

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**News insights for smoked bacon with different woods from
chemical and sensory perspectives**

Thais Cardoso Merlo

Thesis presented to obtain the degree of Doctor
in Science. Area: Food Science and Technology

**Piracicaba
2020**

**Thais Cardoso Merlo
Food Engineering**

News insights for smoked bacon with different woods from chemical and sensory perspectives

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Profa. Dra. **CARMEN JOSEFINA CONTRERAS CASTILLO**

Thesis presented to obtain the degree of Doctor
in Science. Area: Food Science and Technology

**Piracicaba
2020**

**Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP**

Merlo, Thais Cardoso

News insights for smoked bacon with diferent woods from chemical and sensory perspectives / Thais Cardoso Merlo. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - - Piracicaba, 2020.

93 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Defumação 2. HPAs 3. Compostos orgânicos voláteis 4. Antioxidante 5. Antimicrobiano I. Título

Aos meus pais Shirlei e Merlo, por todo amor, suporte e educação

ACKNOWLEDGEMENTS

Querido Deus, te agradeço por sempre me ouvir, abençoar, guardar e cuidar.

Quero agradecer aos meus pais Merlo e Shirlei, que sempre foram os meus maiores incentivadores desde criança, me ensinando o caminho do bem e a lutar pelos meus objetivos sempre com humildade e honestidade acima de tudo. Vocês são os meus heróis da vida real, amo vocês demais! Estendo os meus agradecimentos a toda minha família (avós, tios, primos) que sempre se fizeram presentes apesar da distância.

A minha orientadora, Profa. Dra. Carmen J. Contreras Castillo, por ter me acolhido em seu laboratório desde 2015, pela disponibilidade, paciência, acompanhamento e orientação durante a execução do projeto. Ao Prof. Dr. Erick Saldaña, por todo o conhecimento compartilhado, pela contribuição na qualidade científica da tese, pela paciência e amizade acima de tudo. Ao Prof. Dr. José Manuel Lorenzo, pela disponibilidade e acolhimento em seu laboratório em Ourense, Espanha.

Agradeço a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela bolsa de doutorado, a Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) por financiar o projeto intitulado: “Desenvolvimento de bacons defumados com madeiras reflorestadas: estudo de caracterização sensorial, alterações lipídicas e produção de hidrocarbonetos policíclicos aromáticos (HPAs)” (Contrato: 2016/15012-2). Ao Departamento de Agroindústria, Alimentos e Nutrição (LAN), Escola Superior de Agricultura “Luiz de Queiroz” – ESALQ (ESALQ) e Universidade de São Paulo (USP) por proporcionar estrutura física e acadêmica para a realização do doutorado.

Sem dúvida nenhuma quero agradecer a minha rede de apoio feminino mais incrível que eu poderia ter Mariana, Iliani, Samara, Beatriz, Alais, Ana Clara, Ana Luiza, Carol e Izabella, vocês me ensinaram o verdadeiro sentido da palavra “Sororidade”. Vocês sempre souberam me olhar com afeto quando mais precisei, trabalhando com respeito mútuo e muita união. Vocês me apoiaram independente das nossas diferenças e me ampararam sem julgamentos. Esta tese eu compartilho com vocês com muito orgulho, pois somos feitas de todas. Serei eternamente grata, meus anos morando em Pira não seriam os mesmos sem vocês. E, hoje, vocês não sabem

a falta que me fazem, espero que possamos nos encontrar em breve para colocar o papo em dia e dar muitas risadas. Amo vocês!

Quero também agradecer aos meus colegas de laboratório Giu, Mariana Marinho, Priscilla, Juan Dario, Giovani, Maria Clara, Yudi, Evelyn, Profa. Aline, Leticia, por todo companheirismo dentro e fora do laboratório.

Agradecer ao Heitor, Luciano, Rodrigo e todo o pessoal do Ministério da Agricultura, Pecuária e Abastecimento (Seção Laboratorial Avançada - SLAV - São José - SC) pela acolhida e por todo o ensinamento necessário para o desenvolvimento do método para a análise de HPAs.

Agradeço também aos estagiários Bruna e Matheus da UFSCar que me ajudaram nas análises. Obrigada por toda a disposição, conversas e muitas risadas. Vocês são especiais!

Meu muito obrigada aos professores Dr. Cristian e Dra. Taciana, pela disponibilidade e paciência para me auxiliar no delineamento e análise de dados.

Quero agradecer ao Centro Tecnológico da Carne (CTC), especificamente ao grupo do laboratório de cromatografia (Fatima, Paula, Laura, Patrícia, Olaja, Raquel, Aurora e Giuseppe) por me receber tão bem e permitir aprender em suas instalações. Quero agradecer especialmente ao grupo de brasileiros Paulo, Larissa, Elisa, Allan, Julliane, Marcio e Maristela por me proporcionarem tantos momentos bons, os quais fizeram total diferença na minha estadia em Ourense.

Finalmente, a todos os professores e funcionários do Departamento de “Agroindústria, Alimentos e Nutrição” da “Escola Superior de Agricultura Luiz de Queiroz” da “Universidade de São Paulo” por me proporcionar todo o suporte necessário para o desenvolvimento da tese.

“Conheça todas as teorias, domine todas as técnicas, mas ao tocar uma alma humana, seja apenas outra alma humana”

Carl Jung

CONTENTS

RESUMO.....	10
ABSTRACT	11
1. GENERAL INTRODUCTION AND OBJECTIVES.....	13
2. ALTERNATIVE PRESSURIZED LIQUID EXTRACTION USING A HARD CAP ESPRESSO MACHINE FOR DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SMOKED BACON.....	17
Abstract.....	17
2.1. Introduction	17
2.2. Material and Methods.....	19
2.2.1. Standards, reagents, and samples	19
2.2.2. PAHs extraction.....	21
2.2.3. LC-MS/MS analysis	22
2.2.4. Single-laboratory validation approach.....	23
2.2.4.1. Selectivity	23
2.2.4.2. Linearity, sensitivity and matrix effect.....	24
2.2.4.3. Recovery and precision.....	24
2.2.4.4. Analytical limits.....	24
2.2.4.5. Statistical analysis.....	25
2.2.5. Method applicability	25
2.3. Results and discussion.....	25
2.3.1. Extraction method and LC-MS/MS analysis	25
2.3.2. Method performance evaluation	29
2.3.3. Method applicability	33
2.4. Conclusion	33
Acknowledgements	34
References.....	34
3. EFFECT OF THE SMOKING PROCESS USING BRAZILIAN REFORESTATION WOODS ON LIPID OXIDATION, MICROBIOLOGICAL AND HEDONIC QUALITY OF BACONS DURING SHELF LIFE.....	39
Abstract.....	39
3.1. Introduction	39
3.2. Material and methods.....	40

3.2.1. Material	40
3.2.2. Bacon manufacture	41
3.2.3. Chemical analysis of bacon.....	42
3.2.3.1. pH.....	42
3.2.3.2. Peroxide value (PV).....	42
3.2.3.3. Determination of thiobarbituric acid reactive substances (TBARS).....	43
3.2.4. Microbiological analysis	43
3.2.5. Overall liking.....	44
3.2.6. Statistical analysis.....	44
3.3. Results and discussion	45
3.3.1. pH	45
3.3.2. Lipid oxidation changes during storage	47
3.3.3. Microbial changes during storage	49
3.3.4. Overall liking.....	50
3.4. Conclusions	52
Acknowledgements.....	52
References	52
4. FORMATION PATHWAYS OF FLAVOR IN SMOKED BACON DURING STORAGE: RELATIONSHIP BETWEEN VOLATILE ORGANIC COMPOUNDS, FREE AMINO ACIDS AND SENSORY PROFILE	59
Abstract	59
4.1. Introduction.....	59
4.2. Material and Methods	61
4.2.1. Bacon samples.....	61
4.2.2. Volatile organic compounds profile (VOCs).....	62
4.2.3. Free amino acid profile.....	63
4.2.4. Microbiological analysis	63
4.2.5. Sensory analysis	64
4.2.6. Statistical analysis.....	65
4.3. Results and discussion	65
4.3.1. VOCs profile.....	65
4.3.2. Free amino acid profile.....	72
4.3.3. Overall liking and Rate-all-that-apply	76

4.3.4. Relationship between volatile organic compounds, free amino acids and sensory characteristics	80
4.4. Conclusion	82
Acknowledgments	82
References	82
5. GENERAL CONCLUSION	93

RESUMO

Novas descobertas para bacon defumado com diferentes madeiras do ponto de vista químico e sensorial

O bacon é um produto de carne de porco curado e defumado. A defumação pode ocorrer de duas maneiras, pela queima da madeira ou então pela aspersão de fumaça líquida. Vale ressaltar que a defumação feita com madeira deve empregar espécies reflorestadas, com o intuito de preservar a flora nativa nacional e assim ser ambientalmente amigável. Devido às diferenças na composição das madeiras, os compostos gerados na pirólise podem ser diferentes, formando fenóis, carbonilas, ácidos orgânicos e aldeídos. São estes compostos que dão as características ao bacon, como a cor, sabor e o odor desejável, além de contribuírem para a inibição da oxidação lipídica e redução do crescimento de microrganismos, colaborando para a vida útil do produto. Neste contexto, o objetivo geral deste estudo foi analisar o efeito da queima de diferentes madeiras de reflorestamento sobre a qualidade toxicológica, físico-química e sensorial de bacons. A tese foi dividida em três estudos, o primeiro capítulo foi baseado na análise de compostos carcinogênicos, como os hidrocarbonetos policíclicos aromáticos (HPAs) produzidos pela queima de diferentes madeiras de reflorestamento. O segundo estudo objetivou analisar a vida útil de bacon defumado através de análises físico-químicas, microbiológicas e sensoriais durante o armazenamento refrigerado. E o último estudo buscou entender a formação das características sensoriais através de compostos voláteis e não voláteis. Finalmente, a partir deste estudo, foi possível produzir um bacon defumado com concentrações de HPAs dentro dos limites determinados pela legislação, características físico-químicas e microbiológicas adequadas e perfil sensorial desejado pelos consumidores, ou seja, um produto cárneo saudável e proveniente de uma produção sustentável contribuindo com a indústria brasileira.

Palavras-chave: Defumação, HPAs, Compostos orgânicos voláteis, Antioxidante, Antimicrobiano

ABSTRACT

News insights for smoked bacon with different woods from chemical and sensory perspectives

Bacon is a cured and smoked pork meat product. The smoking can be performed by the direct burn of wood or by aspersion of liquid smoke. The wood smoking requires the use of reforested woods, to be environmentally friendly, and to promote national native flora preservation. Due to different wood compositions, the smoke compounds vary, producing substances like phenols, carbonyls, organic acids, and aldehydes. These compounds contribute positively to bacon quality, providing good taste, color, and desirable flavor, inhibiting lipid oxidation, and decreasing the development of microorganisms, which improves the chemical quality and increases the shelf life of this product. Thus, the general objective of this work was to analyze the effect of burning different reforested woods on the toxicological, physical-chemical, and sensory quality of bacon. The thesis was divided into three studies; the first chapter of this work aimed to analyze carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs) produced by burning wood. The second study aimed to analyze the shelf life of smoked bacon through physical-chemical, microbiological, and sensory analyzes during refrigerated storage. In addition, the last study sought to understand the formation of sensory characteristics through volatile and non-volatile compounds. Finally, as of this study, it was possible to produce smoked bacon with PAHs concentrations within the limits determined by legislation, with appropriate physicochemical and microbiological characteristics, and a sensory profile desired by consumers, that is, a healthy meat product from a sustainable production, contributing to the Brazilian industry.

Keywords: Smoking, PAHs, Volatile organic compounds, Antioxidant, Antimicrobial

1. GENERAL INTRODUCTION AND OBJECTIVES

The consumption of pork occurs mainly in industrialized forms, such as sausages and smoked products. The processing of meat influences cost and shelf life, which can be reduced and increased, respectively (FAO, 2014). Smoked bacon is one of the most consumed pork meat products in the world, being a product of high added value for industries. Industries are continually looking for new technologies and ingredients to develop quality products that meet the needs of consumers (Saldaña et al., 2020; Knipe & Beld, 2014).

Smoked bacon can be mainly obtained from pork belly; it is mandatory to use some ingredients, such as sodium chloride and sodium nitrite/sodium nitrate in the brine, after which, it's submitted to the smoking process (Brasil, 2000; Knipe & Beld, 2014).

The smoking process is one of the oldest technologies for processing and preserving meat products (Stumpe-Viksna et al., 2008) and results from the incomplete burning of wood, which releases aromatic (carbonyl and phenolic), antimicrobial (alcohols, ketones, formic acid, acetic acid) and antioxidants (pyrogallol, resorcinol, 4-methyl-guaiacol, 4-vinyl- guaiacol, guaiacol, syringol, 4-methyl-syringol, and 4-vinyl-syringol) compounds (Ogbadu, 2014). These volatile organic compounds (VOCs) influence the physical-chemical, microbiological, and sensory characteristics of smoked products (Alén, Kuoppala, & Oesch, 1996; Sikorski & Kołakowski, 2010). This process can also be carried out with liquid smoke, resulting from wood pyrolysis. Liquid smoke can also be distilled in order to remove toxic substances, such as polycyclic aromatic hydrocarbons (PAHs), providing greater toxicological safety to smoked bacon (Sikorski & Kołakowski, 2010).

However, since 2012 in Brazil, the new regulation restricted the use of the word "smoked" only for products smoked by wood pyrolysis (Brasil, 2012). This was the main reason for studying the changes in bacon processing, taking into account mainly the smoking type and the variety of woods that could be used for this purpose.

Thus, this study considered the use of reforested wood for smoking, because the last statistical yearbook of the Brazilian tree industry showed that for each hectare (ha) of planted forest, 0.7 ha of native flora was preserved (Ibá, 2017). In Brazil, *Eucalyptus* (*Eucalyptus* spp.) is one of the most used woods for this purpose (Luz, 2013), and

Acacia mangium, *Acacia mearnsii*, *Tectona grandis*, and *Mimosa scabrella* can also be used. However, the literature does not present in-depth studies regarding the influence of smoking with these woods on the formation of carcinogenic compounds, lipid and microbiological changes, and the formation of sensory characteristics.

In this context, the present thesis is organized into three chapters. In the first chapter, a new methodology was studied for extracting, identifying and quantifying PAHs using an espresso machine approach followed by coupled to mass spectrometry in tandem mode (LC-MS/MS). The second chapter discusses how smoking with reforestation woods influences parameters that determine the shelf life of the product. In the third and final chapter of this thesis, the smoked bacon that showed the best results in the previous chapter was chosen to be analyzed from a sensory point of view, that is, on how volatile organic compounds and free amino acids influence the flavor of this type of product.

For that, some specific objectives were intended:

- Develop and validate a methodology to identify and quantify PAHs in smoked bacon (Chapter 1);
- To evaluate the influence of smoking using two types (*Eucalyptus citriodora* and *Acacia mearnsii*) on lipid oxidation, microbiological changes and sensory status of bacon stored under refrigeration (Chapter 2);
- To correlate the volatile organic compounds and free amino acids in the formation of sensory characteristics (flavor) of smoked bacon with eucalyptus and to understand the consumer perception concerning these characteristics (Chapter 3).

References

- Alén, R., Kuoppala, E., & Oesch, P. (1996). Formation of the main degradation compound groups from wood and its components during pyrolysis. *Journal of Analytical Applied Pyrolysis*, 36, 137-148. [https://doi.org/10.1016/0165-2370\(96\)00932-1](https://doi.org/10.1016/0165-2370(96)00932-1).
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 21, de 31 de julho de 2000. Regulamentos Técnicos de Identidade e Qualidade de Patê, de Bacon ou Barriga Defumada e de Lombo Suíno. Diário Oficial da União, Brasília, 03 de agosto de 2000, Seção 1.

- Brasil. Ministério da Agricultura, Pecuária e Abastecimento-MAPA. Memorando 095/2012/DICS/CGI/DIPOA, de 27 de julho de 2012. Esclarecimento quanto ao procedimento de defumação de produtos cárneos e revisão de rotulagem de produtos defumados, 2012.
- FAO (Food Agriculture Organization). (2014). Agriculture and Consumer Protection Department. Meat & Meat products. Retrieved from <http://www.fao.org/ag/againfo/themes/en/meat/home.html>. Accessed date: 25 February 2020.
- Indústria Brasileira De Árvores – IBÁ. (2017). Relatório anual 2017, ano base 2016. Brasília: IBÁ.
- Knipe, C. L., & Beld, J. (2014). BACON PRODUCTION | Bacon BT - Encyclopedia of Meat Sciences (Second Edition) (pp. 53–57). Oxford: Academic Press. <https://doi.org/https://doi.org/10.1016/B978-0-12-384731-7.00110-0>.
- Luz, R. L. F. (2013). Hidrocarbonetos policíclicos aromáticos (HPAs) em queijos defumados e em queijos assados em churrasqueiras. Retrieved from http://repositorio.ufla.br/jspui/bitstream/1/1675/2/DISSERTACAO_Hidrocarbonetos%20polic%c3%adclicos%20arom%c3%a1ticos....pdf. Accessed date: 06 March 2020.
- Ogbadu, L. J. (2014). PRESERVATIVES | Traditional Preservatives – Wood Smoke - Encyclopedia of Food Microbiology (Second Edition) (pp. 141-148). London: Elsevier. <https://doi.org/10.1016/B978-0-12-384730-0.00261-5>.
- Saldaña, E., Martins, M. M., Behrens, J. H., Valentin, D., Selani, M. M., & Contreras-Castillo, C. J. (2020). Looking at non-sensory factors underlying consumers' perception of smoked bacon. *Meat Science*, 163, 108072. <https://doi.org/10.1016/j.meatsci.2020.108072>.
- Sikorski, Z. E., & Kołakowski, E. (2010). Smoking. In F. Toldrá (Ed.), *Handbook of Meat Processing* (pp. 231–245). Oxford: Wiley-Blackwell. <https://doi.org/10.1002/9780813820897.ch12>.

2. ALTERNATIVE PRESSURIZED LIQUID EXTRACTION USING A HARD CAP ESPRESSO MACHINE FOR DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SMOKED BACON

This chapter was published in *Food Control*.

doi: 10.1016/j.foodcont.2020.107565.

