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Technological performance and effects of traditional grilling on the physicochemical characteristics and polycyclic aromatic hydrocarbon contamination of *kilichi*, a grilled meat produced in Niger

Processing and safety of grilled meat from Niger

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Abstract

The physicochemical characteristics and chemical safety of two categories of grilled meat from Niger were assessed, using sun drying and optionally wood fire grilling processes. Contamination factors associated with the critical unit operations were identified and the technological parameters were monitored through these processes. Sixty samples were analysed for dry matter, pH and water activity. Polycyclic Aromatic Hydrocarbons were assessed using HPLC-FLD technique. No effect of the process on the duration of *kilichi* production was observed whereas the yield of *kilichi* was significantly (p=0.015) dependent on the process and *kilichi* category. Production of *kilichi* leads to losses of dry matter (yield <90%). All wood fire grilled samples were highly contaminated with BaP and PAH4, with concentrations exceeding up to 10 times the EU maximum limits set at 2 and 12 µg/kg, respectively. Kiln type, fire wood species and processing practices were the critical factors for an improved quality of *kilichi*.

NOVELTY IMPACT STATEMENT

The two main traditional processes of grilled meat production identified in Niger are characterized for one process, by sun-drying and wood fire grilling of beef meat and for the second by sun-drying only, without wood fire grilling.

Grilled meat obtained through the process not involving wood fire grilling has a level of PAHs contamination lower than the maximal limits for BaP (2 µg/kg) and PAH4 (12 µg/kg), whatever the category of grilled meat.

All the wood fire grilled meat samples are highly contaminated with BaP and PAH4, exceeding up to 10 times the EU maximum limits for both BaP and PAH4.

Keywords: polycyclic aromatic hydrocarbons, kiln grilling, *kilichi* quality

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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1. INTRODUCTION

Kilichi, originated from Hausa people, is the most popular added value meat product manufactured in Sahelian countries, which provides cash income to most producers (Boubacar et al., 2019a). It is a ready-to-eat product consumed in both rural and urban areas, mainly as a snack (Kalilou, 1997; Mgbemere et al., 2011; Isah and Okubanjo, 2012; Olusola et al., 2012). Two main categories of *kilichi* are manufactured in Niger: coated *kilichi* (*kilichi ja)* and uncoated *kilichi* (*rumuzu*). *Kilichi ja* is produced by trimming boneless meat, cutting into pieces of parallelepiped shape before slicing into flat thin straps. The straps are pre-dried in the sun, followed by marinating in a sauce made of complex blend of spices and colorant, before a second sun drying and briefly grilling at wood fire. The process of *rumuzu* production is similar to that of *kilichi ja*, except the slight seasoning of the pre-dried straps before grilling and the second sun drying step.

As *kilichi* process involves a grilling step in which the wood flame is in direct contact with the product, the risk of polycyclic aromatic hydrocarbons (PAHs) contamination is very high (EFSA, 2008; CAC/RCP 68-2009, 2009; Olatunji et al., 2013; Hokkanen et al., 2018). PAHs are toxic compounds produced by incomplete combustion of organic matter during thermal meat processing including grilling. The sum of eight PAHs (PAH8), namely Benzo [a] anthracene (BaA), Chrysene (CHR), Benzo [b] fluoranthene (BbF), Benzo [k] fluoranthene (BkF), Benzo [a] pyrene (BaP), Dibenzo [a, h] anthracene (DhA), Benzo [g, h, i] perylene (BgP), Indeno [1,2,3-cd] pyrene (IcP), as well as the sum of a subgroup of four PAHs (PAH4), including BaA, CHR, BbF and BaP, are used as suitable indicators. Among the PAH4, BaP is the most toxic, due to its carcinogenic effect in humans. The European Commission (rule n°835/2011) had fixed maximal content of 2 µg / kg for BaP and 12 µg / kg for PAH4 in food commodities like meat and meat products (EFSA, 2008).

Previous studies (Boubacar et al., 2019a; 2019b), reported the diversity of processes used in traditional *kilichi* production in Niger, resulting in a diversity of *kilichi* in the markets and a high level of PAHs in different categories of *kilichi,* showing potential health risk for consumers. Monitoring the most important processes would help to identify the critical control points in terms of potential sources of PAHs contamination and to develop efficient technical tools to produce safe and acceptable *kilichi* for consumers. Therefore, the objectives of this study were to characterize the two main processes of *kilichi* production, to identify potential factors of PAHs contamination and to determine the PAHs contamination associated with these factors, with the aim of improving process and sanitary quality of *kilichi*.

2. MATERIALS AND METHODS

2.1 Monitoring of meat processing into *kilichi*

Two processes (without grilling, and kiln grilling on fire of meat pieces) were applied to the most consumed categories of *kilichi* (*rumuzu* and *kilichi ja*) produced in Niger. The monitoring of the processes/*kilichi* category was performed in duplicate at three processing sites of high production (Niamey, Madaoua and Tessaoua) under the supervision of three highly skilled producers per site, as described in Table 1.

2.1.1. Meat pretreatment for *rumuzu* and *kilichi ja* process monitoring

For *rumuzu*, the sun-dried thin straps were sprayed with oil and slightly seasoned with salt and spices. These seasoned thin straps underwent a grilling on a grill placed on a kiln when grilling was applied (Boubacar et al., 2019a).

For *kilichi ja*, the sun-dried thin straps were coated with a sauce composed of defatted peanut paste, combined with a cocktail of ingredients and colorant. These coated straps underwent a second sun drying, followed by grilling on a kiln when applied (Boubacar et al., 2019a).

2.1.2. Description of grilling operation

Grilling was carried out according to the traditional practices (Boubacar et al. 2019a) using a clay kiln (rectangular banco) and a half-barrel kiln. The half-barrel kiln fueled with *Combretum glutinosum* wood was used in Niamey and the clay kiln (rectangular banco) fueled with *Piliostigma reticulatum* wood was used in Madaoua. The production of *kilichi* at Tessaoua did not involve any fire wood grilling.

2.1.3. Assessment of the technological parameters used during processing

Technological parameters (mass of products, time) were registered at each unit operation level during the production of each category of *kilichi*. Fire temperature and attack temperature were measured during the grilling steps. Samples of meat products were taken at each step to determine their physicochemical characteristics. Sixty samples, including 24 intermediary products before grilling and 36 samples of *kilichi* as described in Table 1, were collected from the three processing sites for physicochemical analyses.

