with braised heatreaches twice more of this amount.

So, the development of BaP in the two products is not related to the high temperature of the fire, but it depends on the cooking duration. A Khi2 testisrealized to check the dependence between the variables. We have two qualitativevariables: the BaP quantity in the variety of grilled meat (chicken and beef) and the heattemperature (braised heat, intense). In null hypothesis (Ho), the content of BaP is not linked to theincrease in the fire temperature while in the alternative hypothesis, the two variables depend oneach other. The p-value (0.9546) is superior to significance threshold 0.05. So that, thehypothesis Ho is agreed which means the development of BaP is not related to the intensity offire.

IV. Discussion

The difference in the rate of BaP in these foods is due to: the kind and surface of the piece of meat in contact with the charcoal fire, and the grilling for cooking the meat. The meat in contact with heat for hours provides HAP [9,12]. In addition, the grilled chicken is susceptible to contain BaP because of its amount of fat. The exudation of fat from heat increases the development of HAP [9,10,11]. Skewers are grilled with specific materials. Sometimes, the sellers use 6 cm spaced metal rod (which permits a direct contact of meat and the fire), the dug flat iron also is used to cook the skewers. Figures 4 and 5 show the types of used grill for cooking the meat.

Figure 4 : Type of grill for beefskewers Figure 5 : Type of grill for grilled chicken (Source : author)

Studies were done to limit the contact between the meat fat and the source of heat to decreasethe pyro synthesis of HAP. Using barbecue with a vertical heating system allows to avoid theexuded fat to the fame. It leads to the development of HAP at 10 times to 30 times less thanwith classical barbecue with horizontal system [3,18]. And the content of the most raised BaPis registered for the barbecue grilling (0.09 to 4.86µ/kg) even they were few (about 0.1µg/kgfor that are cooked with spoil or roast). Concerning the variability of the meat, the highestquantity of BaP were measured in the chicken and in the beef [9].

Consequently, it is important to give some suggestions to improve the practice of grilling andto limit the overexposure of the foods to the composition.

- The foods must be cooked with braised heat, not in a direct contact with the fire[12].
- The cooking temperature should not exceed 22O°C and the grill ought to be put at least10 cm from the braised heatin case of horizontal grill or even choose the vertical grill [1, 18].
- It is crucial to avoid the fat to fall in the fire. The leaner is the meat, the lower is the riskof BaP [2].

However, HAP contamination risk increases if the way of cooking is not wellcontrolled. In fact, the failing fat in the fire provokes fumes and flame which creates HAPwhen it is in contact with the meat. Then, it has to be covered with thin ash or to take offthe fat of the meat.For hygienic and security reason, permanent cleaning of all the grilling materials isrequired.

V. Conclusion

The grilled chicken has a high quantity of BaP which is superior to the limit 2µg/kgcompared to beef skewers. Cooking with braised heat fire for 10 to 20 min presents more content ofBaP than cooking with intense fire for 10 min or less. Consuming beef skewers or grilledchicken may cause immediately notified or chronic disease, it depends on the consummationfrequency and the amount of the products. But it provides protein for the young consumers asmeat contains essence amino acid and grill can reduce chronic malnutrition (whose prevalenceis 47.3% in Madagascar). In a word, a good alimentation is a source of better economicdevelopment of a country.

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