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are noxious compounds that can be found in some foods, especially those submitted to the traditional smoking process. In this study, a novel pressurized liquid extraction based on hard cap espresso machine followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was developed to determine PAHs in smoked bacon. Method performance was evaluated in accordance to the Commission Decision 2002/657/EC in terms of selectivity, linearity, sensitivity, matrix effect, recovery and detection capability (CC_{β}). Linearity was satisfactory with regression coefficients (R^2) higher than 0.98. Recoveries ranged from 73.9 to 99.9%. CC_{β} values ranged from 0.30 to 0.90 $\mu\text{g kg}^{-1}$, values below the maximum limits suggested by Commission Regulation 835/2011, which propose 2.0 $\mu\text{g kg}^{-1}$ for benzo[a]pyrene and 12.0 $\mu\text{g kg}^{-1}$ for sum of the four priority PAHs. The method was applied to real samples, with PAHs levels below these limits. The developed method proved to be a fast and cheap approach for the efficient and reliable determination of PAHs in smoked bacon.

Keywords: Carcinogenic compounds; Smoking; Benzo[a]pyrene; LC-MS; Matrix effect

2.1. Introduction

Nowadays, one of the consumer's main concern is understand how certain food will influence their health. Recently, the worries regarding meat and processed foods containing meat are increasing. Meat products are important for the diet due to their nutritional content, but during culinary preparations (by grilling, frying, roasting) and industrial processing (by drying and smoking) carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs) (Bansal & Kim, 2015) can be formed.

PAHs are generated from incomplete combustion processes. They are cyclic hydrocarbons composed strictly by hydrogen and carbon with alternating single and

double bonds, forming two or more condensed aromatic rings. PAHs are potentially carcinogenic and mutagenic (Al-Rashdam et al., 2010; Bertinetti et al., 2018). PAHs that contain up to four aromatic rings are classified as light and volatile; PAHs that contain more than four aromatic rings in their structure, are less volatile and classified as heavy compounds. Heavy PAHs are characterized by their greater stability and toxicity, being found in several food matrices (Olatunji et al., 2014).

In the smoking process, PAHs can be formed due to incomplete combustion or by the thermal decomposition of organic compounds from the wood (Hitzel et al., 2013). Fragments of molecules and free radicals generated by this process react to form firstly the light and then the heavy PAHs, which are retained by fat-rich parts of foods, due to their high hydrophobicity (Bansal & Kim, 2015; Ledesma, Rendueles, & Díaz et al., 2016).

The level of PAHs in smoked products depends on several factors, such as the type of wood using in smoking, the temperature of wood-burning during combustion, the concentration of oxygen, and speed of ventilation inside the smoking chamber (Hitzel et al., 2013). Among these factors, the type of wood has a great influence on the production of PAHs in smoked foods. It is reported that smoking with softwoods (spruce, fir and pine) tends to produce high concentrations of high molar mass PAHs compared to smoking with hardwoods (beech, acacia, eucalyptus) (Stumpe-Viksna et al., 2008; Hitzel et al., 2013; Malarut & Vangnai, 2018).

PAHs are represented by a large number of compounds. The most important is benzo[*a*]pyrene (BaP), due to its elevated toxicity. BaP is classified by the International Agency for Research on Cancer (IARC) as belonging to group 1 (carcinogenic to humans), followed by benzo[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF) and chrysene (Chry), which were classified by the IARC as belonging to group 2B (possibly carcinogenic to humans) (IARC, 2010). Thereafter, regulatory agencies around the world set regulatory limits for PAHs in various types of foods. In the European Union, the maximum allowed limit for BaP has been reduced to 2 $\mu\text{g kg}^{-1}$ in 2014. Similarly, the sum of the four main PAHs was set to 12 $\mu\text{g kg}^{-1}$ (EC, 2011).

Thus, the determination of PAHs in smoked meat products is an important issue for guarantee the consumer's health and food safety. The extraction of PAHs from meat products has been carried out by classical methodologies, such as liquid-solid extraction (Gosetti et al., 2011) and saponification (Lee et al., 2016; Malarut & Vangnai, 2018), and also by alternative methods such as pressurized liquid extraction

(Pöhlmann et al., 2013), accelerated solvent extraction (Wagas et al., 2014), and sonication (De Lima et al., 2017). Analytical determination of PAHs is generally achieved by gas chromatography (GC) with flame ionization detectors (FID) or coupled to mass spectrometry (GC-MS). Less extensively, PAHs can be also determined by high performance liquid chromatography (HPLC) with fluorescence detection (HPLC-FD) and coupled to mass spectrometry in single (LC-MS) or tandem mode (LC-MS/MS).

However, some of those methods require a long extraction times, high solvent consumption and decomposition of thermolabile compounds (Martinez-Sena et al., 2017). Thus, despite the great advances in the analytical methodologies of PAHs, new methods of extraction, identification and quantification of these compounds are needed, aiming at the development of reproducible analytical methods, with high sensitivity and reliability of its results (Molognoni et al., 2019).

In this regard, the use of espresso machines has been studied for the extraction of several organic compounds to reduce the extraction time and amount of organic solvent used. This approach reduces the cost of acquiring laboratory instrumentation, bringing savings to high-frequency analytical laboratories (Gallart-Mateu et al., 2017). This device was initially studied by Armenta, De La Guardia, & Esteve-Turrillas (2016) for the extraction of PAHs from soil and sediments samples, whom obtained satisfactory results. However, no other studies reporting the adaption of this extraction technique to meat products with high-fat content was found.

Considering this scenario, the main goal of this study was to develop a methodology for extracting low concentrations of PAHs from smoked bacon with the aid of a hard cap espresso machine. The analytes were determined by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The proposed method was validated in terms of selectivity, linearity, precision, recovery, matrix effect, limits of detection and quantification, and detection capability.

2.2. Material and Methods

2.2.1. Standards, reagents, and samples

Solvents (methanol, hexane, acetonitrile, ammonium acetate, formic acid, and acetic acid) of chromatographic grade were supplied by Merck KGaA (Darmstadt, Germany). An Integral 10 Milli-Q system (Millipore SAS, Molsheim, France) was used

to obtain ultrapure water (minimum 18.3 MΩ cm resistivity) and to prepare all aqueous solutions. Diatomaceous earth were from Honeywell/Fluka, with a density of 2.4 g cm⁻³ and grain size around 140 mesh.

Analytical standards were purchased from Sigma-Aldrich (St. Louis, USA) and included benzo[a]pyrene (≥ 96% purity) (CAS no. 50-32-8), benzo[b]fluoranthene (CAS no. 205-99-2) and chrysene (CAS no. 218-01-9). Benzo[a]anthracene was included in a PAHs Calibration Mix (Supelco) 10 µg mL⁻¹ of each component in acetonitrile, containing sixteen PAHs (naphthalene, acenaphthalene, acenaphene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[ah]anthracene, benzo[ghi]perylene and indeno (1, 2, 3-cd)pyrene. Stock solutions were prepared at 1000 µg mL⁻¹ in methanol from the individual PAHs standards and stored at -20 °C.

Five smoked bacon samples were used for method development and validation. Four different smoked bacon were processed in a meat processing pilot-plant. The smoked bacon samples have been prepared by injecting pork bellies with brine at 10% ratio in an automated injector (Super Inject Max Power Flavor, Stander model). Samples were vacuum-packed and left for 24 h at 5 °C for brine penetration. Smoked bacon was processed according to the procedures of Brazilian bacon production, as described in Table 1. The smoking time with different reforestation woods (*Acacia mearnsii*, *Acacia mangium*, *Mimosa scabrella*, and *Eucalyptus* sp) was 60 min with 75 °C of temperature. The product final contained the following ingredients in the concentrations shown below: 1.5% sodium chloride, 0.6% refined sucrose, 0.015% sodium nitrite, 0.3% sodium tripolyphosphate and 0.07% sodium erythorbate. A commercial sample obtained from a Brazilian industry under federal inspection was also used.

Table 1. Smoking process.

Process	Time/Local temperature	Equipment description
Drying	30 min/65 °C	Open chimney and high ventilation
Smoking	60 min/75 °C	Half open chimney and low ventilation
Steam cooking	30 min/75 °C	Closed chimney and high ventilation
Steam cooking	--/80 °C	Cooking until the temperature of the thermal center reached 75 °C

All samples were ground, homogenized using an automatic disk mill and stored in plastic bags at -20 °C until analysis.

2.2.2. PAHs extraction

A Nespresso U Milk D55 (Nespresso Magyarország, Budapest, Hungary) hard cap coffee machine was used for the extraction of PAHs from the smoked bacon samples, with a working pressure of 19 bar. The procedures of extraction were done according with the manufacturer instructions to a *ristretto* coffee preparation. As cell extraction, refillable plastic compatible capsules (EmoHome 04T, Zhejiang, China) were used. Firstly, the espresso machine was purged with the extraction solvent (water: acetonitrile, 80:20, v/v, acidified with 0.1% formic acid) using a capsule filled with the dispersant agent (diatomaceous earth). The same solvent was used before each different sample extraction to clean and pre-heat the system. The homogenized samples were weighed (5.0 ± 0.1 g) into 50-mL polypropylene centrifuge tubes (PCT). After that, diatomaceous earth (2.0 ± 0.2 g) was added to each tube. The tubes were shaken again to mix the dispersing agent with the sample. The mixture was then quantitatively transferred to an extraction capsule. Additional diatomaceous earth was added to fill any empty space into the capsule. The mixture was softly compressed with a pestle. After that, the capsule was inserted in the coffee machine and the extraction was performed. The extraction carried out by 10-15 s at 75 ± 2 °C. Then, the eluate (15 ± 2 mL) was collected into 50-mL clean PCT and were kept at -30 ± 5 °C for 30 min. After that, PCTs were submitted to centrifugation (3488 g, 4 °C, 5 min). The upper layer was collected in another PCT and 10 mL of hexane were added. The PCT was stirred for 10 min at 110 rpm. Afterwards, the hexane phase was kept in an ultra-freezer (-80 °C, 30 min), centrifuged (3488 g, 4 °C, 5 min), and concentrated to dryness in a water bath (50 °C) under a gentle nitrogen stream. Then, the dried residue was dissolved in 0.6 mL of acetonitrile with 0.1% of formic acid. Following, an aliquot of florisil was added (200 mg). This extract was stirred in a vortex for 30 s and centrifuged. The upper layer was collected in a microtube and centrifuged again. Finally, a volume of 0.5 mL was transferred to a glass vial for LC-MS/MS analysis.

2.2.3. LC-MS/MS analysis

The LC-MS/MS analyzes were carried out using a 1290 Infinity high-performance liquid chromatography (Agilent Technologies, Waldbronn, Germany) coupled to a 5500 QTrap hybrid triple quadrupole-linear ion trap-mass spectrometer from Sciex (Framingham, USA). The chromatographic separation was achieved with cyanopropyl as stationary phase (Zorbax 300 SB-CN 150 mm x 4.6 mm i.d., 5 μm , 300 \AA , from Agilent Technologies, Santa Clara, USA). The mobile phase was composed by an aqueous solution with 0.1% acetic acid (solvent A) and methanol with 0.1% acetic acid (solvent B). Ammonium acetate (5 mmol L^{-1}) was added to both phases. Gradient elution and instrument parameters were set as follow: 95% A (0-2 min), 15% A (2-4 min), 10% A (4-7 min), 95% A (7-8 min) and 3 min for auto-equilibrium. The mobile phase flow was 500 $\mu\text{L min}^{-1}$. The injection volume was 10 μL and the column was maintained under 40 $^{\circ}\text{C}$. Mass spectrometry analysis were carried out in tandem mode with electrospray ionization (ESI) source in positive mode (ESI⁺) The ionization parameters were as follows: ion spray (IS) voltage: 5500 V; curtain gas: 25 psi; nebulizer gas (GS1): 55 psi; auxiliary gas (GS2): 55 psi; source temperature: 400 $^{\circ}\text{C}$. Nitrogen was used for nebulization and collision. All analytes were evaluated using multiple reaction monitoring (MRM), with two m/z transitions monitored for each compound. The Analyst 1.6.2 and the MultQuant softwares (Sciex, Foster City, CA) were used for data acquisition and processing. Analyte-specific mass spectrometry parameters are described in Table 2.

Table 2. Values of the optimized parameters for monitoring multiple reactions (MRM) using electrospray ionization in positive mode (ESI +) for identification of PAHs by LC-MS/MS.

Analyte	Q1 (Precursor ion) (<i>m/z</i>)	Q3 (Fragment ion) (<i>m/z</i>)	DP (V)	EP (V)	CE (V)	CXP (V)
BaA 1 Q	229.057	226.1	91	10	65	12
BaA 2 I1	229.057	224.1	91	10	93	10
BaA 3 I2	229.057	225.2	91	10	83	12
Chry 1 Q	229.012	226	71	10	65	12
Chry 2 I1	229.012	227.9	71	10	53	14
Chry 3 I2	229.012	223.9	71	10	95	12
BaP 1 Q	252.946	250.1	161	10	73	14
BaP 2 I1	252.946	248	161	10	101	14
BaP 3 I2	252.946	224.1	161	10	93	12
BbF 1 Q	253.047	250.1	171	10	73	14
BbF 2 I1	253.047	248	171	10	101	12
BbF 3 I2	253.047	248.7	171	10	91	12

BaA: Benzo[a]anthracene; Chry: Chrysene; BaP: Benzo[a]pyrene; BbF: Benzo[b]fluoranthene.
 DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision cell exit potential.

2.2.4. Single-laboratory validation approach

The analytical validation followed the criteria established by the Commission Decision 657/2002/EC (European Commission, 2002) to assess selectivity, working range (linearity), precision (in terms of repeatability and intermediate precision), recovery, matrix effect, and detection capability ($CC\beta$) of the proposed method. Additionally, sensitivity in terms of limit of detection (LOD) and limit of quantification (LOQ) was also calculated.

2.2.4.1. Selectivity

Selectivity was evaluated by the analysis of at least 20 samples of smoked bacon. The selectivity evaluated the presence of possible endogenous and exogenous interferences from the matrices, as well as the occurrence of contaminations during analysis. The resulting chromatograms were evaluated for the presence of interfering peaks around the analytes retention times windows, after its establishing by injecting the standards in pure solvent. The retention times were compared in samples with and without fortification at $0.5 \mu\text{g kg}^{-1}$ of each analyte.

2.2.4.2. Linearity, sensitivity and matrix effect

Analytical curves were prepared in pure solvent and in blank matrix using external standardization with six concentration levels (including zero). The analytical curves were prepared by plotting the analytes concentrations (x-axis) *versus* the respective peak area (y-axis). Linearity was assessed by checking the reproducibility of the analytical curves (that ranged from 0 to 4 $\mu\text{g kg}^{-1}$ for each analyte). For this purpose, the measurement variable day of analysis ($n = 3$) was applied. The acceptance criterion was the mean of the regression coefficients (R^2) which should be greater than 0.98.

The LOD corresponded to the lowest response value for each analyte with acceptable reproducibility ($CV < 50\%$). The LOQ was set as the first point of the analytical curve with proven recovery and precision.

The matrix effect was evaluated according to Hoff et al. (2015) by comparing the derivatives of the lines obtained from matrix-matched and pure solvent calibration curves. It was considered that no matrix effects were present if the values ranged from 0.8 to 1.2.

2.2.4.3. Recovery and precision

Recovery and precision were determined by spiking blank samples. Four groups of three blank samples were fortified at 4.0 $\mu\text{g kg}^{-1}$, subjected to measurement variables such as days and samples. The results were obtained by interpolating the analytes peak areas in analytical curves prepared on the same day of each experiment. Recovery was considered acceptable if the recovery rate was among 50 % and 120%. Precision was considered acceptable if the coefficient of variation (CV) was lower than 30%.

2.2.4.4. Analytical limits

The detection capability (CC_β) was calculated through the expanded standard uncertainty ($k = 3.00$) to obtain positive results with a high probability of success (99%), within the limit of detection in error format, considering a rectangular distribution. Thus, the values could only be considered positive when they were higher than the CC_β value calculated for each analyte.

2.2.4.5. Statistical analysis

Statistics analysis of random uncertainties was performed after excluding outliers (Dixon test, 95%), the study of data randomness (scatter plot by points), mean and median (as conventionally true value) and standard deviation of the mean (random standard uncertainty, 68%). The variances related to variability and tendency to recovery (bias), were propagated and expanded considering the t-Student distribution (95%).

2.2.5. Method applicability

To verify the fit-for-purpose of the proposed method, five samples of smoked bacon were analyzed.

2.3. Results and discussion

2.3.1. Extraction method and LC-MS/MS analysis

Bacon is a matrix with high-fat content and is complex such any biological or food sample. Moreover, PAHs are very hydrophobic compounds. Thus, the extraction of PAHs of bacon samples are challenging, due to high affinity between analyte and matrix. In this case, we chosen the use of a technique based on pressurized liquid extraction, where the elevated pressure and temperature allow an increase in the solubility of the PAHs in the solvent. Besides that, the use of diatomaceous earth as adsorbent was selected to removal of matrix interferences with polar nature as well as fatty acids.

Hard cap espresso machine has been good applicability to extract organic compounds from different solid matrices associated with their low cost, easiness and fast (Leiman et al., 2018). The extraction method based on hard cap espresso machines was firstly reported for PAHs analysis by Armenta, De La Guardia, & Esteve-Turrillas (2016), although other compounds such as bioactive compounds, flavonoids, and cannabinoids have also been extracted from a wide variety of complex matrices using the same approach (Just et al., 2016; Martinez-Sena et al., 2017; Corell et al., 2018; Leiman et al., 2018). Thus, the hard cap espresso machine extraction method have been adapted to different analytes and matrices. Recently, some of the authors of the present study have been reported methods based on hard cap espresso

machine extraction for veterinary drugs in feeding tuffs (Hoff et al., 2020) and carcinogens compounds in meat (Molognoni et al., 2020).

In our study, the total time for the PAHs extraction from smoked bacon took less than 20 s, being considered a fast extraction method. High extraction temperature increases the solubility of solutes in the organic solvent, increasing extraction efficiency and recovery. However, temperatures above 300 °C are not recommended for the extraction of PAHs, because they can degrade analytes and extract undesirable components from the matrix (Mukhopadhyay, Dutta, & Das, 2020). Thus, the time and temperature employed in this study were adequate, resulting in good recoveries of the analytes.