2.2 Physicochemical analyses

The physicochemical analyses performed were: pH, moisture and water activity. The pH was measured as described by Mgbemere et al. (2011) and the sample moisture was determined, using the ISO 1442:1997 standard. The water activity (a_w) was measured according to the method described by Anihouvi et al. (2006) using aw sprint (SWISS made NOVASINA TH-500) water activity equipment.

2.3 Polycyclic Aromatic hydrocarbons analyses

The polycyclic aromatic hydrocarbons (15 EU PAHs) were determined in intermediary products (24 dried samples) before grilling and samples of *kilichi* (36 end-products) using a High-Performance Liquid Chromatograph coupled with a fluorescence detector (HPLC-FLD) according to the method described earlier (Brasseur *et al.*, 2007). A Model 600 E solvent delivery system, equipped with a Model 717 automatic injector, a Mistral TM oven and 2475 fluorescence detector (Waters Corporation, Milford, MA, USA) were used. C18 Pursuit 3 PAHs (100 x 4.6 mm, 3 µm) equipped with a ChromGuard (10 x 3 mm) pre-column, both from Varian (Agilent Technologies, Santa Clara, California, USA) were used to separate the PAHs. The excitation and emission wavelengths of the PAHs analyzed are given in Table 2. The PAHs were extracted from the samples using the method described by Veyrand *et al.,* (2007). The samples were frozen in liquid nitrogen and then freeze-dried for 36-48 hours. For the extraction, one g of lyophilized samples was homogenized with a mixture of hexane/acetone (50/50, v/v) by using the Accelerated Solvent Extraction system (ASE 200, Dionex, Sunnyvale, California, USA). Then the solvent was evaporated until 1 ml, and the extract was reconstituted with 5 ml of cyclohexane, before to be purified using a Chromatographic Column (Cartridges, Marchery-Magel Chromabond), previously conditioned with 15 ml of ethyl acetate and 10 ml of cyclohexane. The extract was poured on the column which was then washed twice with 3 ml of cyclohexane/ethanol (70/30, v/v) mixture. The PAHs were eluted tree times using 4 ml of cyclohexane/ethyl acetate (40/60 v/v) mixture. The solvent was evaporated to dryness using a TurboVap system (Zymark), then 90 µl of acetonitrile and 10 µl of the deuterated DiP-D14, used as internal standard (LGC Promochem, France), were added. Five (5) µl of this final extract was injected on the HPLC column, which was set at 36°C.

After checking that the signal to noise ratio was greater than 10, the limits of quantification (LOQ) were set at 0.90 µg/kg of fresh weight for the Benzo(j)fluoranthene and Indeno[1, 2, 3-cd]pyrene and 0.23 µg/kg fresh weight for the rest of the PAHs, those values corresponding to the first point of the calibration curve for each PAH. Recovery of extraction was evaluated for the 15 PAHs and ranged between 62 and 97%, 53 and 92% and 59 and 97 % after spiking at 5, 10 and 15 µg/kg respectively. More validation parameters such as repeatability, intermediate precision, recovery and expanded uncertainty at the three mean introduced concentrations can be found in the paper published by Brasseur et al. (2007). Chromatograms obtained for the 15 PAHs in a reference solution (1 µg/kg PAH except for IcP and BjF: 2 µg/kg PAH) acquired with the different channels of the fluorescence detector are shown in Figure 1.

2.4 Statistical analyses

All analyses were performed in the R software version 3.5.2 (R Core Team, 2018). To assess the effect of the process (with or without grilling) and the type of *kilichi* (*rumuzu* and *kilichi ja*) on the technological (yield and operation duration) and physicochemical parameters (dry matter, pH and water activity), a linear model with mixed effects was built with two principal factors using package *ImerTest* (Kuznetsova et al., 2017) of R software. The fixed factors were technology and the categories of *kilichi* while processor was the random factor.

As the levels of combinations of factors were not found in the dataset, to evaluate the effect of the type of wood and type of kiln on the formation of PAHs in the two categories of *kilichi*, an exploratory study of data was done. So, in order to have a complete and balanced plan, and thereby facilitate the result interpretation, some factors have been merged. The new data following a complete and balanced plan is constituted of the factors: Process (combination of the factors: site, type of kiln, type of wood, *kilichi* at intermediate stages (*kilichi* before and after grilling, *kilichi* without grilling) and type of technology), Processor and Category of *kilichi* (Table 3). The fixed factors were the process and the category of *kilichi* while the processor was the random factor.

For each parameter, a linear model with mixed effect was applied with these factors, using *lmerTest* package (Kuznetsova et al., 2017) of the R software. For each parameter, the global model taking into account all the main factors and their interactions was first considered; following the interaction significance or not, a final simpler model was retained. Moreover, the interactions between the process and the category of *kilichi* were checked. The significance threshold was $\alpha = 5\,\%$. The adjusted means of the significant factors of the final model were calculated with *Emmeans* package (Lenth, 2018). In order to prioritize the different levels of the significant factors, an organization of means was performed with the test of Tukey. For the PAHs, these two determined elements (adjusted mean and organization of means) were represented graphically by histograms, error bars and letters symbolizing the organization of means with *ggplot2* package (Wickham, 2016).

3. RESULTS AND DISCUSSION

3.1 Processing of fresh meat to produce *rumuzu* and *kilichi ja*.

The main operations of the processing of raw meat to produce *rumuzu* and *kilichi ja* are trimming-cutting, production of thin straps, drying, seasoning and grilling. While *rumuzu* production involves a slight seasoning using oil and diverse spices, *kilichi ja* seasoning is made of a strong coating with a sauce composed of peanut paste mixed with a cocktail of spices followed by a second drying before grilling (Fig. 2 and 3).

The duration of the trimming-cutting, production of thin straps and first drying are similar whatever the process used and the category of *kilichi* produced ($p>0.05$). Seasoning and coating steps for *kilichi ja* production was twice more time-consuming than seasoning for *rumuzu* production ($p <0.000$). While only one sun-drying (lasting about 12 h) was applied for *rumuzu* whatever the process used (with or without grilling), a second sun-drying was applied for *kilichi ja* production, with 2.5 more time ($p=0.013$) of exposure to solar light when further fire wood grilling was not applied. No significant difference ($p>0.05$) was observed between the total duration of production for the two categories of *kilichi* whatever the process applied (with or without fire wood grilling) (Table 4).

