Determination of polycyclic aromatic hydrocarbons (PAHs) by liquid chromatography-tandem mass spectrometry in smoked bacon

Thais C. Merlo¹, Luciano Molognoli², Heitor Daguer², Erick M. Saldaña¹, Carolina N. Aroeira¹, Mariana D.B. Dargelio¹ and Carmen J. Contreras-Castillo¹

¹Escola Superior de Agricultura Luiz de Queiroz, USP/Esalq, Av. Pádua Dias 11, 13418-900 Piracicaba/SP, Brazil
²Ministério da Agricultura, Pecuária e Abastecimento, Laboratório Nacional Agropecuário (SLAV/SC/Lanagro/RS), Rua João Grumiché 117, 88102-699 São José/SC, Brazil

*Corresponding author email: ccastill@usp.br

I. INTRODUCTION

The process of smoking meat products is an ancient food preservation technique which influences the color, odor and flavor of these products [1,2]. Traditionally, the process smoking involves from burning wood or addition of liquid smoke. However, undesirable compounds such as polycyclic aromatic hydrocarbons (PAHs) can be produced during the traditional smoking process due to incomplete combustion or by the thermal decomposition of organic compounds (wood) [3,4]. PAHs are divided into groups: group 1 are considered carcinogenic, such as benzo[a]pyrene (BaP) and group 2B are possibly carcinogenic, among them benzo[a]anthracene (BaA), benzo[b]fluorene (BbF) and chrysene (Chry) [5]. The sum content of four PAHs (ΣPAH4) has been used as indicator of the occurrence of PAHs in food. Thus, the objective of this study was to analyze the concentration of BaP, BaA, BbF and Chry in smoked bacons through the woods from reforestation and the addition of liquid smoke.

II. MATERIALS AND METHODS

Three samples of bacon were selected: one negative control (unsmoked bacon), smoked bacon with wood specie (Acacia mearnsii) and artificially smoked bacon (liquid smoke), abbreviated C, AM and LS, respectively. The extraction method was carried out, the samples were firstly hydrolyzed (1,5 mol L⁻¹ KOH methanolic), they were filtered and subjected to the liquid-liquid extraction (C₆H₁₄/CH₃OH/CH₃COOH) (80:20:0,05, v/v). The upper layer was freezing (- 80°C), centrifuged (4000 rpm, 4°C, 10 min) and concentrate the eluate to dryness with nitrogen stream (30°C). Then they were dissolved the dried concentrate in 1 mL of acetonitrile with 0,1% of formic acid and 1 mL was transferred to a vial for LC-MS/MS analysis. The LC-MS/MS analyzes were carried out in the 5500 QTrap hybrid triple quadrupole-linear ion trap-mass spectrometer from Sciex (Framingham, USA), equipped with ESI (electrospray ionization) and APCI (atmospheric pressure chemical ionization) sources. The mass spectrometer was coupled to the 1290 Infinity high performance liquid chromatography binary pump from Agilent Technologies, Deutschland GmbH (Waldbronn, Germany). The study was a randomized block design, with two blocks (each block corresponding to an independent bacon processing). And analysis of variance (ANOVA) was carried out to analyze the results and the comparisons of treatments were performed by the Tukey’s test (P < 0.05), using R environment (R Core Team, 2017) [6].

III. RESULTS AND DISCUSSION

From Table 1, it is apparent that the concentration of the compound BaP and ΣPAH4 were below at the all treatments. The maximum limit for BaP and ΣPAH4 in smoked meat products is 2 μg/kg and 12 μg/kg, respectively [7]. The C treatment not demonstrated the presence of PAHs as expected and the Chry was the only compound with significant difference (P < 0.05) between both treatments (AM and LS). In Brazil, LS is widely used in smoked meat products, it has some advantages in relation to traditional smoking, such as the reduction of smoking time, environmentally friendly and at the production of LS there is the possibility of the reduction toxic or carcinogenic compounds as the PAHs [8]. The Acacia mearnsii wood used in this study is considered like hardwood used for the smoking process, which have a low amount of resin,
influencing the low concentrations of PAHs. According Stumpe-Viksna [1], the softwood contains larger amounts of resin which can intensify the soot production and thus influence the higher production of PAHs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BaP</th>
<th>BaA</th>
<th>BbF</th>
<th>Chry</th>
<th>ΣPAH4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>&lt; 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LS</td>
<td>0.21±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.63±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AM</td>
<td>0.12±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.74±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.89±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ab</sup> Different letters in the same column differ significantly (P < 0.05) by the Tukey’s test.

IV. CONCLUSION

The use of *Acacia mearnsii* wood and liquid smoke for the smoking process in bacons produce PAHs, however the concentrations are below the maximum limit established by legislation. It is important that the PAHs concentration decrease in smoked meat products, due to its carcinogenic potential.

ACKNOWLEDGEMENTS

The authors acknowledge the FAPESP (2016/15012-2) for the financial support, T.C. Merlo and C. N. Aroeira thanks CAPES for the scholarship and E. Saldaña thanks the CONCYTEC (Contract 104-2016-FONDECYT).

REFERENCES