Espresso machines were originally designed to extract coffee with hot water, which is not an adequate solvent for the extraction of non-polar organic compounds such as PAHs (Gallart-Mateu et al., 2017). In this way, the use of miscible organic solvents with water can improve the extraction efficiency of non-polar compounds (Just et al., 2015). Previously, the acetonitrile: water mixture was employed in a hard cap espresso machine for the PAHs extraction (Armenta, De La Guardia, & Esteve-Turrillas, 2016). The same authors reported that the use of acetonitrile as a solvent for extracting PAHs increased the analytes recovery. Thus, the concentration of acetonitrile varies according to the analyte to be extracted, ranging from low concentrations (for the extraction of smaller PAHs) to higher concentrations (for the extraction of more hydrophobic PAHs).

Despite its advantages such as speed, low cost, and easiness to operate, the use of hard cap espresso machines in sample preparation present some limitations. Critical parameters such as pression, temperature and extraction solvent volume are fixed, according to the manufacturer configuration. Even if pressure and solvent volume can be slightly changed according to a lower or a higher compression of the mixture sample plus adsorbent inside the capsule, we observed that the more the sample is compacted, the greater the frequency of extraction failures.

To aid the removal of fat content, the sample was firstly dispersed with an adsorbent. Considering the low cost and the ability to adsorb polar compounds such as free fatty acids and triglycerides, the chosen adsorbent was diatomaceous earth. Sea sand and activated carbon were also evaluated but these adsorbents did not promote an adequate matrix dispersion. Two densities of diatomaceous earth were evaluated: Honeywell/Fluka with a density of 2.4 g cm⁻³ and grain size around 140

mesh; Macherey-Nagel, with a density of 0.33 g cm^{-3} and grain size around 80 mesh. Both kinds have promoted similar results, but the first one (Honeywell/Fluka) was easier to disperse the matrix.

In this study, a mixture of water and acetonitrile (80:20, v/v) acidified with 0.1% formic acid was employed for the extraction of PAHs from smoked bacon. The solvent composition was optimized using different ratios of water and acetonitrile, with or without organic acid as modifier. The use of acetonitrile instead of methanol or other water-miscible organic solvents was chosen based on literature references and in the limited compatibility of the hard cap espresso machine (Gallart-Mateu et al., 2017; Armenta, De La Guardia, & Esteve-Turrillas, 2016; Hoff et al., 2020). Again, due to the machine limitations, just percentages of 0, 10, 20, and 30% of acetonitrile were evaluated. The use of pure water and 10% of acetonitrile resulted in lower recoveries. The results obtained with 30% were slightly better than those obtained with 20%. Thus, in order to avoid a decrease in the machine lifetime, the use of 20% of acetonitrile was chosen. The use of a slight amount of organic solvent in the extractant, provided acceptable recoveries with values ranging from 73.9 to 99.8%. Acetic and formic acids were evaluated, both at 0.05, 0.1 and 0.2%. The signal intensity was similar for both acids at 0.2 and 0.1%, with slightly cleaner supernatants obtained with formic acid. After the initial extraction, ultra-freezing and refrigerated centrifugation were used as clean-up, eliminating large amount of fat micelles and other co-extractives (Molognoni et al., 2018). The absence of the two steps of low temperature clean-up results in visible fat content after the extract evaporation. The use of hexane to perform a liquid-liquid extraction was useful to remove PAHs from the water: acetonitrile extract without the removal of matrix hydrophobic interferents, which were previously cleaned up by the hard cap espresso machine step. Ethyl acetate was also evaluated, but due to formation of emulsion between the phases, *n*-hexane was chosen. To remove residual fat after the evaporation of all solvent, a second adsorbent was used. To avoid the undesirable sorption of PAHs, just polar adsorbents were evaluated. The comparison between florisil and activated alumina have demonstrated that florisil use resulted in cleaner extracts. Thus, a small amount of florisil was used to remove some remaining co-extractives, which were perceived by the characteristic odor of wood in the final extract.

Chromatographic separation and mass spectrometry analysis (LC-MS/MS) were optimized to achieve maximum sensitivity in the quadrupole (Molognoni et al., 2018).

This set up allowed for the efficient differentiation of some mass isomers, such as BaA/Chry (m/z 228) and BbF/BaP (m/z 252).

The determination of PAHs in LC-MS/MS are often achieved using C18 columns and APCI or APPI ionization sources. However, we chosen the use of a cyano column, a reversed-phase able to rapidly separate hydrophobic compounds. Besides, the optimization of the target PAHs was successfully done even with the ESI source (Figure 1).

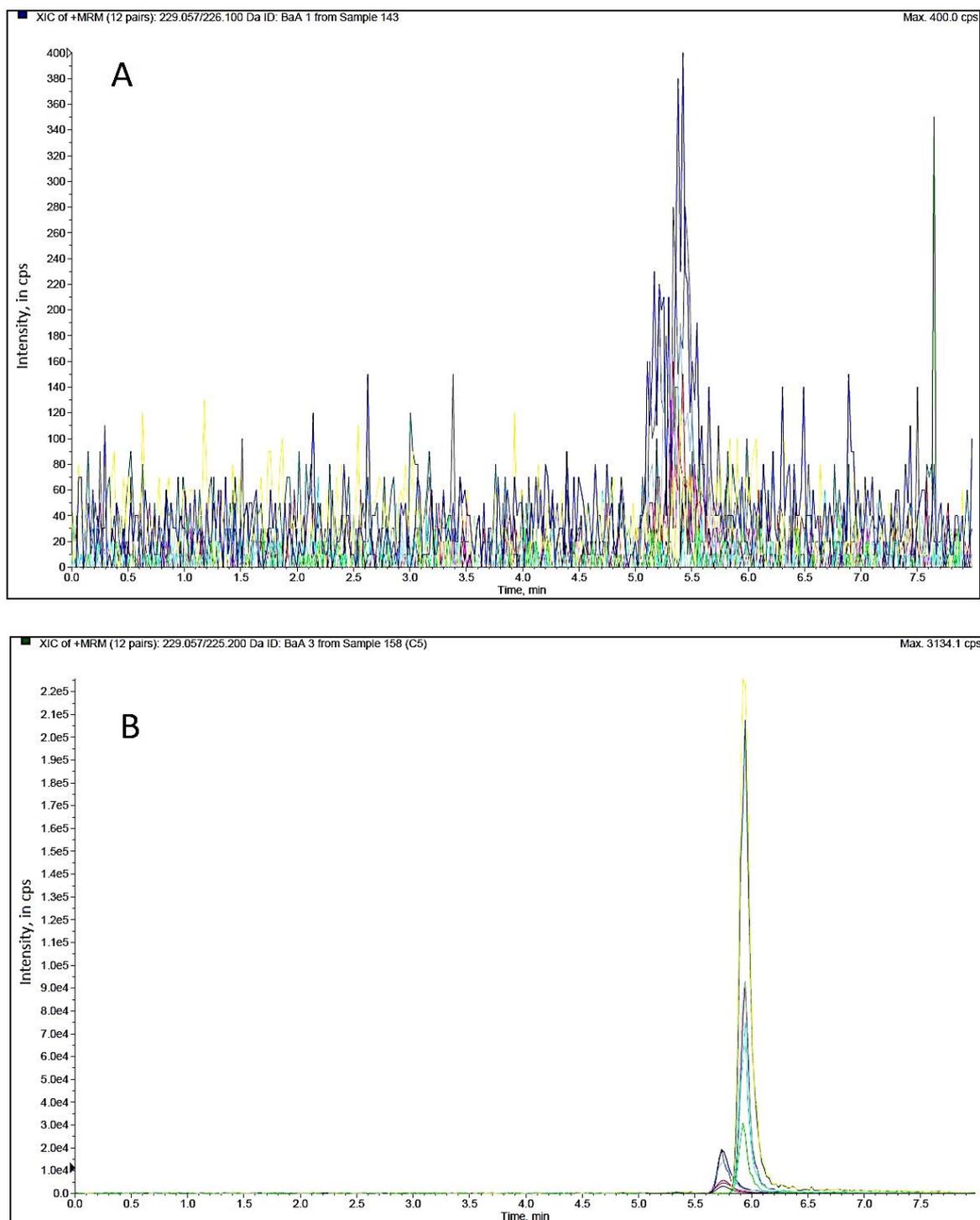


Figure 1. Chromatograms of a blank bacon sample (Part A) and of a PAHs-fortified bacon sample (Part B).

2.3.2. Method performance evaluation

Selectivity is an essential parameter for the reliability of the results, because compounds with low molecular weight, which may come from the food matrix, are susceptible to interferences during LC-MS/MS analysis. As shown in Table 3, the

relative ion intensities for PAHs compounds were all above 20%, which prove method sensitivity and reproducibility, avoiding false-positive results (Molognoni et al., 2018).

Table 3. Validation results of the Espresso machine-LC-MS/MS method for the analysis of PAHs in smoked bacon.

Parameters	BaA	Chry	BaP	BbF
Means values ($\mu\text{g kg}^{-1}$)	3.65	3.94	3.04	2.69
Selectivity (ionic ratio %)	32.61	91.87	35.94	35.63
Linearity (R^2)	0.98	0.98	0.98	0.98
Limits of detection (LOD, $\mu\text{g kg}^{-1}$)	0.10	0.10	0.10	0.25
Limits of quantification (LOQ, $\mu\text{g kg}^{-1}$)	0.50	0.50	0.50	0.50
Matrix effect (ME)	0.80	0.07	0.41	0.23
Recovery (%)	91.17	99.79	76.04	73.90
Relative repeatability (%)	25.95	11.06	15.62	10.10
Relative intermediate precision (%)	28.79	22.11	19.11	25.55
Detection capability ($\text{CC}\beta$, $\mu\text{g kg}^{-1}$) ($k= 3.00$)	0.34	0.34	0.35	0.88

BaA: Benzo[a]anthracene; Chry: Chrysene; BaP: Benzo[a]pyrene; BbF: Benzo[b]fluoranthene.

The linearity of individual analytes was evaluated as the regression coefficient (R^2). All analytes presented good linearity with $R^2 \geq 0.98$, as shown in Table 3.

In terms of sensitivity, the LOD and LOQ obtained for smoked bacon were in accordance the criteria given by Commission Regulation (EC) 333/2007, which recommends values of LODs to be lower than $0.30 \mu\text{g kg}^{-1}$ and LOQs to be lower than $0.90 \mu\text{g kg}^{-1}$ for PAHs analysis (Table 3). These results were also in agreement with PAHs detection and quantitation limits obtained by other validation studies (Gosetti et al., 2011; Lee et al., 2016).

The LC-MS/MS techniques usually provides better analytical sensitivity but it is subjected to strong matrix effect (ME) (Gosetti et al., 2011). Our results demonstrated a strong suppression of analytical signal to Chry, BaP, and BbF compounds. This is possible because bacon contains high amounts of fat and protein in its composition, which are responsible for signal suppression in the electrospray ionization (ESI) mode (Molognoni et al., 2018; Hoff et al., 2015). In this case, the use of florisil in the final extraction stage was not enough. Thus, the use of other sorbents for the clean-up process, such as solid-phase extraction (SPE), primary-secondary amine (PSA), gel permeation chromatography (GPC) (Gosetti et al., 2011; Pöhlmann et al., 2013; Lee et al., 2016; Malarut et al., 2018) could help to minimize the observed ME.

Recovery rates ranged from 73.9 to 99.8% (Table 3). In other studies, PAHs recovery values ranged from 83.4 to 110.7% for grilled pork and 84.4 to 109.3% for smoked sausages (Lee et al., 2016; Malarut & Vangnai, 2018). Based on European regulation (EC, 2007), recovery rate should be in the range of 50 – 120%. Therefore, the recovery values found were acceptable. Precision in terms of relative repeatability and relative intermediate precision yielded CV values below 30%, being acceptable for all analytes.

In this study, the $CC\beta$ was estimated to increase the reliability of the positive results and to statistically avoid wrong results. As shown in Table 3, $CC\beta$ values ranged from 0.3 to 0.9 $\mu\text{g kg}^{-1}$. These values were considered satisfactory due to being below the maximum values allowed by legislation, which are 2.0 and 12.0 $\mu\text{g kg}^{-1}$ for BaP and the sum of the main four PAHs, respectively.

To the best of our knowledge, this is the first report of the use of a hard cap espresso machine as an extraction method for PAHs determination in smoked bacon. Thus, an appropriate comparison with similar methods are not available yet. However, the results obtained of validation parameters met the criteria proposed by the Commission Regulation (EC) 333/2007. So, it was concluded that this method fit the purpose and can be used for the determination of four priority PAHs in smoked bacon. The extracted ion chromatogram of each analyte is shown at Figure 2.

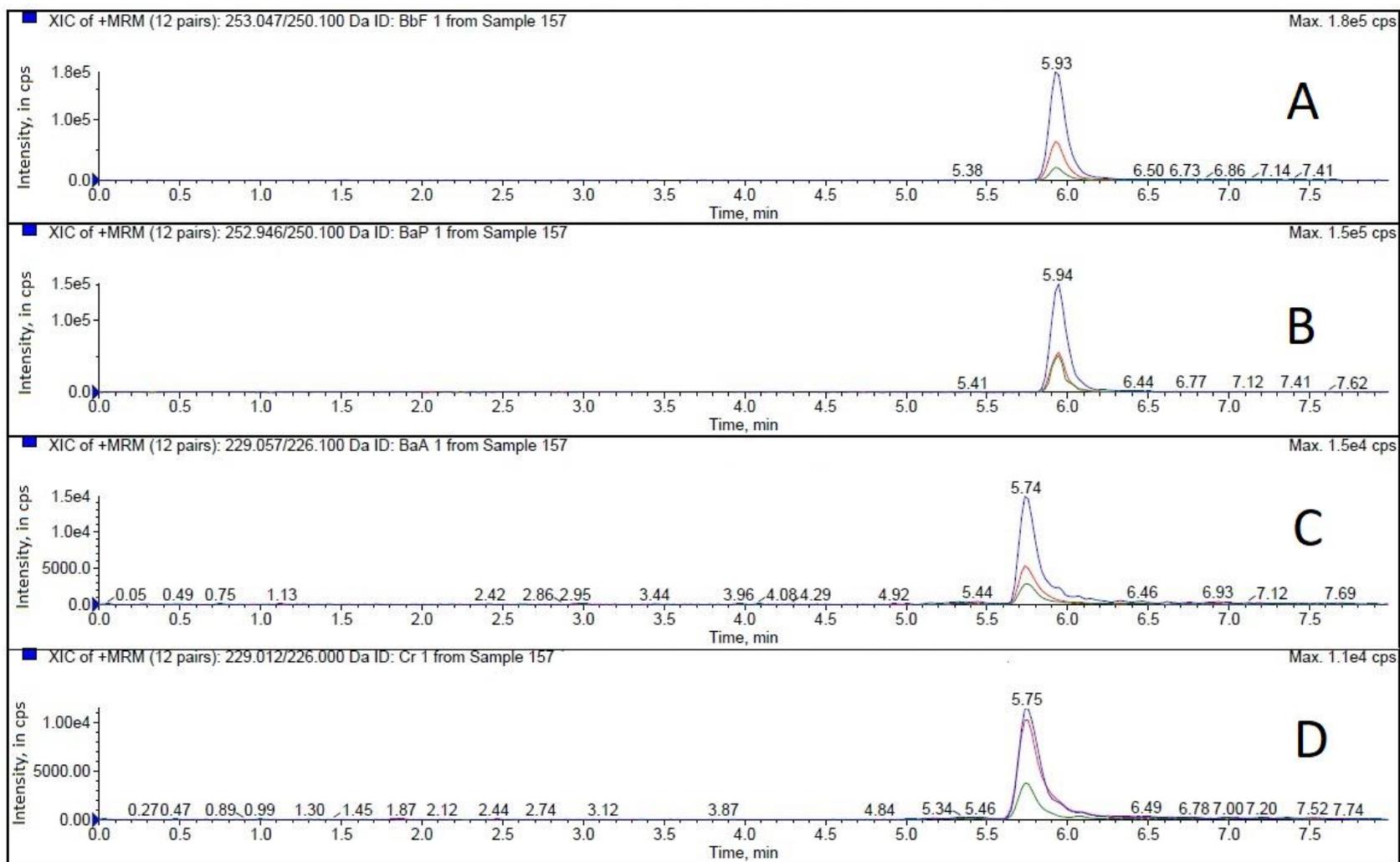


Figure 2. Chromatograms of a bacon sample fortified with PAHs: benzo[*b*]fluoranthene (Part A), benzo[*a*]pyrene (Part B), benzo[*a*]anthracene (Part C), and chrysene (Part D).

2.3.3. Method applicability

The developed method was applied to the determination of PAHs in smoked bacon samples, and the concentrations of BaA, BaP, Chry, and BbF are shown in Table 4. BaA and Chry were the main compounds quantified in the samples, and their concentrations are below the maximum values established by regulatory authorities (EC, 2011). BaP and BbF were not detected in most samples. Smoked sausages (smoked with the same wood, *Acacia* and *Eucalyptus* and similar smoking time/temperature) also yielded low concentrations of PAHs. BaP content did not exceed $0.43 \mu\text{g kg}^{-1}$ (Malarut & Vangnai, 2018). By the other hand, meat products smoked with soft wood (spruce) showed a high content of BaP ($32.34 \mu\text{g kg}^{-1}$) (Stumpe-Viksna et al., 2008).

Table 4. PAHs concentrations (mean \pm standard deviation) in smoked bacon.

Samples	PAHs ($\mu\text{g kg}^{-1}$)				
	BaA	BaP	Chry	BbF	Σ 4PAHs
<i>A. mearnsii</i>	0.10 \pm 0.00	Nd	Nd	Nd	0.10
<i>A. mangium</i>	0.10 \pm 0.00	Nd	0.10 \pm 0.00	Nd	0.20
<i>M. scabrella</i>	0.60 \pm 0.00	Nd	0.50 \pm 0.00	Nd	1.10
<i>Eucalyptus</i>	0.17 \pm 0.09	Nd	0.10 \pm 0.00	Nd	0.27
Commercial	0.10 \pm 0.00	0.10 \pm 0.00	1.17 \pm 0.19	0.10 \pm 0.00	1.47

BaA: Benzo[a]anthracene; BaP: Benzo[a]pyrene; Chry: Chrysene; BbF: Benzo[b]fluoranthene.

Σ 4PAHs: BaA + BaP + Chry + BbF.

Nd: Not detected.