Furthermore, while the yield of *kilichi ja* was similar for both processes used (about 71% dry weight basis), *rumuzu* yield was affected by the process, the one with fire wood grilling being significantly ($p=0.015$) lower than when grilling was not applied. However, no matter the process used, the traditional production of *kilichi* leads to losses of dry matter (yield <90%). The major losses were observed during trimming-cutting and drying. This can be explained by the large quantity of residue (12 to 21%) produced during this operation and the matter loss on the mats during sun drying. The yield of *kilichi* (wet weight basis) varies between 19.5 and 22.9% for *rumuzu* and between 34.8 and 38.1% for *kilichi ja*. Similar values were reported by Kalilou et al. (1998) on *kilichi ja* (30% - 45%). A significant difference ($p=0.028$) of yield (dry weight basis) after the first drying stage was found between both processes of *kilichi* production, although the grilling yield (dry weight basis) depends on *kilichi* categories ($p=0.020$).

3.2 Influence of the unit operations on the physicochemical quality of *kilichi*

Tables 5 and 6 show the evolution of pH, water activity and dry matter during the two processing technologies of *rumuzu* and *kilichi ja*. The pH values of fresh meat (5.5 - 5.7) did not change significantly ($p>0.05$) throughout the process for the *rumuzu* production whatever the technology applied (Table 5). The same trend was noticed for *kilichi ja* produced without fire wood grilling. As far as *kilichi ja* involving fire wood grilling is concerned, although the pH of the final product was similar to the initial pH of fresh meat, a significant ($p<0.05$) difference of pH was observed between the fresh straps (FS) (pH = 5.46) and the coated dried straps (CDS) (pH = 5.78). The higher pH of the CDS was probably due to the coating ingredients used (Table 6). The pH values recorded in this study are similar to that obtained by Salifou et al. (2013). Boubacar et al. (2019b) also observed similar pH values on market *kilichi* of Niger.

First drying, and grilling when applied, affected significantly water activity and dry matter content of the intermediary and end products of the two categories of *kilichi,* whatever the technology used. The water activity was reduced from 0.9 to around 0.5 in the dried thin straps and to about 0.4 after grilling when the dry matter has increased 3 to 4 times in dried and grilled meat compared to fresh meat. The values of the water activity in the dried thin straps and in the end products were far lower than 0.7, value under which microbial growth is minimized (Prescott et al. 2003). The values of dry matter content and water activity recorded in this study are similar to those measured in *kilichi* produced in West and East African countries such as Niger, Nigeria and Cameroun (Kalilou, 1997; Yacouba, 2009; Boubacar et al., 2019b; Mgbemere et al., 2011; Olusola et al., 2012; Apata et al., 2013; Jones et al., 2001).

3.3 PAHs contamination

3.3.1 Distribution of the PAH concentrations in *rumuzu* and *kilichi ja*

Fig. 4 shows the distribution of PAH concentrations with the median, Q1 and Q3 quartiles, outliers, minimum and maximum values (boxplots). There were higher concentrations of PAHs in *kilichi ja,* in comparison with *rumuzu*. The median concentration values of the sum of PAHs, BbF, BaA, CHR, BaP, PAH2, PAH4 and PAH8 were 15.9, 0.9, 3.3, 3.1, 1.2, 4.5, 8.3 and 11.1 µg/kg, respectively for *rumuzu* against 18.9, 0.8, 8.7, 3.9, 1.1, 4.6, 13.7 and 15.3 µg/kg, respectively, for *kilichi ja*. Boubacar et al. (2019b) reported similar PAHs concentrations and distribution in the two categories of *kilichi* collected in the markets. Chrysene and BaA were found as the most prominent PAHs, an observation similar to that made by Rozentāle et al. (2015).

Whereas PAHs were significantly affected by the applied process ($p < 0.05$; Table 7) the category of *kilichi* affected only the formation of BaA (Prob. < 0.05). Indeed, concentration of BaA in *kilichi ja* was significantly higher than that in *rumuzu*, probably as a result of the coating ingredients used, mainly peanut paste which has undergone a roasting to obtain grilled peanut before being mixed with spices.

In order to prioritize the different levels of the significant factors, an organization of the adjusted means was performed with the test of Tukey. The effects of the process and category of *kilichi* on the formation of PAHs revealed that, as a whole, the lowest concentrations of PAHs were observed in the *kilichi* from Niamey before grilling (BaPmax 0.90 µg/kg and PAH4 max 14.33 µg/kg), the *kilichi* of Madaoua before grilling (BaPmax 1.03 µg/kg and PAH4 max 17.75 µg/kg) and in the *kilichi* of Tessaoua without grilling (BaPmax 1.93 µg/kg and PAH4 max 11.23 µg/kg). There was no significant difference between these low concentrations. The highest concentrations of PAHs (BaP max 19.68 µg/kg and PAH4 max 145.64 µg/kg) were recorded in the *kilichi* of Madaoua for which clay kiln has been used as grilling material and *P. reticulatum* as fire wood; and the *kilichi* of Niamey using a barrel kiln as grilling material and *C. glutinosum* as fire wood (BaPmax 19.07 µg/kg and PAH4 max 98.5 µg/kg). These results also showed that *kilichi* from Madaoua (n=12) contained higher concentrations of PAHs than grilled *kilichi* from Niamey (n=12) (Fig. 5 and 6). The higher concentrations of PAHs in Madaoua *kilichi* samples with *P. reticulatum* as fire wood may be due to the lower moisture content of this wood (8.33%) (Sotelo *et al.* (2017), compared to PAHs in the Niamey *kilichi* samples using *C. glutinosum* as firewood (moisture content = 9.15 %). Indeed, the CAC/ RCP 68-2009 (2009) reported that the combustion of wood with lower moisture content is rapid and leads to high PAHs production. Sotelo et al. (2017) and Sotelo et al. (2012) also reported that *P. reticulatum* had low quality fuel wood properties (low wood basic density and fuel value index). Denser wood has more energy per unit volume and burns more slowly. The difference of PAHs concentrations in the *kilichi* before grilling or without grilling and the *kilichi* after grilling was significant ($p<0.05$). High variations of PAHs concentrations were observed between the grilled *rumuzu* samples ranging between 2.2 and 19.7 µg/kg for BaP and between 13.2 and 145.6 µg/kg for PAH4 whereas in *kilichi ja*, these concentrations varied for BaP and PAH4 from 2.9 to 19.1 µg/kg and from 22.7 to 98.5 µg/kg respectively. Rozentāle et al. (2018) also reported high variations of BaP (0.05 to 166 µg/kg) and PAH4 (0.4 to 628 µg/kg) in smoked meat. Similar concentrations of 7.8 µg/kg and 6.9 µg/kg for BaP were reported respectively in *slavonska kobasica* (smoked dry fermented sausage) and smoked *kitoza* (traditional Malagasy smoked meat) respectively (Mastanjevic et al., 2020; Ratsimba et al., 2019). All the grilled samples of the two categories of *kilichi* (100%) exceeded the recommended limit for BaP (2 µg/kg) and PAH4 (12 µg/kg), maximal content fixed by the European Commission (rule n°835/2011) in food commodities like meat and meat products (EFSA, 2008). Several studies reported that food products generally get chemical contamination through thermal processing such as smoking, roasting, grilling, frying and drying where they are in direct contact with the combustion source (Farhadian et al., 2010; Roseiro et al., 2012; Martin et al., 2015; Hokkanen et al., 2018).