From that, two hypotheses were elaborated to justify the obtained results. The first hypothesis is related to the type of wood used. In this study, hardwood which contains low lignin content compared to softwood, was used. This type of wood decreases the formation of smoke and consequently forms less noxious compounds (Stumpe-Viksna et al., 2008). The second hypothesis is related to the manufacturing process. During cooking and smoking, the bacon does not come into direct contact with the heat source, which prevents the direct deposition of PAHs produced by the pyrolysis of the wood (Chung et al., 2011).

2.4. Conclusion

A new method based on pressurized liquid extraction performed by a hard cap espresso machine as a sample preparation step was developed and validated for determination of PAHs in smoked bacon. The optimized extraction followed by

analytical determination with LC-MS/MS achieved satisfactory validation parameters, such as good selectivity and low LOD values. This method is advantageous due to the use of low-cost instruments during extraction, with small amounts of organic solvents. Method applicability to real samples revealed low concentrations of PAHs. This demonstrates that the woods used and the smoking process applied to those bacon samples have produced a small amount of these carcinogenic compounds. Therefore, our results showed a fast, easy and cheap method for PAHs analysis, able to be applied in routine laboratories of food quality control. In addition, it may contribute to Brazilian policy development, since it does not yet established limits for PAHs in smoked meat products.

Acknowledgements

Authors acknowledge the São Paulo Research Foundation (FAPESP, Brazil) for financing the project N° 2016/15012-2. Thais C. Merlo received the PhD scholarships from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001. Iliani Patinho is grateful to CAPES for the master scholarship.

References

- Al-Rashdan, A., Helaleh, M. I. H., Nlsar, A., Ibtisam, A., & Al-Ballam, Z. (2010). Determination of the levels of polycyclic aromatic hydrocarbons in toasted bread using gas chromatography mass spectrometry. *International Journal of Analytical Chemistry*, 2010, 1-8. <https://doi.org/10.1155/2010/821216>.
- Armenta, S., de la Guardia, M., & Esteve-Turrillas, F. A. (2016). Hard cap espresso machines in analytical chemistry: What else?. *Analytical Chemistry*, 88, 6570–6576. <https://doi.org/10.1021/acs.analchem.6b01400>.
- Bansal, V., & Kim, K. H. (2015). Review of PAH contamination in food products and their health hazards. *Environmental International*, 84, 26-38. <https://doi.org/10.1016/j.envint.2015.06.016>.

- Bertinetti, I. A., Ferreira, C. D., Monks, J. L. F., Sanches-Filho, P. J., & Elias, M. C. (2018). Accumulation of polycyclic aromatic hydrocarbons (PAHs) in rice subjected to drying with different fuels plus temperature, industrial processes and cooking. *Journal of Food Composition and Analysis*, 66, 109-115. <https://doi.org/10.1016/j.jfca.2017.12.009>.
- Chung, S. Y., Yettella, R. R., Kim, J. S., Kwon, K., Kim, M. C., & Min, D. B. (2011). Effects of grilling and roasting on the levels of polycyclic aromatic hydrocarbons in beef and pork. *Food Chemistry*, 129, 1420–1426. <https://doi.org/10.1016/j.foodchem.2011.05.092>.
- Corell, L., Armenta, S., Esteve-Turrillas, F. A., & De La Guardia, M. (2018). Flavonoid determination in onion, chili and leek by hard cap espresso extraction and liquid chromatography with diode array detection. *Microchemical Journal*, 140, 74-79. <https://doi.org/10.1016/j.microc.2018.04.014>.
- De Lima, R. F., Dionello, R. G., Peralba, M. C. R., Barrionuevo, S., Radunz, L. L., & Reichert Júnior, F. W. (2017). PAHs in corn grains submitted to drying with firewood. *Food Chemistry*, 215, 165-170. <https://doi.org/10.1016/j.foodchem.2016.07.164>.
- EC. (2002). European Commission, Commission Decision 2002/657/EC of 12 August 2002. Implementing Council Directive 96/23/EC concerning performance of analytical methods and the interpretation of results, Off. J. of the Eur. Communities, Brussels, L221/8, 2002.
- EC. (2007). Commission regulation (EC) No. 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. Official Journal of the European Union, 88, 29.
- EC. (2011). Commission regulation (EC) No. 835/2011 of 19 August 2011 amending Regulation (EC) 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. Official Journal of the European Union, 215, 7–8.
- Gallart-Mateu, D., Pastor, A., De La Guardia, M., Armenta, S., & Esteve-Turrillas, F. A. (2017). Hard cap espresso extraction-stir bar preconcentration of polychlorinated biphenyls in soil and sediments. *Analytica Chimica Acta*, 952, 41-49. <http://dx.doi.org/10.1016/j.aca.2016.11.051>.

- Gosetti, F., Chiuminatto, U., Mazzucco, E., Robotti, E., Calabrese, G., Gennaro, M. C., & Marengo, E. (2011). Simultaneous determination of thirteen polycyclic aromatic hydrocarbons and twelve aldehydes in cooked food by an automated on-line solid phase extraction ultra high performance liquid chromatography tandem mass spectrometry. *Journal of Chromatography A*, 1218, 6308-6318. <https://doi.org/10.1016/j.chroma.2011.06.085>.
- Hitzel, A., Pöhlmann, M., Schwägele, F., Speer, K., & Jira, W. (2013). Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in meat products smoked with different types of wood and smoking spices. *Food Chemistry*, 139, 955–962. <https://doi.org/10.1016/j.foodchem.2013.02.011>.
- Hoff, R. B., Rübensam, G., Jank, L., Barreto, F., Peralba, M. D. C. R., Pizzolato, T. M., & Barceló, D. (2015). Analytical quality assurance in veterinary drug residue analysis methods: matrix effects determination and monitoring for sulfonamides analysis. *Talanta*, 132, 443-450. <http://dx.doi.org/10.1016/j.talanta.2014.08.046>.
- Hoff, R. B., Molognoni, L., Deolindo, C. T. P., 568 Vargas, M. O., Kleemann, C. R., & Daguier, H. (2020). Determination of 62 veterinary drugs in feedingstuffs by novel pressurized liquid extraction methods and LC-MS/MS. *Journal of Chromatography B*, 1152, 122232. <https://doi.org/10.1016/j.jchromb.2020.122232>.
- IARC. (2010). International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans (vol. 92). Lyon, France. <http://monographs.iarc.fr/ENG/Monographs/vol92/mono92.pdf>.
- Just, J., Jordan, T. B., Paull, B., Bissember, A. C., & Smith, J. A. (2015). Practical isolation of polygodial from *Tasmania lanceolata*: A viable scaffold for synthesis. *Organic and Biomolecular Chemistry*, 13, 11200–11207. <https://doi.org/10.1039/C5OB01573A>.
- Just, J., Bunton, G. L., Deans, B. J., Murray, N. L., Bissember, A. C., & Smith, J. A. (2016). Extraction of eugenol from cloves using an unmodified household espresso machine: An alternative to traditional steam-distillation. *Journal of Chemical Education*, 93, 213–216. <https://doi.org/10.1021/acs.jchemed.5b00476>.

- Ledesma, E., Rendueles, M., & Díaz, M. (2016). Contamination of meat products during smoking by polycyclic aromatic hydrocarbons: Processes and prevention. *Food Control*, 60, 64-87. <https://doi.org/10.1016/j.foodcont.2015.07.016>.
- Lee, J. G., Kim, S-Y., Moon, J-S., Kim, S-H., Kang, D-H., & Yoon, H-J. (2016). Effects of grilling procedures on levels of polycyclic aromatic hydrocarbons in grilled meats. *Food Chemistry*, 199, 632-638. <https://doi.org/10.1016/j.foodchem.2015.12.017>.
- Leiman, K., Colomo, L., Armenta, S., De La Guardia, M., & Esteve-Turrillas, F. A. (2018). Fast extraction of cannabinoids in marijuana samples by using hard-cap espresso machines. *Talanta*, 190, 321-326. <https://doi.org/10.1016/j.talanta.2018.08.009>.
- Malarut, J., & Vangnai, K. (2018). Influence of wood types on quality and carcinogenic polycyclic aromatic hydrocarbons (PAHs) of smoked sausages. *Food Control*, 85, 98-106. <https://doi.org/10.1016/j.foodcont.2017.09.020>.
- Martinez-Sena, M. T., De La Guardia, M., Esteve-Turrillas, F. A., & Armenta, S. (2017). Hard cap espresso extraction and liquid chromatography determination of bioactive compounds in vegetables and spices. *Food Chemistry*, 237, 75-82. <http://dx.doi.org/10.1016/j.foodchem.2017.05.101>.
- Molognoni, L., Daguer, H., Ploêncio, L. A. S., Lindner, J. D. D. (2018). A multi-purpose tool for food inspection: simultaneous determination of various classes of preservatives and biogenic amines in meat and fish products by LC-MS. *Talanta*, 178, 1053–1066. <http://dx.doi.org/10.1016/j.talanta.2017.08.081>.
- Molognoni, L., Daguer, H., Motta, G. E., Merlo, T. C., & Lindner, J. D. D. (2019). Interactions of preservatives in meat processing: Formation of carcinogenic compounds, analytical methods, and inhibitory agents. *Food Research International*, 125, 108608. <https://doi.org/10.1016/j.foodres.2019.108608>.
- Molognoni, L., Daguer, H., Hoff, R. B., Rodrigues, J., Joussef, A. C., & Lindner, J. D. D. (2020). Assessing the mutagens ethylnitrolic acid and 2-methyl-1,4-dinitro-pyrrole in meat products: Sample preparation and simultaneous analysis by LC–MS/MS. *Journal of Chromatography*, 1609, 460512. <https://doi.org/10.1016/j.chroma.2019.460512>.

- Mukhopadhyay, S., Dutta, R., & Das, P. A critical review on plant biomonitors for determination of polycyclic aromatic hydrocarbons (PAHs) in air through solvent extraction techniques. *Chemosphere*, 251, 126441. <https://doi.org/10.1016/j.chemosphere.2020.126441>.
- Olatunji, O. S., Fatoki, O. S., Opeolu, B. O., & Ximba, B. J. (2014). Determination of polycyclic aromatic hydrocarbons [PAHs] in processed meat products using gas chromatography – Flame ionization detector. *Food Chemistry*, 156, 296-300. <https://doi.org/10.1016/j.foodchem.2014.01.120>.
- Pöhlmann, M., Hitzel, A., Schwagele, F., Speer, K., & Jira, W. (2013). Influence of different smoke generation methods on the contents of polycyclic aromatic hydrocarbons (PAH) and phenolic substances in frankfurter-type sausages. *Food Control*, 34, 347-355. <https://doi.org/10.1016/j.foodcont.2013.05.005>.
- Stumpe-Viksna, I., Bartkevics, V., Kukare, A., & Morozovs, A. (2008). Polycyclic aromatic hydrocarbons in meat smoked with different types of wood. *Food Chemistry*, 110, 794-797. <https://doi.org/10.1016/j.foodchem.2008.03.004>.
- Waqas, M., Khan, S., Chao, C., Shamshad, I., Qamar, Z., & Khan, K. (2014). Quantification of PAHs and health risk via ingestion of vegetable in Khyber Pakhtunkhwa Province, Pakistan. *Science of The Total Environment*, 497-498, 448-458. <https://doi.org/10.1016/j.scitotenv.2014.07.128>.

3. EFFECT OF THE SMOKING PROCESS USING BRAZILIAN REFORESTATION WOODS ON LIPID OXIDATION, MICROBIOLOGICAL AND HEDONIC QUALITY OF BACONS DURING SHELF LIFE

This chapter forms part of the article published in *Meat Science*.

doi:10.1016/j.meatsci.2020.108110.

Abstract

The aim of this study was to investigate the effect of using different reforestation woods (*Acacia mearnsii* and *Eucalyptus citriodora*) on changes in the lipid fraction (peroxide value and thiobarbituric acid reactive substances), microbiological counts and overall liking in bacons stored for 60 days at 5 ± 1 °C. Smoking with *Eucalyptus citriodora* wood reduced the concentration of malonaldehyde and PV values. Although smoking with different reforestation woods did not impact negatively on the acceptability of products, no changes on antimicrobial activity was observed in the developed products. Thus, the use of *Eucalyptus citriodora* wood may be an alternative for smoking meat products as it reduced lipid oxidation and provided higher antioxidant activity. The information generated herein can be used as a platform for companies to test the viability of producing meat products smoked with reforestation woods.

Keywords: Smoked bacon; *Eucalyptus citriodora*; Antioxidant; Antimicrobial

3.1. Introduction

Smoked products have unique sensory traits, such as color, aroma, taste and flavor; and their manufacturing process employs a smoking step, which is one of the oldest conservation methods. Bacon is a cured and smoked product that is among the most consumed meat products in the world, and is mainly made with pork belly (Soladoye et al., 2017; Saldaña et al., 2019a).

The smoking process can be performed by thermal combustion of wood (Stumpe-Viksna et al., 2008; Essumang, Doodoo, & Adjei, 2013) or by the direct application of liquid smoke into foods (Lingbeck et al., 2014; Soares et al., 2016). However, in Brazil, the Ministry of Agriculture, Livestock and Supply states that smoked products should go through the process of natural smoking, i.e smoking using woods (Brazil, 2012). Considering the legal aspect, the study of different reforestation woods for the smoking

of meat has become an alternative to replace the use of liquid smoke (Soares et al., 2016).

The latest statistical report of the Brazilian tree industry in 2016 showed that for each hectare of planted forest, 0.7 hectare was destined for preservation (Ibá, 2017). Among the several planted species, Eucalyptus (*Eucalyptus* spp.) is the main planted species, representing roughly 72% of the total tree plantation in the country. Eucalyptus has been used for pulp and paper production but its use as raw material to smoke meat product has increased (Ibá, 2017; Luz, 2013). Other species can be used for smoking, such as Acacia, a species planted mainly in the southern region of Brazil and represents a source of polyphenolic compounds (Ibá, 2017; Hoong et al., 2009).

The chemical composition of wood influences not only the formation of volatile organic compounds but also the physicochemical (pH, color, lipid oxidation), microbiological and sensory characteristics of the product (Lingbeck et al., 2014; Soladoye et al., 2017). The formation of antioxidant and antimicrobial components during wood burning and the amount of these substances that adhere to the product surface may have a significant impact on its shelf life (Ogbadu, 2014; Sikorski & Sinkiewicz, 2014).

Considering the consumer's preferences for more natural foods and the use of different reforestation woods to develop meat products, (Saldaña et al., 2018; Saldaña et al., 2019b), the present study proposed to evaluate the influence of smoking using two wood species (*Eucalyptus citriodora* and *Acacia mearnsii*) on lipid oxidation, microbiological counts and overall liking of bacon stored under refrigeration for 60 days.

3.2. Material and methods

3.2.1. Material

Pork belly was purchased from a local slaughterhouse (Capivari/SP, Brazil). The brine additives were donated by Ibrac (Rio Claro/SP, Brazil). *Eucalyptus citriodora* wood was obtained from the experimental station of Forest Sciences of Itatinga (Itatinga/SP, Brazil) and *Acacia mearnsii* wood was obtained in a local company (Rio Grande/RS, Brazil).

3.2.2. Bacon manufacture

A total of 18 pork bellies with an average weight of 5 ± 1 kg were used in the experiment. Brine was injected in pork bellies (Super Inject Max Power Flavor, Stander model) at random points at a ratio of 10% w/w. The ingredients and their concentrations of brine in the product final were: sodium chloride (1.5%), refined sucrose (0.6%), sodium nitrite (0.03%), sodium tripolyphosphate (0.3%) and sodium erythorbate (0.07%). Injected pork bellies were packed in vacuum shrinkable packages and refrigerated at 5 °C for 24 h for homogeneous brine penetration. After the curing process, pork bellies were randomly divided into three treatments (Table 1).

Table 1. Description of treatments.

Treatments	Coding	Description
Control	CT	Bacon without smoking
<i>Acacia mearnsii</i>	AME	Smoked bacon with <i>Acacia mearnsii</i>
<i>Eucalyptus citriodora</i>	EUC	Smoked bacon with <i>Eucalyptus citriodora</i>

The smoking process was carried out in four stages using a smoke-free automatic smoking machine (Verinox, Italy): (1) dry heat drying at 65 °C/30 min with open chimney and high ventilation; (2) smoking at 75 °C/60 min with half open chimney and low ventilation; (3) humid heat cooking at 75 °C/30 min with closed chimney and high ventilation and (4) humid heat cooking at 80 °C until the temperature of the thermal center reached 75 °C. The manufactured bacons are shown in Figure 1.

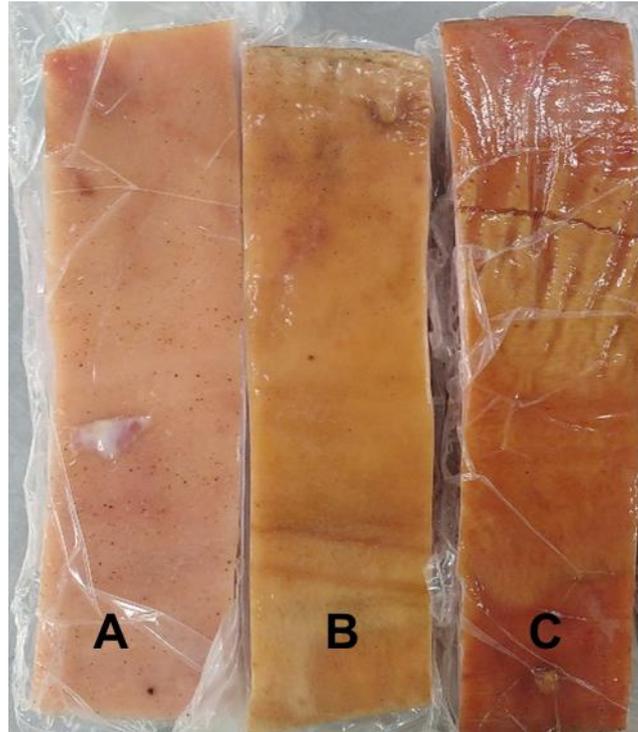


Figure 1. Bacons: control (Part A), *Acacia mearnsii* (Part B) and *Eucalyptus citriodora* (Part C).

After smoking, the samples were cooled in aqueous medium and refrigerated at 2 °C for 12 h, after which they were vacuum packed in heat shrinkable packages and stored under refrigeration (5 ± 1 °C). For physicochemical and microbiological analysis, bacon samples were evaluated at days 0, 15, 30, 45 and 60.

3.2.3. Chemical analysis of bacon

3.2.3.1. pH

pH of each bacon sample was determined using potentiometric measurements (pH1140 model, Mettler-Toledo, Switzerland) on three different areas.