The adsorption of PAHs at food product surface during smoking or grilling processes is influenced by many factors including wood species, temperature of combustion (400-1000°C), the amount of fat, the time of grilling, the distance between the heat source or the grilling equipment (e.g. kiln type). (Adeyeye, 2017; Ledesma et al., 2014; Olatunde, 2013; Hitzel et al., 2013; Farhadian et al., 2010; Stumpe-Visksna, 2008). The mean high temperatures of grilling (720.5 ± 151.9°C and 724.9 ± 142.7°C) recorded during this study justify the high PAHs concentrations in the grilled *kilichi.* The two types of wood and the traditional kiln used for grilling *kilichi* in this study are not suitable to protect processors from heat and to procure safe end products. Several authors reported that the choice of wood for smoking is one of the critical parameters to be controlled because wood nature has a significant effect on the concentration of PAHs in smoked meat (Hitzel et al., 2013; EFSA, 2008; Stumpe-Visksna, 2008). The extreme values of BaP (6 to 10 times the standard limit) and PAH4 (6 to 12 times the standard limit) determined in the grilled *kilichi* (Fig. 4A and 4B) are certainly due to the oil spray on the thin straps observed before and during the grilling. In fact, one of the sources of the formation of PAHs is the pyrolysis of organic matter such as proteins, fat and carbohydrates. The added oil falls on the combustible thereby causing their pyrolysis and leading to the formation of PAHs (Duedahl-Olesen et al., 2015; Santos et al., 2011). For *kilichi ja*, the results show that 16% (1/6) of Niamey samples and 50% (3/6) of Madaoua samples before grilling were not compliant for PAH4.

4. CONCLUSION

This study revealed that the processes used to produce *rumuzu* and *kilichi ja* in Niger are not efficient and lead to lower yields (<90% dry matter basis), meaning 10% of dry matter loss but gives a probable microbial stability (a_w<0.7). The results reported that there were higher concentrations of PAHs in *kilichi ja,* as compared with *rumuzu*, and do not change significantly with the type of kiln. All *kilichi* obtained by the process without grilling comply with the standard established for PAHs whereas, all the grilled samples of the two categories of *kilichi* (100%) exceeded the recommended limit for BaP (2 µg/kg) and PAH4 (12 µg/kg) contents set by the European Commission (rule n°835/2011). Because of these high concentrations of PAHs in the final products, *kilichi* produced through the current wood fire grilling processes is unsafe for human consumption. The workplace organization, kiln and species of wood used, the inappropriate manufacturing practices during the processing are the major factors that contribute to the high concentrations of PAHs. To reduce these PAHs contamination, the grilling process should be technically improved through the development of efficient dryer and kiln, with appropriate fuel source and application of good manufacturing practice principles.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest in this article.

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Table 1: Experimentation plan for the monitoring of *kilichi* production

Technology/ category of *kilichi*	Number of trials per processor and per site			
	Niamey	**Madaoua**	**Tessaoua**	**Total**
Grilling/ *Rumuzu*	6	6	0	12
Grilling/ *Kilichi ja*	6	6	0	12
Without grilling/ *Rumuzu*	0	0	6	6
Without grilling/ *Kilichi ja*	0	0	6	6
Total	12	12	12	36

Table 2: Maximum excitation (λexc) and emission (λemis) wavelengths of the 15 EU priority PAHs.

HAPs	λ_{exc} max (nm)	λ_{em} max (nm)
BcL	304	353
BaA	275	389
CHR	260	381
5MC	260	376
BjF	300	512
BbF	280	438
BkF	290	412
BaP	281	407
DlP	295	424
DhA	285	396
BgP	285	416
IcP	290	499
DeP	285	398
DiP	380	434
DhP	290	452

Table 3: Description of the three experiment factors

Factors	Modalities	Nature
Processor	**3 (2 repetitions per processor)**	**Randomly**
Process	- NAGr (Niamey before grilling) - NGrBC (Niamey Barrel *C.glutinosum* Grilled) - MAGr (Madaoua before grilling) - MBanPGr (Madaoua Banco rectangular *P. reticulatum* Grilled) - TSG (Tessaoua without grilling)	Fixed
Categories of *kilichi*	- *Rumuzu* - *Kilichi ja*	Fixed

Table 4: Comparison of the performance parameters of the two processes of *kilichi* production

	Process with grilling		Process without grilling		
Parameters	***Rumuzu***	***kilichi ja***	***Rumuzu***	***kilichi ja***	**Pvalue**
Yield (% dry weight basis)	68.83 ± 3.25 b	72.34 ± 3.13 ab	83.51 ± 3.54 a	69.56 ± 4.55 ab	0,015
Yield (% wet weight basis)	19.47 ± 1.11 b	34.75 ± 1.11 a	22.92 ± 1.57 b	36.33 ± 1.57 a	0.073
Time (min)	849 ± 74.9 a	906.9 ± 74.9 a	945.2 ± 105.9 a	1123.3 ± 105.9 a	0.466

Table 5: Effects of the unit operations on the physicochemical quality of *rumuzu*

Phys-chem	Process with grilling					Process without grilling			
Characteristics	TCFM	FS	FDS	CDS	GS	TCFM	FS	FDS	CDS
pH	5.70±0.23 a	5.51±0.25 a	5.55±0.20 a	5.51±0.25 a	5.55±0.29 a	5.41±0.53 a	5.70±0.13 a	5.60±0.31 a	5.56±0.47 a
a_w	0.91±0.08 a	0.88±0.09 a	0.49±0.11 b	0.46±0.13 b c	0.35±0.08 c	0.94±0.03 a	0.93±0.03 a	0.39±0.12 b	0.32±0.01b
Dry matter content (%)	26±2.09 c	25.75±2.01 c	89.67±2.31 b	90±2.26 b	92.83±1.75 a	25.67±2.66 b	25.67±1.51 b	93.83±0.75 a	94.83±0.75 a

Table 6: Effects of the unit operations on the physicochemical quality of *kilichi ja*

Physico-chemical Characteristics	**Process with grilling**					**Process without grilling**			
	TCFM	**FS**	**FDS**	**CDS**	**GS**	**TCFM**	**FS**	**FDS**	**CDS**
pH	5.53±0.2 ab	5.46±0.3 b	5.53±0.25 ab	5.78±0.18 a	5.77±0.37 ab	5.75±0.19 a	5.59±0.25 a	5.63±0.17 a	5.64±0.48 a
a_w	0.89±0.09 a	0.89±0.09 a	0.47±0.12 b	0.56±0.13 b c	0.42±0.121 c	0.93±0.031 a	0.91±0.036 a	0.41±0.085 b	0.33±0.02 b
Dry matter content (%)	25.5±2.07 c	25.33±1.37 c	87.58±5.4 a	76.33±9.54 b	85.08±5.02 a	24.83±0.75 c	25.17±1.72 c	93.5±1.64 a	89.67±2.34 b

Table 7: Results of analysis of variance on the mixed effect models testing the effects of the *kilichi* category, the process applied and their interaction on the formation of PAHs

Variable		Sum Sq	Mean Sq	NumDF	DenDF	F	Prob	
BbF	Category of *kilichi* (C)	5.90	5.90	1.00	48.00	0.44	0.509	ns
	Process (P)	489.26	122.32	4.00	48.00	9.19	0.000	***
	PxC	63.06	15.77	4.00	48.00	1.18	0.330	ns
BaA	Category of *kilichi* (C)	221.81	221.81	1.00	18.00	4.43	0.496	*
	Process (P)	1747.06	436.76	4.00	18.00	8.73	0.000	***
	PxC	172.57	43.14	4.00	18.00	0.86	0.505	ns
CHR	Category of *kilichi* (C)	3.11	3.11	1.00	20.00	0.10	0.755	ns
	Process (P)	1528.00	382.00	4.00	20.00	12.33	0.000	***
	PxC	107.29	26.82	4.00	20.00	0.87	0.501	ns
BaP	Category of *kilichi* (C)	0.01	0.01	1.00	48.00	0.00	0.977	ns
	Process (P)	406.24	101.56	4.00	48.00	11.09	0.000	***
	PxC	35.11	8.78	4.00	48.00	0.96	0.439	ns
Sum PAHs	Category of *kilichi* (C)	55.00	54.50	1.00	48.00	0.04	0.837	ns
	Process (P)	45232.00	11307.90	4.00	48.00	8.87	0.000	***
	PxC	5406.00	1351.50	4.00	48.00	1.06	0.387	ns
2PAHs	Category of *kilichi* (C)	3.20	3.21	1.00	48.00	0.04	0.835	ns
	Process (P)	3806.70	951.68	4.00	48.00	13.09	0.000	***
	PxC	285.20	71.30	4.00	48.00	0.98	0.427	ns
4PAHs	Category of *kilichi* (C)	215.70	215.70	1.00	48.00	0.60	0.444	ns
	Process (P)	15999.40	3999.90	4.00	48.00	11.06	0.000	***

	PxC	1411.20	352.80	4.00	48.00	0.98	0.430	ns
8PAHs	Category of *kilichi* (C)	246.10	246.10	1.00	48.00	0.47	0.494	ns
	Process (P)	23194.60	5798.70	4.00	48.00	11.18	0.000	***
	PxC	2082.50	520.60	4.00	48.00	1.00	0.415	ns

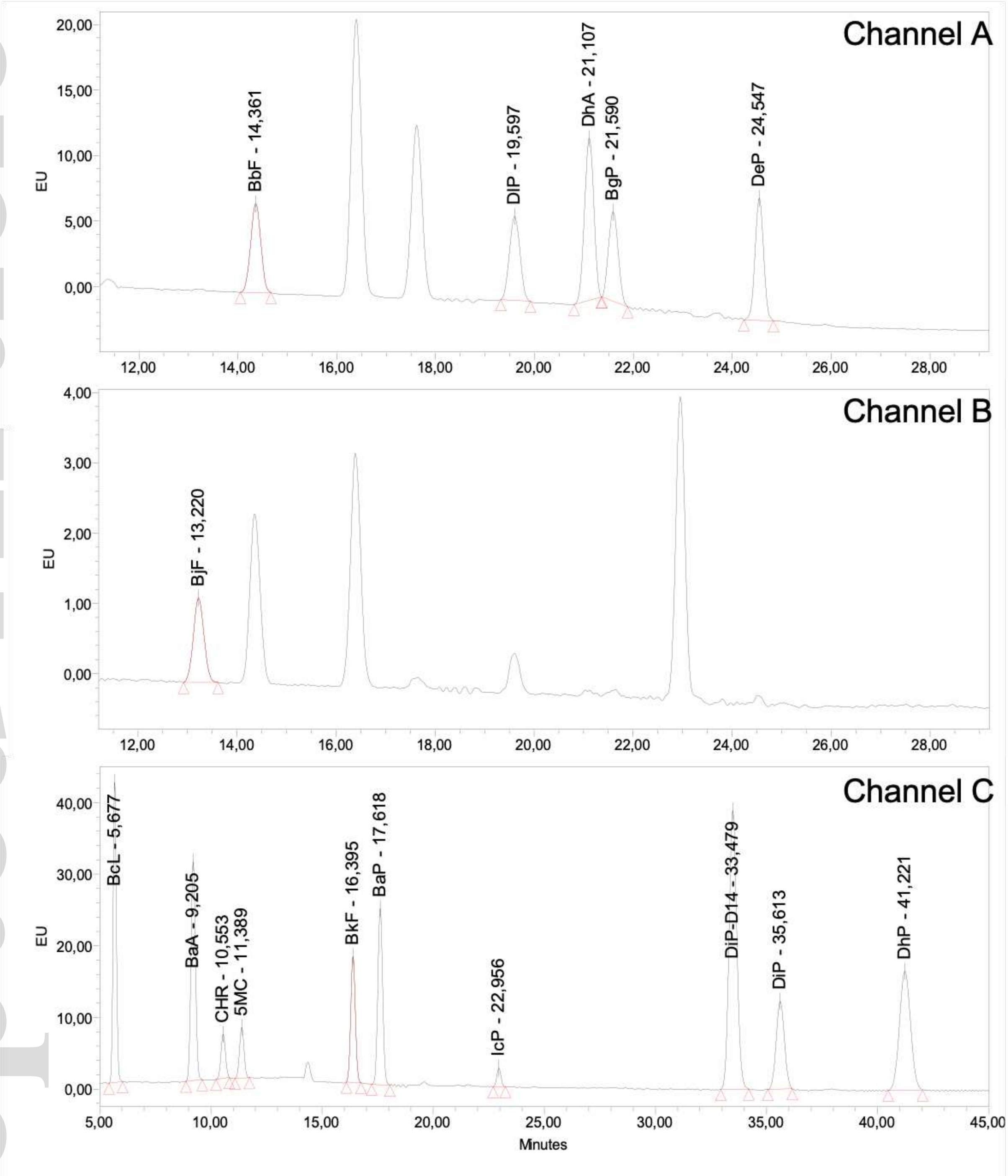


Figure 1 Chromatograms (HPLC-fluorescence) obtained for the 15 PAHs in a reference solution (1 µg/kg PAH except for IcP and BjF: 2 µg/kg PAH) acquired with the A, B and C channels of the fluorescence detector.