3.2.3.2. Peroxide value (PV)

Primary lipid oxidation analysis was performed by quantifying peroxides. For this purpose, the lipids were extracted following the Bligh & Dyer (1959) methodology and the levels of peroxides were estimated using the methodology proposed by the American Oil Chemists' Society AOCS - method Cd 8-53 (1990) and results were expressed as milli-equivalents oxygen per kg fat.

3.2.3.3. Determination of thiobarbituric acid reactive substances (TBARS)

The extent of secondary lipid oxidation (TBA) was analyzed by malonaldehyde quantification (MDA). MDA was extracted and quantified by the method Cd 19-90 (AOCS, 1990), with modifications. Seven grams of sample were weighed into a polypropylene tube (50 mL) with 0.015 g of ethylenediamine tetraacetic acid (EDTA) and 0.015 g of propyl gallate. After this, 15 mL of 7.5% trichloroacetic acid were added to the tubes followed by homogenization (1 min) and addition of 15 mL of 7.5% trichloroacetic acid. The solution was homogenized for 1 min and filtered through a qualitative filter and 2.5 mL of this solution were transferred to a test tube along with 2.5 mL of 46 mM thiobarbituric acid reagent. A blank containing 2.5 mL 7.5% TCA and 2.5 mL 46 mM TBA reagent was also prepared. Tubes were incubated in a water bath at 95 ± 5 °C for 35 min and then chilled in an ice bath for approximately 5 min. Absorbance was measured at 532 nm (Shimadzu UV – Vis mini 1240, Chiyoda-ku, Tokyo, Japan). Results were calculated using an analytical curve of 1,1,3,3-tetraethoxypropane (TEP) and expressed as mg malonaldehyde (MDA) per kg sample.

3.2.4. Microbiological analysis

Microbiological analyses of bacon samples were conducted to assess the counts of deteriorating bacteria in the course of storage. Twenty-five grams of each sample were diluted in 225 mL of 0.1% sterile peptone water (Difco Laboratories, Detroit, MI, USA). Decimal dilutions were made and the following microbiological analyzes were performed in duplicate: the total mesophilic bacteria count (TMVC) was assessed by the standard plate count method – PCA - Plate Count Agar (Kasvi™) (Morton, 2001); total psychrotrophic count (TPVC) was analyzed by surface plating using PCA (Difco PCA™) (Cousin, Jay, & Vasavada, 2001); lactic acid bacteria counts (LAB) were enumerated using De Man Rogosa & Sharpe's medium (MRS) (Hall, Ledenbach, & Flowers, 2001). Bacterial counts were expressed as colony-forming units per gram of sample (\log CFU g^{-1} of sample).

3.2.5. Overall liking

Eighty four regular bacon consumers (70% women and 30% men) were recruited from students and employees of the *Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (ESALQ/USP)*. Bacon samples were cut into slices (2 mm thick) and prepared in hot plate (250 °C) (Edanca, Brazil) for about 2 min on each side to achieve the temperature of 80°C. After cooking, the bacon samples were cut into strips of approximately 2 cm and kept in an oven until they were offered to consumers. In a single session, consumers evaluated nine samples of bacon corresponding to the three treatments (CT, AME and EUC) stored at 0, 30 and 60 days. Consumers were installed in individual sensory booths under artificial white light and samples were presented monadically in 50 mL disposable plastic cups coded with three-digit random numbers following a Williams Latin Square Design. Consumers rated their overall liking using a 9-point hedonic scale, ranging from "disliked extremely" (1) to "liked extremely" (9). Water and biscuit were offered to consumers to clean the palate between samples. Data collection was conducted entirely in Compusense Cloud (Compusense Inc., Guelph, Ontario, Canada) using tablets (Samsung Galaxy Tab E, T560, screen 9.6").

3.2.6. Statistical analysis

The experiment was conducted using a randomized block design with three replications (3 blocks) in which the treatments were arranged according to a 3 x 5 factorial scheme (3 smoking processes and 5 storage times). The statistical model is given by:

$$Y_{ijk} = \mu + b_k + \beta_i + \gamma_j + \beta \gamma_{ij} + \varepsilon_{ijk}$$

where: Y_{ijk} is the value of the response in the k -th block ($k = 1, 2, 3$) that received the i -th type of smoking ($i = 1, 2, 3, 4$) at the j -th time of storage ($j = 1, 2, 3, 4, 5$); μ is a constant inherent in all observations; b_k is the effect of the k -th block; β_i is the effect of the i -th type of smoking; γ_j is the effect of the j -th storage time; $\beta \gamma_{ij}$ is the effect of the interaction between the i th smoking type and i -th storage time; ε_{ijk} is the experimental error associated with the response variable Y_{ijk} that is normally distributed ($\mu=0$, constant σ^2).

Data from physicochemical and microbiological analyzes were submitted to analysis of variances (ANOVA) and the effects were tested considering the significance level of 5% ($\alpha = 0.05$). For the type of smoke, when significant in the ANOVA, samples were compared using the Tukey test. For the storage time, when significant, polynomial regression models were adjusted and the model with the highest significant degree ($\alpha = 0.05$) was chosen. The adherence to the Normal distribution of the residuals was verified by the Shapiro-Wilk test.

For the variables whose model assumptions were not met (peroxides and counts of LAB), the Box-Cox transformation was used (Box & Cox, 1964) to choose the λ value that minimized the residual. This method produces a transformation of the response variable (Y) in a way that the assumptions of homoscedasticity and normality of the residuals are simultaneously satisfied. The transformation indicated for both variables was $Y_t = 1/Y^2$, whereby Y_t is the transformed variable and Y is the original variable.

The overall liking results were analyzed by mixed ANOVA at 5% significance. When appropriate, the Tukey's test was performed for pairwise comparison.

3.3. Results and discussion

3.3.1. pH

Changes in the pH of smoked bacons manufactured with different reforestation woods are presented in Table 2. There was no significant difference between the types of smoking, but the pH decreased over the storage times for all treatments (Figure 2). The decrease in pH of smoked bacon during storage has also been reported elsewhere (Li et al., 2019; Wang, Zhang & Ren, 2017).

Table 2. pH and lipid oxidation (Peroxide values and TBARS) of bacons during storage time at 5 ± 1 °C.

Variable	Treatments	Storage time (days)				
		0	15	30	45	60
pH	CT	6.37±0.18 ^a	6.29±0.14 ^a	6.27±0.11 ^a	6.25±0.11 ^a	6.28±0.11 ^a
	AME	6.31±0.02 ^a	6.30±0.02 ^a	6.24±0.00 ^a	6.18±0.02 ^a	6.17±0.01 ^a
	EUC	6.47±0.07 ^a	6.44±0.07 ^a	6.39±0.04 ^a	6.34±0.10 ^a	6.31±0.09 ^a
Peroxide values (mEq O ₂ Kg ⁻¹ fat)	CT	0.252±0.00 ^a	0.063±0.00 ^b	0.028±0.00 ^b	0.011±0.00 ^c	0.064±0.00 ^b
	AME	0.252±0.00 ^a	0.252±0.00 ^a	0.063±0.00 ^a	0.063±0.00 ^b	0.063±0.00 ^b
	EUC	0.253±0.00 ^a	0.063±0.00 ^b	0.063±0.00 ^a	0.112±0.00 ^a	0.254±0.00 ^a
TBARS (mg MDA Kg ⁻¹)	CT	0.248±0.03 ^a	0.251±0.01 ^a	0.436±0.02 ^a	0.741±0.01 ^a	0.715±0.02 ^a
	AME	0.240±0.01 ^a	0.223±0.01 ^b	0.267±0.01 ^b	0.305±0.02 ^b	0.381±0.01 ^b
	EUC	0.176±0.00 ^b	0.201±0.00 ^b	0.222±0.01 ^c	0.268±0.01 ^c	0.296±0.00 ^c

CT: control (without smoking); AME: smoked bacon with *Acacia mearnsii*; EUC: smoked bacon with *Eucalyptus citriodora*.

The results were expressed as mean \pm standard deviation. Different lower case letters indicate significant differences between the analyzed treatments (column), using Tukey test ($\alpha = 0.05$).

The decrease in the pH is probably associated to the growth of lactic acid bacteria and the respective formation of organic acids, such as lactic acid (Wang et al., 2015; O'Neill et al., 2018). However, the pH of the CT treatment increased between 45 and 60 days of storage, which may be related to lipid and protein degradation, which generates free fatty acids and amino acids (Huang et al., 2014).

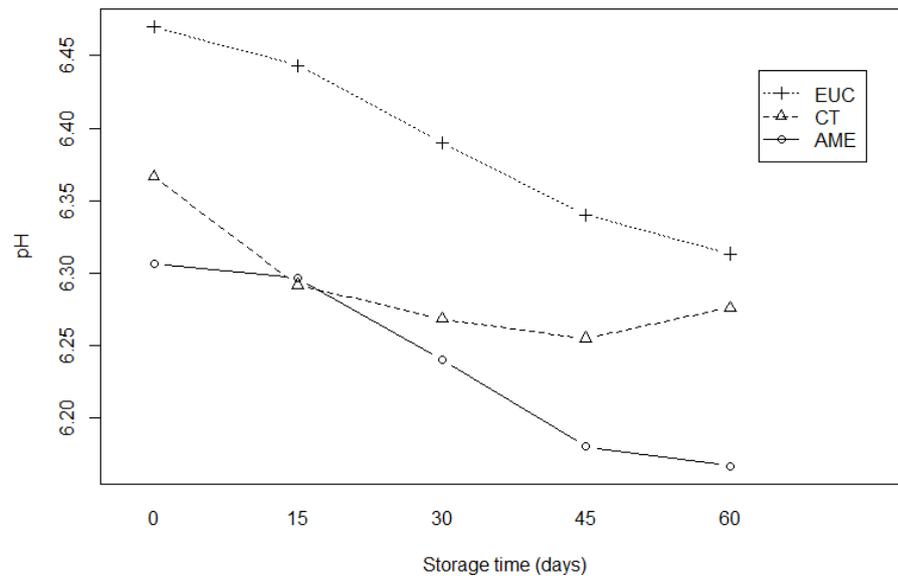


Figure 2. Effect of storage time on the pH of different types of smoking.

3.3.2. Lipid oxidation changes during storage

The transformed PV values ($\text{Peroxide}_T = 1/\text{Peroxide}^2$) are presented in Table 2. Overall, there was no significant difference between treatments at time 0 but PV values were lower in EUC, AME and CT samples. The initial PV values are mainly due to the thermal process to which the bacon was submitted: the temperature increase accelerates the breakdown of peroxides, thus enhancing the increase of free radicals and being the basis for chain lipid oxidation reactions (Reginato-D'arce, 2006).

In general, PV increased in the first three stages of storage and decreased until the end (Figure 3), similar behavior was reported by other authors during storage of the same product (Huang et al., 2014; Soares et al., 2016). The AME treatment remained stable from day 30, while CT treatment values decreased until day 45 (Table 2). EUC treatment reached maximum PV at day 30, decreasing until the end of storage (60 days). The decrease in PV in CT and EUC treatments is related either to the decomposition of hydroperoxides in carbonyls or to reactions with other compounds (Shahidi, 2001; Soyer et al., 2010; Bou et al., 2008).

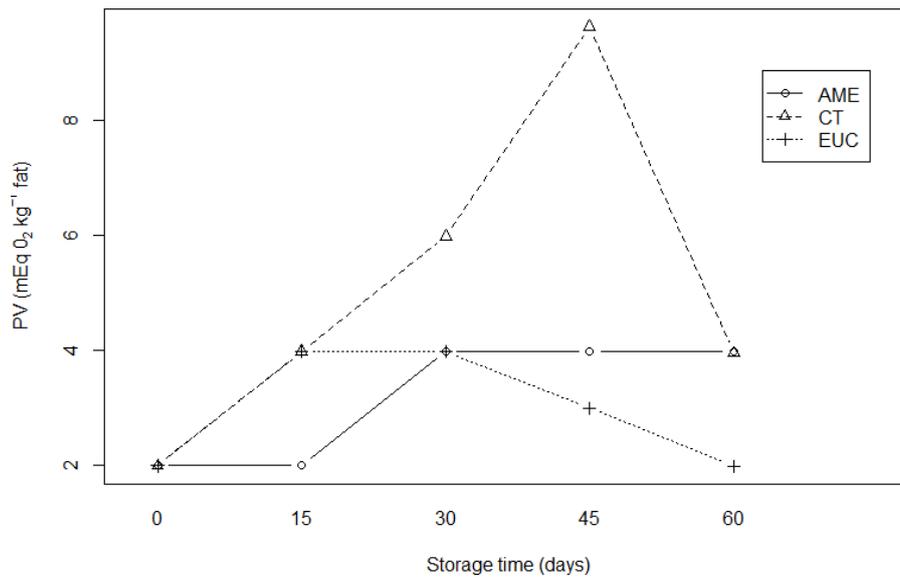


Figure 3. Effect of storage time on the peroxide levels ($\text{Peroxide}_T = 1/\text{Peroxide}^2$; $\text{mEq O}_2 \text{ kg}^{-1} \text{ fat}$) for the different types of smoking.

The TBARS values shown in Table 2 indicate that the EUC treatment had lower lipid oxidation ($\alpha = 0.05$) compared to the other treatments. In addition, from Figure 4, it is possible to observe that the CT treatment had a greater increase in TBARS values from day 15 compared to other treatments, with a slight decline on the last day of storage (60 days).

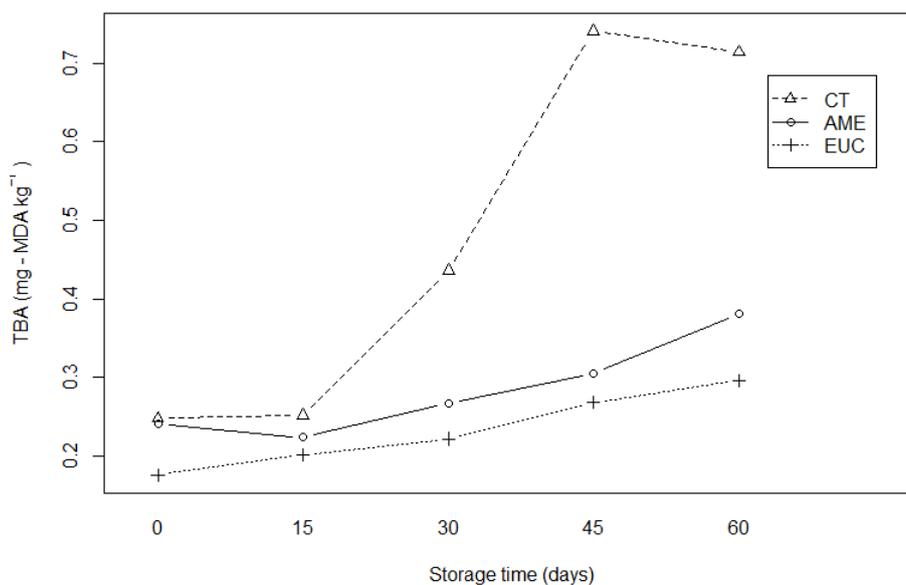


Figure 4. Effect of storage time on the TBA (mg MDA Kg^{-1}) for the different types of smoking using different regression models: cubic (CT); quadratic (AME) and linear (EUC).

The increase in the extent of lipid oxidation (TBARS) generally became more evident from day 30, while PV tended to decrease, thus indicating that hydroperoxides were degraded to TBARS (Shahidi, 2001).

However, the lowest TBARS values were found for the EUC treatment, which presented a high proportion of phenolic VOCs (Table*) compared to the other treatments. This result indicates that some phenols can be antioxidants in smoked bacon, reducing lipid oxidation and contributing to its conservation (Ogbadu, 2014; Sikorski & Sinkiewicz, 2014). Similar TBARS values were found in bacon manufactured with natural antioxidants, such as grape seed extract and green tea (Wang et al., 2015). The CT treatment showed a slight decrease in TBARS on the 60th day of storage, indicating lipid degradation in the bacon sample (Shahidi, 2001; Schwert et al., 2011). Finally, the lipid oxidation data are correlated with the VOCs results, where some VOCs, such as pentanal, hexanal, heptanal, benzaldehyde, octanal and nonanal were generated from the oxidation reaction (Table*).

3.3.3. Microbial changes during storage

Table 3 shows the real and the transformed values of mesophilic and psychrotrophic counts and the values for lactic acid bacterial counts ($LAB_T = 1/LAB^2$) for the different bacon samples. There was no significant difference between the treatments analyzed, and a gradual increase in those counts was observed during storage, which is in-line with the data reported by Wang, Zhang & Ren (2017). However, microbiological counts for the microorganisms studied did not exceed the acceptability limit of 7 log CFU g⁻¹ (Kreyenschmidt et al., 2010).

*This Table was published in:

Merlo TC, Antonio JC, Savian TV, et al (2020). Effect of the smoking using Brazilian reforestation woods on volatile organic compounds, lipid oxidation, microbiological and hedonic quality of bacons during shelf life. Meat Sci 164:108110.

Table 3. Microbial counts of smoked bacons during storage time at 5 ± 1 °C.

Variable	Treatment	Storage time (days)				
		0	15	30	45	60
TMVC (log CFU g ⁻¹)	CT	1.96±0.26 ^a	2.30±1.06 ^a	2.49±0.49 ^a	3.16±2.09 ^a	3.26±1.57 ^a
	AME	1.90±0.23 ^a	2.24±0.25 ^a	3.48±0.90 ^a	3.90±0.85 ^a	5.64±2.64 ^a
	EUC	1.39±0.39 ^a	2.68±2.18 ^a	2.81±0.40 ^a	3.97±2.61 ^a	3.80±2.57 ^a
TPVC (log CFU g ⁻¹)	CT	1.99±0.13 ^a	2.20±0.39 ^a	2.68±1.03 ^a	3.31±2.01 ^a	3.58±1.67 ^a
	AME	2.10±0.17 ^a	3.10±0.78 ^a	4.00±2.89 ^a	4.34±3.14 ^a	3.55±2.95 ^a
	EUC	2.20±0.39 ^a	3.26±1.10 ^a	3.42±2.46 ^a	4.26±2.61 ^a	3.77±3.07 ^a
LAB (log CFU g ⁻¹)	CT	0.26±0.02 ^a	0.23±0.04 ^a	0.20±0.09 ^a	0.17±0.13 ^a	0.19±0.11 ^a
	AME	0.24±0.02 ^a	0.15±0.11 ^a	0.17±0.13 ^a	0.12±0.12 ^a	0.10±0.13 ^a
	EUC	0.25±0.00 ^a	0.13±0.11 ^a	0.17±0.13 ^a	0.17±0.13 ^a	0.16±0.12 ^a

CT: control (without smoking); AME: smoked bacon with *Acacia mearnsii*; EUC: smoked bacon with *Eucalyptus citriodora*.