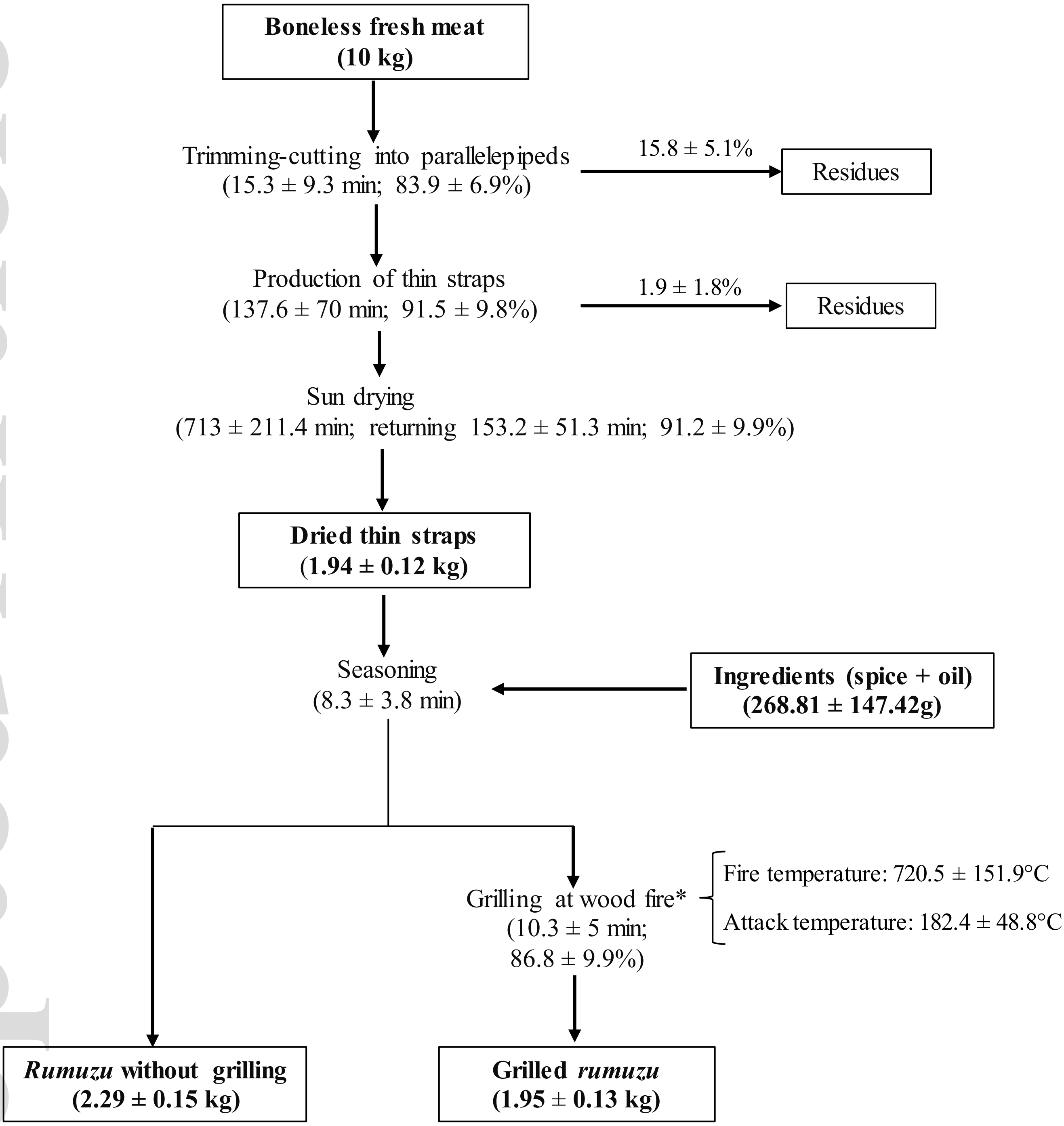


Figure 2 Flow diagram for *rumuzu* production indicating risk of PAHs contamination (*)

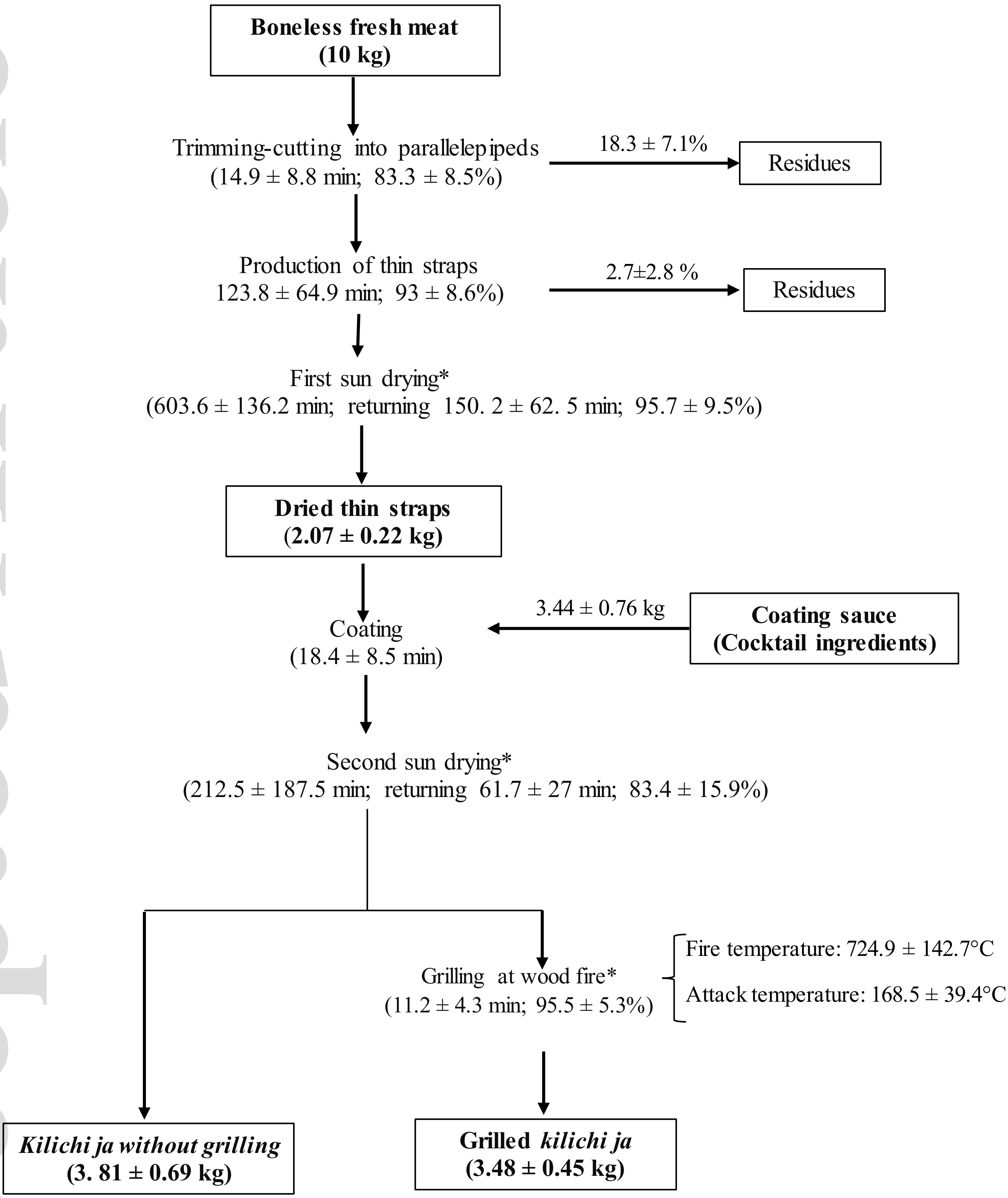
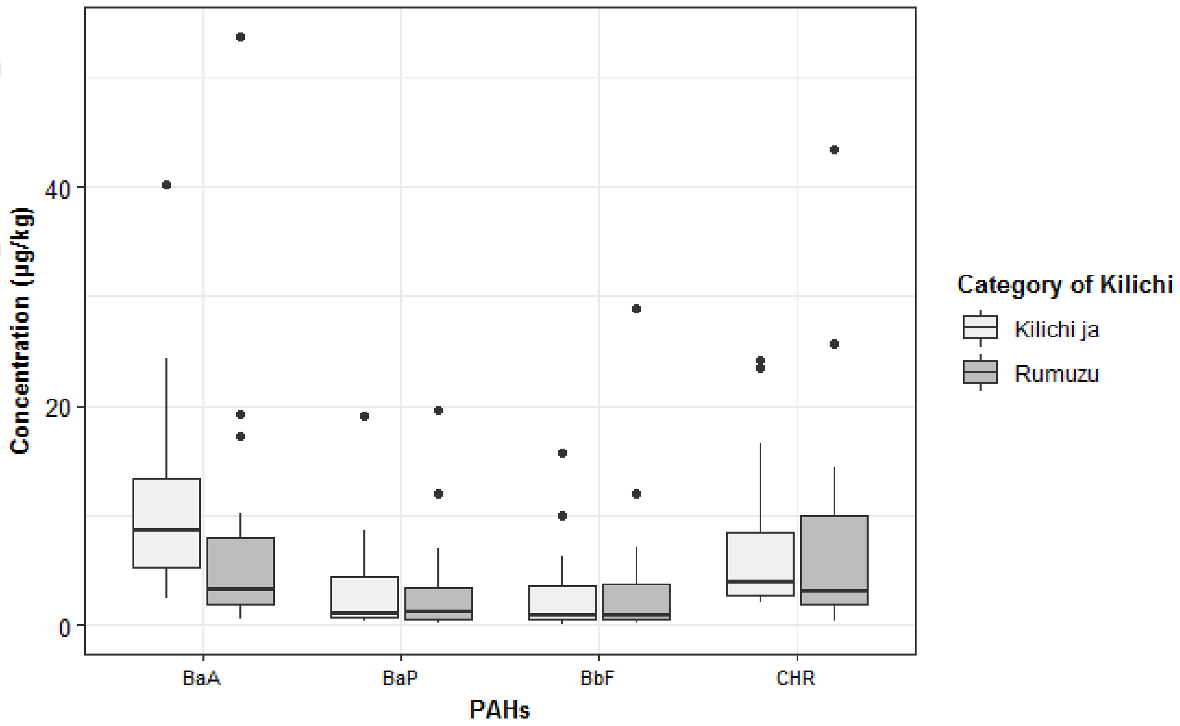
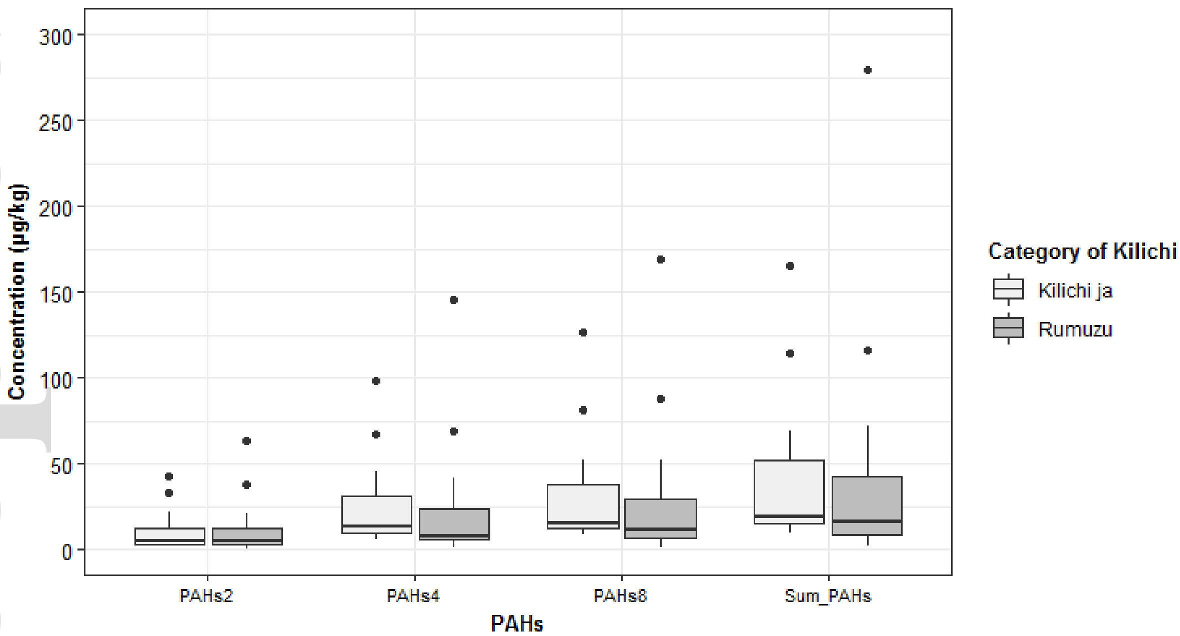