TMVC: Total mesophilic viable count; TPVC: Total psychrotrophic viable count; LAB: Lactic acid bacteria.

The results were expressed as mean \pm standard deviation. Different lower case letters indicate significant differences between the analyzed treatments (column), using Tukey test ($\alpha = 0.05$).

Two main factors may have influenced the microbiological growth: 1) the concentration of the VOCs (creosol and guaiacol) generated in the smoking process was not enough to change the membrane permeability of microorganisms (Gram-positive bacteria), which would cause intracellular damage and inhibit their growth (Martin et al., 2010; Davidson, Critzer & Taylor, 2013); and 2) Bacons were vacuum packed, which favours the growth of psychrotrophic microorganisms, including LAB (Kalschne et al., 2015).

3.3.4. Overall liking

According to the overall liking of samples, a significant increase in the course of storage time (Figure 5) was observed. Bacons smoked with *Acacia mearnsii* (AME30) and *Eucalyptus* (EUC30) from day 30 of refrigerated storage had scores higher than the means acceptance value (6.42). This indicates that the smoking process has a significant influence on the generation of sensory attributes that drive the liking of the products (Saldaña et al., 2019a).

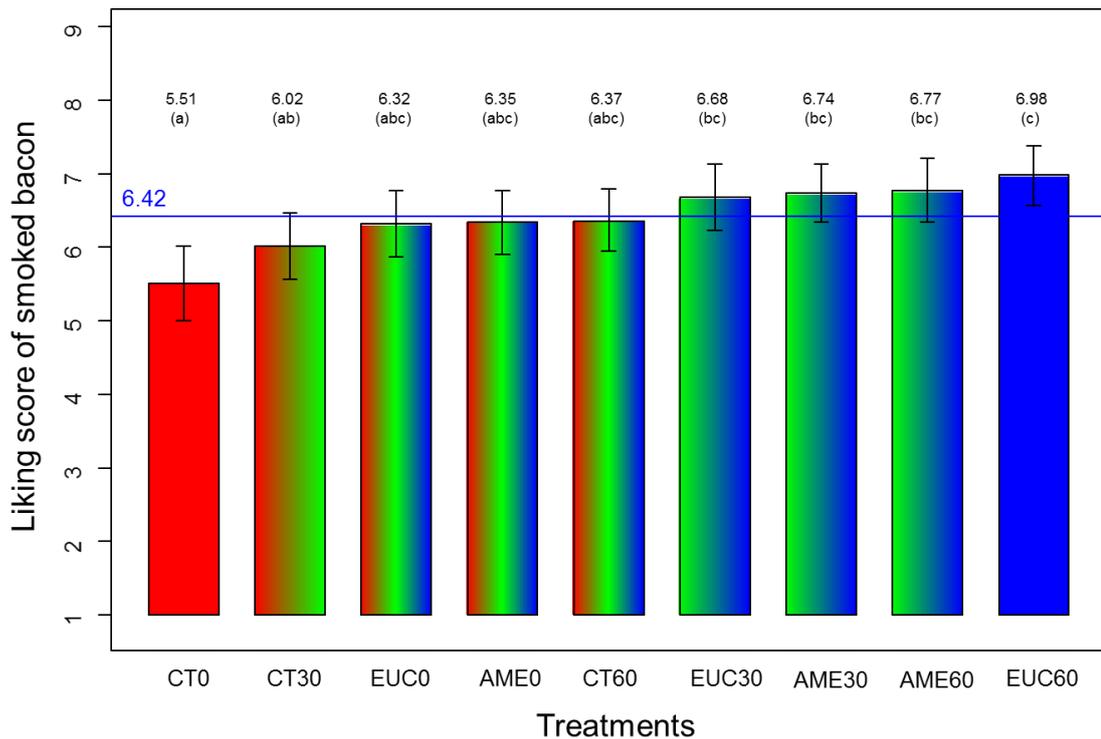


Figure 5. Mean scores of Liking by smoked bacon.

*Means with the same letter or with the same color are not significantly different.

*Error bars are the standard error of the mean.

So, compared with the control treatment, bacon smoked with *Eucalyptus* (EUC60) on the last day of storage (60 days) was the most accepted by the consumers. This behavior is expected because smoking with eucalyptus wood increase the formation of phenolic compounds, such as guaiacol and creosol (Table*). These compounds are essential for the appreciation of smoked products, because they are responsible for the smoked flavor (Yu et al., 2008; Stojkovic et al., 2015; Saldaña et al., 2019a). On the other hand, CT samples presented the lowest liking maybe related to the fact that the CT had a higher lipid oxidation, as previously demonstrated, leading to the formation of aldehydes that in high concentrations can confer rancid flavor (Lorenzo, Carballo & Franco, 2013), unpleasant for the consumers.

*This Table was published in:

Merlo TC, Antonio JC, Savian TV, et al (2020). Effect of the smoking using Brazilian reforestation woods on volatile organic compounds, lipid oxidation, microbiological and hedonic quality of bacons during shelf life. Meat Sci 164:108110.

3.4. Conclusions

The smoking process of bacon using *Eucalyptus citriodora* wood promoted a significant decrease on oxidative parameters (peroxide value and TBARS). Thus, the differences found between the smoked bacon samples are strongly associated to the chemical composition of the woods, impacting on the consumer's overall liking. Therefore, the smoking process of bacon using eucalyptus wood seems to be a suitable approach to be considered by the meat industry.

Acknowledgements

Authors acknowledge the São Paulo Research Foundation (FAPESP, Brazil) for financing the project N^o 2016/15012-2. Thais C. Merlo received the PhD scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001. Iliani Patinho is also acknowledge for her support in the sensory data collection.

References

- AOCS. (1990). Determination of peroxide value. Cd 8-53. Official methods and recommended practices of the American oil Chemists' Society (4th ed.), American Oil Chemists' Society, Champaign.
- AOCS. (1990). 2-Thiobarbituric Acid Value, Direct Method. Cd 19-90. Official methods and recommended practices of the American oil Chemists' Society (7th ed.), American Oil Chemists' Society, Champaign.
- Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Journal of Biochemistry and Physiology*, 37, 911-917. <https://doi.org/10.1139/o59-099>.
- Brasil. (2012). Ministério da Agricultura, Pecuária e Abastecimento – MAPA. Memorando 095/2012/DICS/CGI/DIPOA, de 27 de julho de 2012. Esclarecimento quanto ao procedimento de defumação de produtos cárneos e revisão de rotulagem de produtos defumados.

- Bou, R., Codony, R., Tres, A., Decker, E. A., & Guardiola, F. (2008). Determination of hydroperoxides in foods and biological samples by the ferrous oxidation–xylenol orange method: A review of the factors that influence the method's performance. *Analytical Biochemistry*, 377, 1 – 15. <https://doi.org/10.1016/j.ab.2008.02.029>.
- Box. G. E. P., Cox, D. R. (1964). An analysis of transformations. *Journal of the Royal Society*, 26, 211-252.
- Cousin, M. A., Jay, J. M., & Vasavada, P. C. (2001). Psychrotrophic microorganisms. In: F. P. Downes, & K. Ito (Eds.), *Compendium of Methods for the Microbiological Examination of Foods* (pp. 159-166). Washington: American Public Health Association.
- Davidson, P. M., Critzer, F. J., & Taylor, T. M. (2013). Naturally occurring antimicrobials for minimally processed foods. *Annual review of food science and technology*, 4, 163-190. <https://doi.org/10.1146/annurev-food-030212-182535>.
- Essumang, D. K., Dodoo, D. K., & Adjei, J. K. (2013). Effect of smoke generation sources and smoke curing duration on the levels of polycyclic aromatic hydrocarbon (PAH) in different suites of fish. *Food and Chemical Toxicology*, 58, 86-94. <https://doi.org/10.1016/j.fct.2013.04.014>.
- Hall, P. A., Ledenbach, L., & Flowers, R. S. (2001). Acid-producing microorganisms. In: F. P. Downes, & K. Ito (Eds.), *Compendium of Methods for the Microbiological Examination of Foods* (pp. 201-207). Washington: American Public Health Association.
- Hoong, Y. B., Paridah, M. T., Luqman, C. A., Koh, M. P., & Loh, Y. F. (2009). Fortification of sulfited tannin from the bark of *Acacia mangium* with phenol–formaldehyde for use as plywood adhesive. *Industrial Crops and Products*, 30, 416-421. <https://doi.org/10.1016/j.indcrop.2009.07.012>
- Indústria Brasileira De Árvores – IBÁ. (2017). Relatório anual 2017, ano base 2016. Brasília: IBÁ.

- Kalschne, D. L., Womer, R., Mattana, A., Sarmiento, C. M. P., Colla, L. M., & Colla, E. (2015). Characterization of the spoilage lactic acid bacteria in “sliced vacuum-packed cooked ham”. *Brazilian Journal of Microbiology*, 46, 173–181. <http://dx.doi.org/10.1590/S1517-838246120130019>.
- Kreyenschmidt, J., Hübner, A., Beierle, E., Chonsch, L., Scherer, A., & Petersen, B. (2010) Determination of the shelf life of sliced cooked ham based on the growth of lactic acid bacteria in different steps of the chain. *Journal of Applied Microbiology*, 108, 510-520. <https://doi:10.1111/j.1365-2672.2009.04451.x>.
- Li, X., Li, C., Ye, H., Wang, Z., Wu, X., H, Y., & Xu, B. (2019). Changes in the microbial communities in vacuum-packaged smoked bacon during storage. *Food Microbiology*, 77, 26-37. <https://doi.org/10.1016/j.fm.2018.08.007>.
- Lingbeck, J. M., Cordero, P., O'bryan, C. A., Johnson, M. C., Ricke, S. C., & Crandall, P. G. (2014). Functionality of liquid smoke as an all-natural antimicrobial in food preservation. *Meat Science*, 97, 197-206. <https://doi.org/10.1016/j.meatsci.2014.02.003>.
- Lorenzo, J. M., Carballo, J., & Franco, D. (2013). Effect of the inclusion of chestnut in the finishing diet on volatile compounds of dry-cured ham from celta pig breed. *Journal of Integrative Agriculture*, 12, 2002-2012. [https://doi.org/10.1016/S2095-3119\(13\)60638-3](https://doi.org/10.1016/S2095-3119(13)60638-3).
- Luz, R. L. F. (2013). Hidrocarbonetos policíclicos aromáticos (HPAs) em queijos defumados e em queijos assados em churrasqueiras. Retrieved from http://repositorio.ufla.br/bitstream/1/1675/2/DISSERTACAO_Hidrocarbonetos%20polic%c3%adclicos%20arom%c3%a1ticos....pdf.
- Martin, E. M., O'bryan, C. A., Lary, R. Y. Jr, Griffis, C. L., Vaughn, K. L., Marcy, J. A., ... Crandall, P. G. (2010). Spray application of liquid smoke to reduce or eliminate *Listeria monocytogenes* surface inoculated on frankfurters. *Meat Science*, 85, 640-644. <https://doi.org/10.1016/j.meatsci.2010.03.017>.
- Morton, R. D. (2001). Aerobic plate count. In: F. P. Downes, & K. Ito (Eds.), *Compendium of Methods for the Microbiological Examination of Foods* (pp. 63-67-166). Washington: American Public Health Association.

- Ogbadu, L. J. (2014). In: Encyclopedia of Food Microbiology. PRESERVATIVES | Traditional Preservatives – Wood Smoke (pp. 141–148). [s.l.] Elsevier.
- O' Neill, C. M., Cruz-Romero, M. C., Duffy, G., & Kerry, J. P. (2018). Shelf life extension of vacuum-packed salt reduced frankfurters and cooked ham through the combined application of high pressure processing and organic acids. *Food Packaging and Shelf life*, 17, 120-128. <https://doi.org/10.1016/j.fpsl.2018.06.008>.
- Reginato-D'arce, M. A. B. (2006). Deterioração de lipídios–ranço. In: M. Oetterer, R. - D.'a. Mab, & S. MHF (Eds.), Fundamentos de ciência e tecnologia de alimentos (Chapper 6). Brazil: Manole.
- Saldaña, E., Castillo, L. S., Sánchez, J. C., Siche, R., Almeida, M. A., Behrens, J. H., ... Contreras-Castillo, C. J. (2018). Descriptive analysis of bacon smoked with Brazilian woods from reforestation: methodological aspects, statistical analysis, and study of sensory characteristics. *Meat Science*, 140, 44–50. <https://doi.org/10.1016/j.meatsci.2018.02.014>.
- Saldaña, E., Saldarriaga, L., Cabrera, J., Siche, R., Behrens, J. H., Selani, M. M., ... Contreras-Castillo, C. J. (2019a). Relationship between volatile compounds and consumer-based sensory characteristics of bacon smoked with different Brazilian woods. *Food Research International*, 119, 839–849. <https://doi.org/10.1016/j.foodres.2018.10.067>.
- Saldaña, E., Soletti, I., Martins, M. M., Menegali, B. S., Merlo, T. C., Selani, M. M., ... Contreras-Castillo, C. J. (2019b). Understanding consumers' dynamic sensory perception for bacon smoked with different Brazilian woods. *Meat Science*, 154, 46-53. <https://doi.org/10.1016/j.meatsci.2019.04.006>.
- Schwert, R., Verlindo, R., Cichoski, A. J., Oliveira, D., & Valduga, E. (2011). Comparative evaluation of liquid and traditional smoke on oxidative stability, color and sensory properties of Brazilian calabrese sausage. *CyTA Journal of Food*, 9, 131-134. <https://doi.org/10.1080/19476337.2010.491581>.
- Shahidi, F. (2001). In J. Li & G. B. Zhu (Eds.). Flavor of meat, meat products and seafoods (pp. 54–73). Beijing: China Light Industry Press.
- Sikorski, Z. E., Sinkiewicz, I. (2014). In: Encyclopedia Of Meat Sciences. Smoking | Traditional (pp. 321-327). [s.l.] Elsevier.

- Soares, J. M., da Silva, P. F., Puton, B. M. S., Brustolin, A. P., Cansian, R. L., Dallago, R. M., & Valduga, E. (2016). Antimicrobial and antioxidant activity of liquid smoke and its potential application to bacon. *Innovative Food Science & Emerging Technologies*, 38, 189 – 197. <https://doi.org/10.1016/j.ifset.2016.10.007>.
- Soladoye, O. P., Shand, P., Dugan, M. E. R., Gariépy, C., Aalhus, J. L., Estévez, M., & Juárez, M. (2017). Influence of cooking methods and storage time on lipid and protein oxidation and heterocyclic aromatic amines production in bacon. *Food Research International*, 99, 660–669. <https://doi.org/10.1016/j.foodres.2017.06.029>.
- Soyer, A., Özalp, B., Dalmis, Ü., & Bilgin, V. (2010). Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food Chemistry*, 120, 1025 – 1030. <https://doi.org/10.1016/j.foodchem.2009.11.042>.
- Stojkovic, S., Grabez, V., Bjelanovic, M., Mandic, S., Vucic, G., Martinovic, A., ... Egelanddal, B. (2015). Production process and quality of two different dry-cured sheep hams from Western Balkan countries. *LWT-Food Science and Technology*, 64, 1217–1224. <https://doi.org/10.1016/j.lwt.2015.07.022>.
- Stumpe-Viksna, I., Bartkevics, V., Kukare, A., & Morozovs, A. (2008). Polycyclic aromatic hydrocarbons in meat smoked with different types of wood. *Food Chemistry*, v. 110, 794-797. <https://doi.org/10.1016/j.foodchem.2008.03.004>.
- Wang, Y. L., Li, F., Zhuang, H., Chen, X., Li, L. H., Qiao, W. W., & Zhang, J. H. (2015). Effects of plant polyphenols and α-tocopherol on lipid oxidation, residual nitrites, biogenic amines, and N-nitrosamines formation during ripening and storage of dry cured bacon. *LWT - Food Science and Technology*, 60, 199–206. <https://doi.org/10.1016/j.lwt.2014.09.022>.
- Wang, X., Zhang, Y., & Ren, H. (2017). Effects of grape seed extract on lipid oxidation, biogenic amine formation and microbiological quality in Chinese traditional smoke-cured bacon during storage. *Journal of Food Safety*, 38, e12426. <https://doi.org/10.1111/jfs.12426>.

Yu, A. N., Sun, B. G., Tian, D. T., & Qu, W. Y. (2008). Analysis of volatile compounds in traditional smoke-cured bacon (CSCB) with different fiber coatings using SPME. *Food Chemistry*, 110, 233–238. <https://doi.org/10.1016/j.foodchem.2008.01.040>.

4. FORMATION PATHWAYS OF FLAVOR IN SMOKED BACON DURING STORAGE: RELATIONSHIP BETWEEN VOLATILE ORGANIC COMPOUNDS, FREE AMINO ACIDS AND SENSORY PROFILE

Abstract

Flavor is one of the most important characteristics of smoked bacon, strongly affecting its acceptance by consumers. The smoking process, lipid oxidation and proteolysis contribute to bacon quality by means of odor and taste compound generation. In this study, the relationships between free amino acids (FAAs), volatile organic compounds (VOCs) and sensory characteristics on flavor pathway was investigated for smoked bacon stored for 60 days at 5°C. Samples stored for up to 30 days were characterized by having VOCs produced through the smoking process and lipid oxidation. Meanwhile, after 30 days of storage, the samples presented an increase in FAAs produced by proteolysis. Smoked bacon was characterized by having smoky, crunchy, salty, soft and bright attributes. This study demonstrated that VOCs and FAAs are associated with positive effects on the flavor of smoked bacon. Additionally, multi-block analysis showed the relationship between sensory attributes and VOCs and FFAs.