Figure 3 Flow diagram for *kilichi ja* production indicating risk of PAHs contamination (*)



A**.** Boxplots of the PAHs (BaA, BaP, BbF, and CHR) in *rumuzu* and *kilichi ja*.



B. Boxplots of the PAH2, PAH4, PAH8 and sum PAHs in *rumuzu* and *kilichi ja*.

Figure 4. Distribution of PAHs contaminations in *rumuzu* and *kilichi ja*.

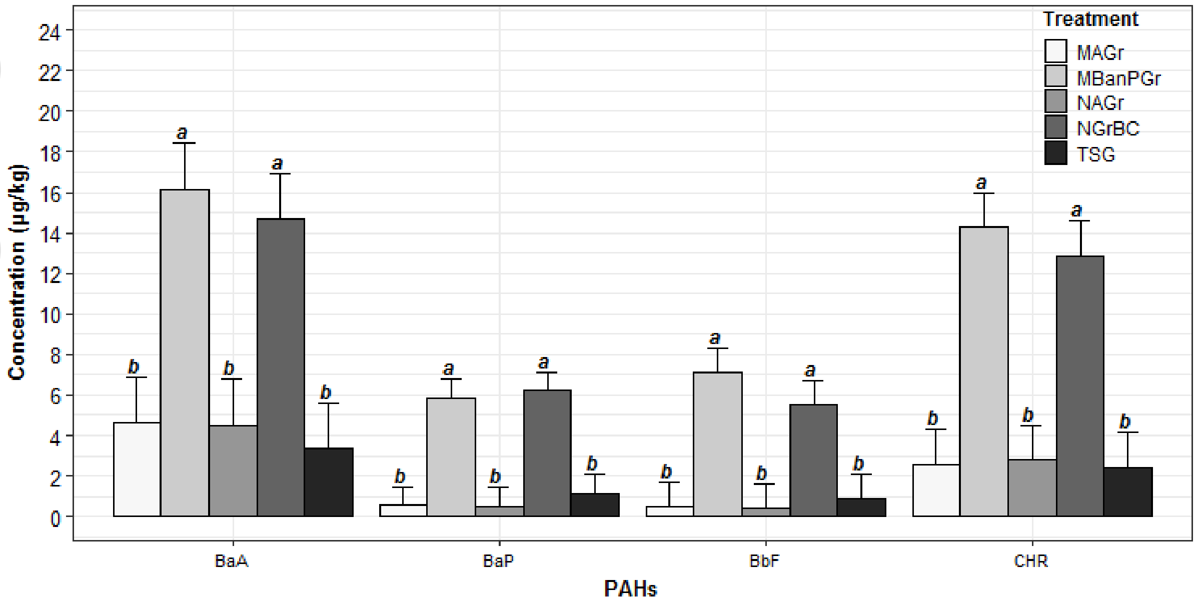


Figure 5. Effects of treatment on the formation of BaA, BaP, BbF and CHR

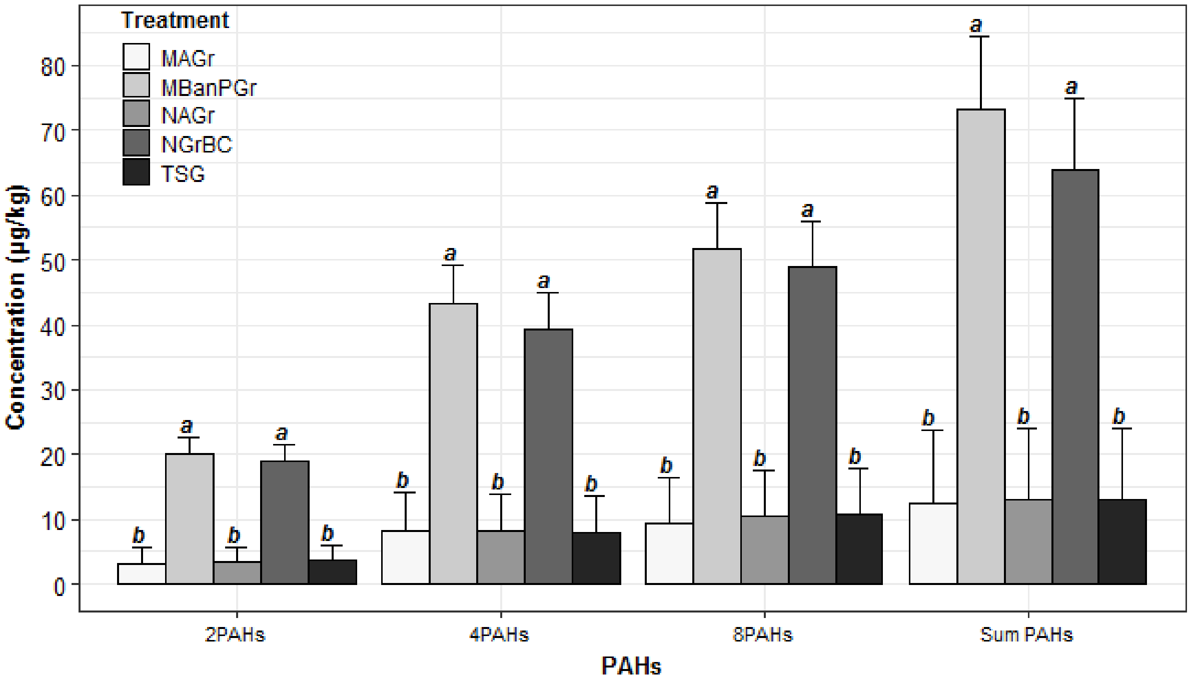


Figure 6. Effects of treatment on the formation of PAH2, PAH4, PAH8 and Sum of PAHs