Keywords: Bacon; Meat product; Taste; Aroma; VOCs; Rate-all-that-apply; Sensory analysis

4.1. Introduction

Smoking process have been used since ancient times to preserve and to extend the shelf-life of meat products (Hitzel, Pöhlmann, Schwägele, Speer, & Jira, 2013). However, in recent years, this technique has also been used to provide some desirable sensory characteristics to products (Saldaña, Saldarriaga, Cabrera, Siche, et al., 2019). One of the most consumed smoked meat products worldwide is smoked bacon, as it presents particular chemical and sensory characteristics (Saldaña, Saldarriaga, Cabrera, Behrens, et al., 2019), originating from its processing as well as pork belly and additives used during its manufacture (Leroy, Geyzen, Janssens, De Vuyst, & Scholliers, 2013; Montanari et al., 2018). The generation of volatile organic compounds (VOCs) and free amino acids (FAA) (Marušić, Petrović, Vidaček, Petrak, & Medić, 2011; Saldaña, Saldarriaga, Cabrera, Siche, et al., 2019; S. Zhang et al., 2017) responsible for bacon flavor are associated with complex biochemical reactions in the meat matrix,

specifically lipid and protein oxidation (Domínguez, Purriños, et al., 2019; Flores, 2010).

In this regard, several groups of VOCs have been reported in bacon and can be grouped according to their chemical family. Phenolic (e.g. guaiacol, creosol, 2-methylphenol) and furan (e.g. 2-penthyl, 2-ethyl furan) compounds are the main VOCs formed during the smoking process that result in woody/smoky flavors and green, fruity, sweet, vegetable aromas with roasted notes, respectively (García-González, Tena, Aparicio-Ruiz, & Morales, 2008; Li et al., 2018; Lorenzo, Franco, & Carballo, 2014; Saldaña, Saldarriaga, Cabrera, Siche, et al., 2019). Other VOC groups such as aldehydes are linked to lipid oxidation and are mainly related to a fatty aroma (Domínguez, Purriños, et al., 2019; Gomez, Dominguez, Fonseca, & Lorenzo, 2015; Lorenzo, Carballo, & Franco, 2013). Alcohols such as 1-hexanol and 1-pentanol are produced from lipid and amino acid oxidation, and are responsible for the herbal/fatty and sweet/fruity odor, respectively (Calkins & Hodgen, 2007; de Carvalho et al., 2020; Yang et al., 2017). The origin of ketonic compounds is diverse; most commonly they are formed by the oxidation of lipids; however they can also be formed by the Maillard reaction and the esterification of microorganisms. These compounds have a great influence on the aroma of meat products and, when present in high concentrations can generate butter, blue cheese and spicy notes (García-González et al., 2008; Narváez-Rivas, Gallardo, & León-Camacho, 2012; Novelli, Gandemer, Meynier, Zanardi, & Chizzolini, 1995). Therefore, analysis of volatile compounds during storage of the meat product can help to understand the formation of flavor in the final product.

Considering that bacon is stored before consumption, it is necessary to analyze the compounds (VOCs and FAA) generated during this period and, even more relevant, associate them with its sensory profile (Li et al., 2018). During the storage of meat products endogenous enzymes (calpain and cathepsin) break down proteins (sarcoplasmic and myofibrillar) into small peptides and FAA (non-volatiles compounds) (Cittadini et al., 2020; Garrido, Domínguez, Lorenzo, Franco, & Carballo, 2012; Lorenzo, Fonseca, Gómez, & Domínguez, 2015; Toldrá, 2008). In many studies, FAAs were positively correlated with the aforementioned sensory properties (Lorenzo, Cittadini, Bermúdez, Munekata, & Domínguez, 2015; Zhang et al., 2017; Zhou et al., 2017). The formation of FAAs in meat products during storage is accelerated and their concentration generally exceeds the identification threshold, influencing the final flavor

of the product (Zhao, Schieber, & Gänzle, 2016). These compounds can be associated with specific notes; for example, alanine, glycine and serine are related to a sweet taste, while aspartic and glutamic acid are typically related to acidic and an umami taste (Zhang & Zhao, 2017).

It is known that the VOCs and FAA generated during smoking and storage improve the sensory profile, which is the main driver of liking of bacon. Therefore, to deeply understanding the characteristics of bacon, its necessary make an association between chemical compounds and sensory data. To do so, new sensory methodologies have been used, enabling quick and effective capturing of product characteristics. The Rate-all-that-apply (RATA) method is an evolution of the Check-all-that-apply (CATA) questionnaire, which makes it possible to characterize the product based on a predefined attribute list, and to obtain attribute intensity information considering the applicability to the product (Ares et al., 2014; Varela & Ares, 2016).

The objectives of this research were to investigate changes in VOCs and FAA during the refrigerated storage of bacon smoked with *Eucalyptus citriodora*. In addition, the study aimed to find associations between these changes, the bacon's sensory characteristics and overall liking of bacon samples using the RATA questionnaire and a hedonic scale. From the presented objectives, the hypotheses proposed are:

- i) The main VOCs responsible for the sensory characteristics of bacon are related to the smoking process and lipid oxidation;
- ii) FAA content increases during storage as a result of proteolysis, contributing significantly to the bacon's flavor development;
- iii) Changes in the VOC and FAA profiles modify the sensory profile and the liking of smoked bacon.

4.2. Material and Methods

4.2.1. Bacon samples

Bacon smoked with *Eucalyptus citriodora* was chosen for this research because our previous study (Saldaña, Saldarriaga, Cabrera, Siche, et al., 2019) indicated that the use of this wood for smoking reduced lipid oxidation and improved the profile of volatile organic compounds, resulting in a high sensory acceptance (Merlo et al., 2020). For this reason, smoked bacon was kept at 5 °C and then evaluated at five storage

intervals (0, 15, 30, 45 and 60 days). The temperature and storage time were chosen based on shelf-life (60 days) and storage temperature (up to 8 °C) commonly used by the Brazilian pork meat industry.

Smoked bacon samples were manufactured with pork bellies, which were injected with a brine (1.5% salt, 0.6% sucrose, 0.03% sodium nitrite, 0.3% sodium tripolyphosphate and 0.07% sodium erythorbate) corresponding to 10% of the weight of the pork bellies. Samples were cooled for 24 h at 5 °C. Then, they were put through the natural smoking process using *Eucalyptus citriodora* wood under the same conditions reported in a previous study (Saldaña, Saldarriaga, Cabrera, Behrens, et al., 2019). Finally, the bacon samples were cooled (12 h at 2 °C), cut into pieces of ~500 g, vacuum sealed and refrigerated (5 °C) for 60 days.

As shown in Figure 1, a reverse storage design was applied to evaluate the stored samples at the same time (Hough, 2010). After each storage interval was reached, the smoked bacon was removed from refrigerated storage (5 ± 1 °C) and was stored at -20 °C, without exposure to light to ensure minimal changes. The day before the chemical and sensory analysis, the bacon samples were thawed at 5 °C.

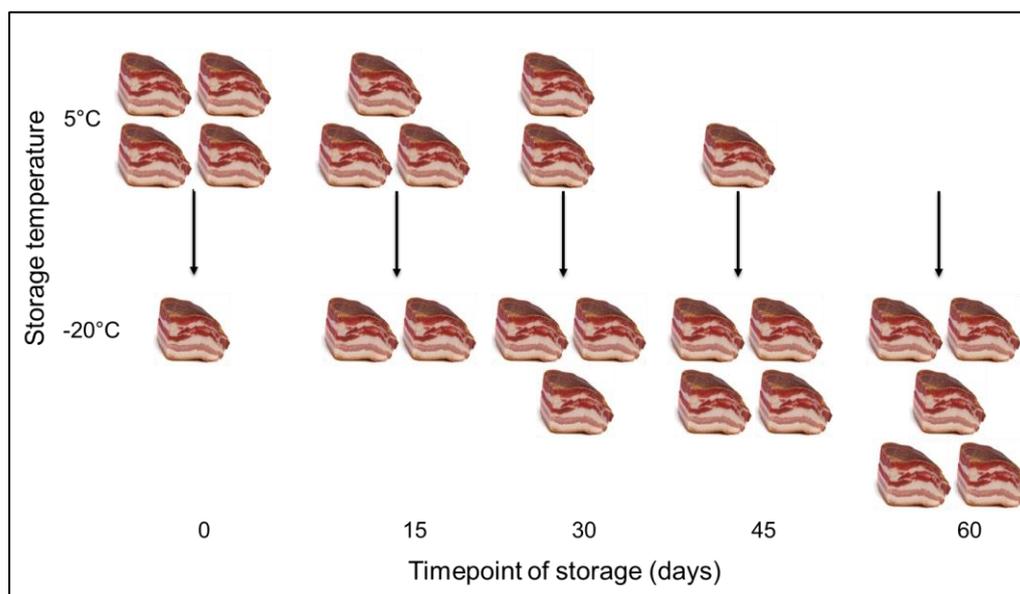


Figure 1. Reverse storage design for smoked bacon samples.

4.2.2. Volatile organic compounds profile (VOCs)

Extraction of VOCs was performed using solid phase microextraction (SPME) and identification was done using gas chromatography (CG) (7890B model, Agilent Technologies, Santa Clara, CA, USA) coupled with mass spectrometry (MS) (5977B

model, Agilent Technologies). A SPME device containing a fused silica fiber (10 mm length), coated with a 50/30 ratio divinylbenzene/carboxen/polydimethylsiloxane (Supelco, Bellefonte, USA), was used. Ground samples (~1g) were put into 20 mL vials and sealed using a laminated Teflon-rubber disc. Extraction, conditioning, injection, chromatographic conditions and identification of volatiles compounds was performed as described by Domínguez et al. (2019). The results were expressed as quantifier area units (AU)·10³ g⁻¹ of sample.

4.2.3. Free amino acid profile

FAA extraction was performed following the protocol described by (Lorenzo, Cittadini, et al., 2015), with some modifications. Five grams of sample were homogenized with 25 mL 0.1N HCl in Ultra-Turrax (T25 digital, IKA) for 8 min, maintaining the homogenate under refrigerated conditions. The homogenized samples were centrifuged (20 min, 4 °C, 10.000 g) and the supernatant was filtered through glass wool. Deproteinization was performed by adding 800 µL of acetonitrile to 200 µL of the filtrate, leaving the mixture to stand for 30 min under refrigeration. The deproteinized sample was then centrifuged (5 min, 4 °C, 10.000 g) and filtered through 0.45 µm filters. FAAs were derivatized using a Waters AccQ-Fluor reagent kit (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and their concentration was determined using high-pressure liquid chromatography (2695 model, Waters).

Chromatographic separation was performed using a Waters Accq-Tag column (3.9 x 150 mm) as the stationary phase. Methods used for gradient elution, HPLC conditions and fluorescence detector parameters for amino acid analysis were previously published (Munekata et al., 2020). The results were expressed as mg of FAA per 100 g of dry matter.

4.2.4. Microbiological analysis

Before the sensory analysis, the pork bellies and smoked bacon (0 day) were subjected to microbiological analysis to verify their hygienic quality. The presence or absence of *Salmonella* sp. was performed by using the Biocontrol™ 1-2 test kit and coagulase positive *Staphylococcus* (CFU g⁻¹) counts were performed using the conventional method described by Lancette & Bennett (2001).

4.2.5. Sensory analysis

A total of 109 habitual bacon consumers participated voluntarily in the study (61% woman and 39% man, ages ranging from 18 to 65 years). Participants consisted of undergraduate and graduate students, as well as employees from the *Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ)*, Piracicaba - SP (Brazil). Consumers were recruited through social media advertisements and the criterion to participate were being a frequent consumer of bacon and availability to participate in the sensory tests.

Smoked bacon samples were cut into slices (2 mm thick) and then cooked using an electric plate (250 °C) (Edanca, Brazil) for about 2 min on each side to achieve the temperature of 80 °C. After cooking, the bacon samples were cut into pieces of 2 cm and kept in an oven at 50 °C to maintain the temperature prior to the sensory test. Before starting the formal test, participants received instructions for 10 min to understand the mechanism behind the RATA questionnaire, using the same attribute list and data collection software.

The formal test was performed in a single session. Samples were monadically presented to the consumers using 50 mL disposable cups coded with three-digit random numbers following a balanced way. Water and biscuits were offered to consumers to clean their palate. First, the consumers rated their overall liking using a 9-point hedonic scale ranging from 1 = dislike extremely - 9 = like extremely (Peryam & Pilgrim, 1957). Subsequently, they selected the attributes they considered appropriate for describing the target sample and then rated its applicability using a 5-point scale, with anchors at 1 = slightly applicable and 5 = very applicable (Varela & Ares, 2016; Ventanas, González-Mohino, Estévez, & Carvalho, 2020). The attributes (smoky, crunchy, salty, soft, bright, fatty, dry, hard, rancid, woody, succulent and fibrous) were selected based on previous studies in the same product category (Saldaña et al., 2018; Saldaña, Saldarriaga, Cabrera, Behrens, et al., 2019; Saldaña, Saldarriaga, Cabrera, Siche, et al., 2019) and were presented following a Williams' Latin square design; that is, the order of presentation of attributes was different for each sample and participant (Ares et al., 2014; Williams, 1949). Data collection was performed using tablets (Samsung Tab E/SM-T560/9.6") and *Compusense Cloud* software (Compusense Inc., Guelph, Ontario, Canada).

4.2.6. Statistical analysis

In this study, bacon samples were evaluated from the five different storage intervals, following 0 (SB0), 15 (SB15), (SB30), 45 (SB45) and 60 (SB60) days of storage using a reverse design. The univariate analyzes were performed in the R software (R Development Core Team, 2017), while the multivariate analyses were performed in XLSTAT. Data from the VOCs and FAAs were submitted to an analysis of variance (ANOVA) considering sample as a factor, and when applicable, the Tukey test was used at 5% significance. A Principal Component Analysis (PCA), based on the Pearson correlation matrix, was applied to multidimensionally study the evolution over time of both VCOs and FAAs (de Almeida et al., 2018).

For the RATA data, intensities ranging from 0 to 5 were treated as continuous data, allowing for the application of parametric methods as recommended by Meyners, Jaeger, & Ares, (2016). Liking and RATA results were evaluated through a mixed ANOVA, considering consumers as a random factor and samples as a fixed factor, followed by a Tukey test ($P < 0.05$) for post-hoc comparison. To complement the ANOVA of RATA data, a PCA was performed to obtain a multivariate relationship between samples and sensory attributes, using a Pearson correlation matrix as well. Multiple factor analysis (MFA) was applied to investigate the relationship between the VOCs, FAAs, Sensory, and liking considering each data type as an independent matrix, eliminating any differences inherent to the nature of each table (Rios-Mera et al., 2020).

4.3. Results and discussion

4.3.1. VOCs profile

At the end of the storage period, a total of 69 volatile organic compounds were found in the smoked bacon samples. These compounds were formed from the smoking process and the degradation of lipids and proteins through chemical reactions (Gomez et al., 2015; Nachtigall et al., 2019; Saldaña, Saldarriaga, Cabrera, Siche, et al., 2019; Toldrá & Flores, 1998). The volatile compounds were classified as part of some of the main chemical families according to previous studies (Domínguez, Purriños, et al., 2019; Li et al., 2018; Lorenzo & Carballo, 2015). As shown in Table 1, the chemical families are comprised of 15 aldehydes, 10 alcohols, 8 acids, 11 ketones, 3 alkanes, 7

hydrocarbons, 2 esters, 2 furans and others. It was not possible to observe a linear increasing or decreasing trend for the different individual VOCs. The same fluctuations in bacon VOCs during refrigerated storage were previously observed by other authors (Li et al., 2018). The aldehydes, alcohols, ketones and acids were the chemical families with the highest concentration. Aldehydes comprised the most numerous chemical family among the different classifications. This family is among the main volatile compounds that contribute to the flavor of cured and smoked products, due to their high (rancid aroma) or low (grass aroma) concentrations (Domínguez, Gómez, Fonseca, & Lorenzo, 2014; Domínguez, Purriños, et al., 2019; Lorenzo et al., 2013; Nachtigall et al., 2019). In this study, 15 aldehydes (mainly the linear aldehydes including hexanal, butanal, propanal, octanal, nonanal and heptanal) were present in large amounts in the early and middle stages of storage, which ranged from 58.56% (minimum) to 64.54% (maximum) of the total area. Linear aldehydes can be produced by lipid oxidation; for example, hexanal is formed by the oxidation of linoleic acid, while the oxidation of oleic acid generates octanal and nonanal (Domínguez, Gómez, Fonseca, & Lorenzo, 2014; Domínguez, Pateiro, et al., 2019; Lorenzo & Domínguez, 2014; Parker, Elmore, & Methven, 2015; Sun, Zhao, Zhao, Zhao, & Yang, 2010). A previous study also identified high aldehyde concentrations in bacon smoked with eucalyptus (Saldaña, Saldarriaga, Cabrera, Siche, et al., 2019).

Ketones are also one of the major chemical families that influence the flavor of the product as result from lipid oxidation, alkane degradation and dehydrogenation of alcohols by bacteria (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015; Resconi, Escudero, & Campo, 2013). However, in this study, these compounds had low concentrations, ranging from 4.72 to 8.33% of the total area, being below the concentrations previously reported by other researchers (Li et al., 2018; Saldaña, Saldarriaga, Cabrera, Siche, et al., 2019). Even at low concentrations, these compounds can intensify the flavor and confer butter, spice and blue cheese notes to the product (Lorenzo & Domínguez, 2014; Lustre & Issenberg, 1970; Novelli et al., 1995).

Table 1. Volatile organic compounds (VOCs) of smoked bacon with *Eucalyptus citriodora* wood during refrigerated storage (5 ± 1 °C) expressed as area units (AU) x 10^3 /g of sample.

VOCs	Code	RT	LRI	m/z	Storage time (days)					SEM
					Day 0	Day 15	Day 30	Day 45	Day 60	
Aldehydes										
Hexanal***	A1	18.57	856	56	31794.75 ^a	21016.89 ^b	29107.22 ^a	20719.62 ^b	18254.29 ^b	874.05
Butanal**	A2	5.68	594	72	418.08 ^{ab}	218.95 ^c	427.93 ^a	259.59 ^c	281.20 ^{bc}	31.75
Propanal**	A3	3.09	541	58	7394.62 ^a	5837.34 ^b	7089.51 ^a	7413.24 ^a	5957.57 ^b	172.60
Octanal**	A4	27.78	1043	55	139.48 ^a	51.71 ^b	157.34 ^a	64.48 ^b	70.37 ^b	13.44
Nonanal*	A5	31.54	1120	98	38.50 ^{ab}	25.46 ^b	75.50 ^a	42.54 ^{ab}	44.25 ^{ab}	8.37
Heptanal**	A6	23.54	957	70	1256.58 ^{ab}	613.92 ^c	1469.45 ^a	907.55 ^{bc}	972.70 ^{bc}	99.10
2-Hexenal, (E)-*	A7	21.89	923	69	220.75 ^a	78.09 ^b	242.21 ^a	77.00 ^b	132.55 ^{ab}	30.60
Butanal, 3-methyl-**	A8	9.04	662	58	4.83 ^c	14.89 ^{bc}	21.48 ^{ab}	8.40 ^c	29.59 ^a	2.82
6-Nonenal, (E)-*	A9	33.97	1169	96	20.16 ^{ab}	3.86 ^b	40.54 ^a	14.32 ^b	13.56 ^b	4.89
2-Nonenal, (E)-**	A10	33.96	1169	55	38.46 ^b	9.02 ^b	81.60 ^a	28.81 ^b	26.33 ^b	9.60
2-Pentenal, (E)-**	A11	16.67	817	84	526.19 ^{ab}	251.91 ^c	707.69 ^a	745.15 ^a	417.80 ^{bc}	50.62
tert-Butyl Hydroperoxide***	A12	19.88	883	59	965.01 ^{bc}	835.97 ^c	1432.32 ^b	2462.23 ^a	1115.24 ^{bc}	125.86
2-Butenal, 2-ethyl-*	A13	20.52	896	98	24.95 ^{ab}	19.68 ^{ab}	16.57 ^b	35.43 ^a	11.45 ^b	3.58
2,4-Heptadienal, (E,E)-**	A14	28.35	1055	81	426.38 ^a	159.40 ^b	389.11 ^a	383.24 ^a	178.35 ^b	44.25
2-Heptenal, (E)-***	A15	26.46	1016	83	2812.33 ^a	833.11 ^b	3021.83 ^a	1443.09 ^b	1267.95 ^b	161.73
Total					46081.07	29970.20	44280.30	34604.69	28773.20	
Total area (%)					58.56	65.09	59.35	61.55	64.54	
Alcohol										
1-Butanol, 2-methyl-**	AL1	21.14	908	55	51.56 ^a	12.27 ^b	22.67 ^b	12.83 ^b	17.71 ^b	3.69
Glycidol**	AL2	1.97	519	44	450.75 ^b	307.23 ^b	694.08 ^a	447.30 ^b	423.18 ^b	39.43
1-Hexanol***	AL3	22.65	939	56	141.39 ^a	41.82 ^b	134.50 ^a	64.26 ^b	47.15 ^b	9.19
1-Butanol**	AL4	11.23	707	56	94.82 ^{ab}	44.19 ^c	113.57 ^a	69.08 ^{bc}	56.66 ^{bc}	8.63
1-Pentanol***	AL5	17.70	838	55	2454.46 ^a	960.13 ^b	2106.28 ^a	1206.16 ^b	796.43 ^b	94.61
2-Propanol, 1-methoxy-**	AL6	10.94	701	45	120.39 ^b	155.42 ^b	157.43 ^b	728.80 ^{ab}	945.46 ^a	137.73
5-Methyl-1-heptanol ^{ns}	AL7	29.85	1085	83	73.54 ^a	20.93 ^a	91.88 ^a	26.14 ^a	22.94 ^a	21.30
4-Ethylcyclohexanol**	AL8	29.51	1078	81	185.66 ^a	33.62 ^b	41.93 ^b	32.10 ^b	59.53 ^b	14.50
1-Heptanol, 4-methyl-*	AL9	30.57	1100	84	14.88 ^a	4.47 ^c	13.50 ^{ab}	8.38 ^{abc}	6.17 ^{bc}	1.80
1-Octen-3-ol***	AL10	27.09	1029	57	4838.15 ^a	1368.38 ^c	3354.36 ^b	1534.30 ^c	1492.08 ^c	158.51
Total					8425.60	2948.46	6730.20	4129.35	3867.31	
Total area (%)					10.69	6.39	9.00	7.33	8.67	
Acids										
Acetic acid***	AC1	10.44	691	60	708.53 ^b	107.79 ^c	1399.20 ^a	673.70 ^b	450.87 ^b	61.46

Butanoic acid ^{ns}	AC2	20.94	904	60	29.02 ^a	22.20 ^a	65.10 ^a	45.80 ^a	27.57 ^a	9.93
Hexanoic acid [*]	AC3	28.61	1060	60	1967.96 ^{ab}	81.45 ^b	4663.82 ^a	921.55 ^b	877.44 ^b	816.79
Pentanoic acid ^{**}	AC4	24.96	986	60	221.48 ^{ab}	9.08 ^c	396.17 ^a	100.59 ^{bc}	57.46 ^{bc}	41.14
Propanoic acid ^{***}	AC5	16.69	818	74	994.50 ^b	77.93 ^e	1345.93 ^a	657.29 ^c	278.25 ^d	27.53
Acetic anhydride ^{**}	AC6	3.19	543	43	752.20 ^a	794.01 ^a	615.59 ^{ab}	537.60 ^{bc}	412.55 ^c	43.89
Hexanoic acid, anhydride ^{ns}	AC7	31.03	1109	99	154.14 ^a	21.80 ^a	275.04 ^a	49.72 ^a	36.21 ^a	59.82
Propanoic acid, 2-methyl-, anhydride ^{ns}	AC8	3.84	557	71	3.81 ^a	11.85 ^a	6.10 ^a	7.35 ^a	4.99 ^a	2.70
Total					4831.64	1126.11	8766.95	2993.60	2145.34	
Total area (%)					6.13	2.44	11.73	5.32	4.81	
Ketones										
1-Penten-3-one [*]	K1	11.62	715	55	1500.52 ^a	339.90 ^c	1322.54 ^{ab}	555.92 ^{bc}	567.13 ^{bc}	207.62
3-Penten-2-one [*]	K2	15.79	799	69	400.14 ^a	255.97 ^{ab}	498.25 ^a	430.67 ^a	135.49 ^b	58.59
2-Pentanone ^{***}	K3	11.85	719	86	103.80 ^a	35.86 ^b	104.12 ^a	41.98 ^b	38.05 ^b	7.59
2,3-Pentanedione ^{**}	K4	12.53	733	100	492.02 ^b	1210.86 ^a	457.93 ^b	1302.99 ^a	489.54 ^b	123.79
Cyclobutanone, 2-methyl- ^{***}	K5	30.57	1100	69	16.61 ^a	5.86 ^c	13.45 ^a	9.47 ^b	5.20 ^c	0.74
3,5-Octadien-2-one ^{***}	K6	30.93	1107	95	2264.62 ^a	980.37 ^b	1153.91 ^b	1819.44 ^a	473.55 ^c	103.47
2-Heptanone ^{**}	K7	23.23	951	58	313.98 ^a	47.34 ^c	192.19 ^{ab}	73.35 ^{bc}	49.98 ^{bc}	32.53
1-Octen-3-one ^{**}	K8	26.86	1024	55	604.42 ^a	179.90 ^b	466.35 ^a	224.68 ^b	205.90 ^a	42.47
2-Butanone [*]	K9	6.10	603	72	80.21 ^a	82.04 ^a	64.19 ^{ab}	58.54 ^{ab}	40.52 ^b	8.39
Methyl Isobutyl Ketone ^{***}	K10	15.30	790	58	83.58 ^a	17.81 ^b	6.09 ^b	5.95 ^b	11.67 ^b	6.16
Acetophenone ^{ns}	K11	31.08	1110	105	192.91 ^a	119.56 ^a	138.23 ^a	158.48 ^a	87.88 ^a	25.32
Total					6079.51	3275.47	4417.25	4681.47	2104.91	
Total area (%)					7.73	7.11	5.92	8.33	4.72	
Alkanes										
Pentane ^{**}	AK1	2.67	533	57	196.95 ^b	475.06 ^b	441.58 ^b	948.73 ^a	1301.74 ^a	96.72
Octane ^{***}	AK2	16.55	815	85	1346.34 ^a	437.49 ^b	493.35 ^b	349.63 ^b	504.40 ^b	65.09
Heptane ^{***}	AK3	9.74	677	71	809.51 ^a	282.66 ^c	444.76 ^b	396.33 ^{bc}	465.56 ^b	34.59
Total					2352.80	1195.21	1379.69	1694.69	2271.70	
Total area (%)					2.99	2.59	1.85	3.01	5.09	
Hydrocarbons										
Dimethyl ether ^{***}	H1	2.72	534	45	1175.53 ^b	2204.30 ^a	859.01 ^c	1208.19 ^b	777.96 ^c	61.75
Cyclopropane, pentyl- ^{**}	H2	16.24	809	55	27.16 ^a	10.48 ^b	29.48 ^a	16.29 ^b	13.05 ^b	2.02
Hexane, 2,2-dimethyl- ^{ns}	H3	8.90	659	57	45.43 ^a	84.94 ^a	41.67 ^a	89.67 ^a	47.30 ^a	13.22
Butane, 2-cyclopropyl- ^{***}	H4	23.28	952	55	156.17 ^a	20.54 ^c	71.95 ^b	32.50 ^{bc}	24.42 ^c	9.81
Cyclobutane ^{ns}	H5	1.85	516	56	6.79 ^a	10.70 ^a	7.84 ^a	8.61 ^a	9.59 ^a	2.50
1-Heptene [*]	H6	9.36	669	55	20.83 ^{ab}	10.68 ^b	24.85 ^a	14.35 ^{ab}	12.76 ^{ab}	3.07
1-Hexene ^{***}	H7	4.53	571	55	15.83 ^b	11.68 ^b	45.47 ^a	18.96 ^b	13.85 ^b	2.58

Total					1447.74	2353.32	1080.27	1388.57	898.93	
Total area (%)					1.84	5.11	1.45	2.47	2.02	
Esters										
Acetic acid, methyl ester*	E1	3.65	553	74	25.40 ^a	24.99 ^a	19.07 ^{ab}	18.12 ^{ab}	16.74 ^b	1.70
Ethanedioic acid, dimethyl ester*	E2	21.37	913	59	111.36 ^{ab}	64.92 ^b	77.15 ^b	157.22 ^a	68.72 ^b	17.35
Total					136.76	89.91	96.22	175.34	85.46	
Total area (%)					0.17	0.19	0.13	0.31	0.19	
Furans										
2-n-Butyl furan*	F1	22.15	929	81	52.36 ^a	15.72 ^b	42.75 ^{ab}	22.36 ^{ab}	16.29 ^b	7.23
Furan, 2-ethyl- ^{ns}	F2	11.06	703	81	212.83 ^a	135.51 ^a	243.83 ^a	250.94 ^a	152.17 ^a	42.46
Total					265.19	151.23	286.58	273.30	168.46	
Total area (%)					0.34	0.33	0.38	0.49	0.38	
Others										
2-Propanamine ^{ns}	O1	12.18	726	58	4924.86 ^a	2528.44 ^a	4825.40 ^a	2837.80 ^a	2613.51 ^a	595.41
2,3-Butanedione**	O2	5.85	597	86	315.77 ^b	915.52 ^a	389.52 ^b	962.19 ^a	584.63 ^{ab}	101.16
Methanethiol**	O3	2.09	521	48	25.52 ^b	51.16 ^a	16.39 ^b	27.36 ^b	15.95 ^b	3.64
2(3H)-Furanone, ethyldihydro-***	5-O4	32.00	1129	85	175.83 ^a	42.93 ^d	140.50 ^b	99.28 ^c	47.71 ^d	3.90
3-Octen-2-one**	O5	29.63	1081	111	3005.66 ^a	1189.07 ^{bc}	1613.17 ^b	1766.55 ^b	676.16 ^c	190.97
1H-Imidazole, 4,5-dihydro-2-methyl-**	O6	15.96	803	83	143.60 ^{abc}	49.77 ^c	190.38 ^{ab}	231.63 ^a	95.01 ^{bc}	24.40
4-Hexen-3-one, 5-methyl- ^{ns}	O7	27.62	1040	83	24.47 ^a	5.62 ^a	33.65 ^a	12.66 ^a	6.69 ^a	9.45
4-Pyridinecarboxamide*	O8	31.87	1126	82	53.29 ^{ab}	25.28 ^{abc}	23.30 ^{bc}	57.57 ^a	10.53 ^c	7.49
4-Penten-1-ol, propanoate ^{ns}	O9	18.11	847	67	19.74 ^a	10.95 ^a	12.99 ^a	12.77 ^a	11.89 ^a	2.34
5-Ethylcyclopent-1-enecarboxaldehyde ^{ns}	O10	29.28	1074	67	106.53 ^a	60.75 ^a	89.76 ^a	53.35 ^a	119.69 ^a	19.33
(E)-4-Oxohex-2-enal***	O11	31.40	1117	83	264.50 ^a	54.41 ^b	231.18 ^a	211.66 ^a	83.08 ^b	13.81
Total					9059.77	4933.90	7566.24	6272.82	4264.85	
Total area (%)					11.51	10.73	10.14	11.17	9.57	

^{a-d} Mean values in the same row with different letters indicate significant difference according to Tukey's test at 5% significance (*:p<0.05; **:p<0.01; ***:p<0.001; ns: non significant). SEM: Standard error of the mean.

RT: retention time; LRI: Lineal Retention Index calculated for DB-624 capillary column (J&W scientific: 30m×0.25mm id, 1.4 μm film thickness) installed on a gas chromatograph equipped with a mass selective detector; m/z: Quantification ion.

The first two dimension of PCA explained 78.25% of the variance (Figure 2). The right side of the first dimension (59.86%) showed a high VOC content to be correlated with the SB0 and SB30 samples. The major chemical families of these compounds that resulted from the smoking process and lipid oxidation were thus found to be aldehydes, alcohols, acids, ketones, and hydrocarbons (Yang et al., 2017), confirming the first hypothesis.

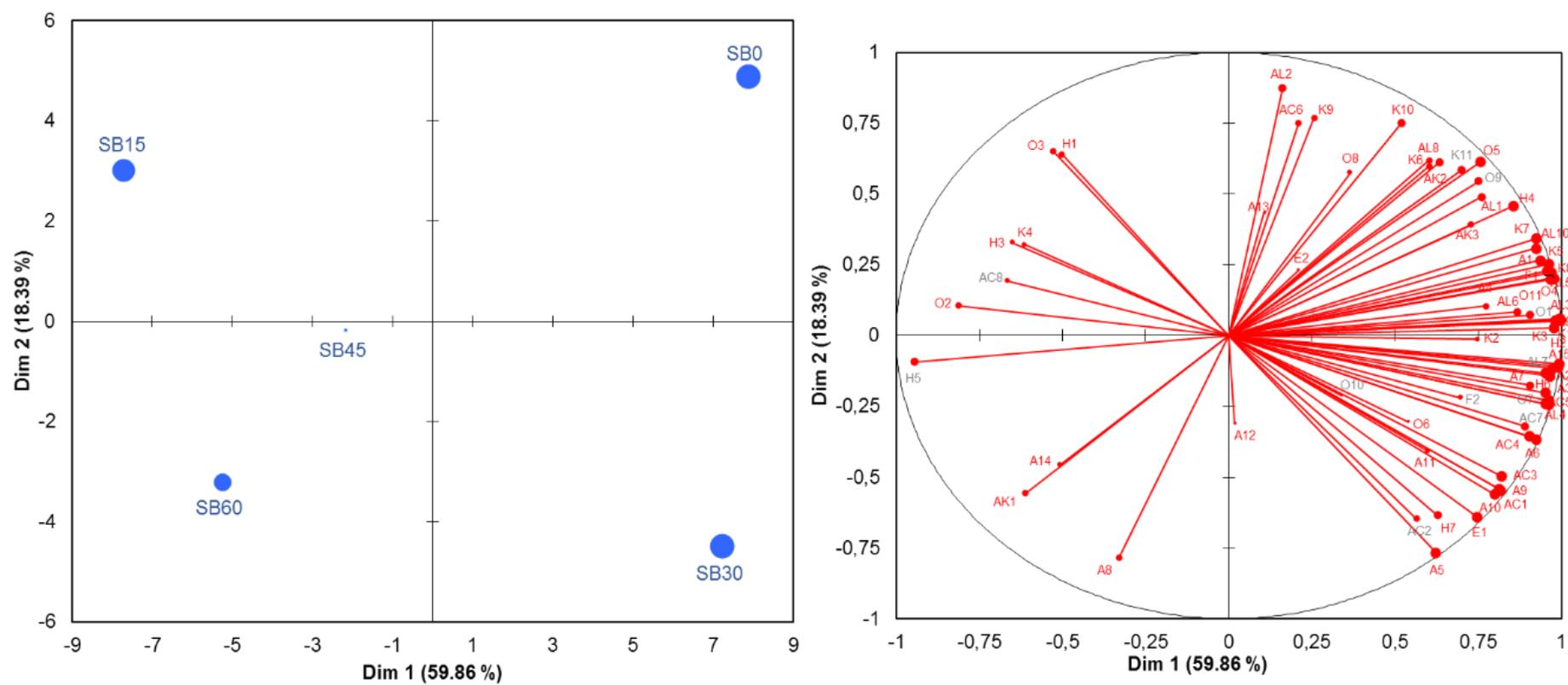


Figure 2. Representation of volatile organic compounds based on the PCA.

Since aldehydes with more than five carbons (hexanal, heptanal, octanal, nonanal) are found in samples SB0 and SB30, these may have a more pronounced fatty aroma compared to the other samples (García-González et al., 2008). It is well-known that alcohols contribute to the characteristic flavor of meat products (Domínguez, Purriños, et al., 2019; Narváez-Rivas et al., 2012). According to the PCA, the SB0 sample accounting the highest content of alcohols. The 1-Octen-3-ol (AL10) was the most abundant alcohol produced through the oxidation of polyunsaturated fatty acids, such as linoleic acid. Some authors report that this compound is associated with mushroom, fatty and rancid notes (García-González et al., 2008; Lorenzo & Domínguez, 2014; Novelli et al., 1995).

The hexanoic and acetic acids showed high concentrations in the SB30 sample. Acetic acid is one of the main acids found in meat products and is responsible for the cured flavor of meat products (Domínguez, Purriños, et al., 2019; Montanari et al., 2018; Yang et al., 2017).

Overall, samples stored up to 30 days showed more VOCs than the other storage times. A possible explanation of this behavior is that by 30 days, the main biochemical changes such as lipid oxidation, which increase the formation of VOCs, have already occurred (Alfaia et al., 2010; Domínguez, Pateiro, et al., 2019). However, the concentration of VOCs was maintained stable in the SB45 and SB60 samples, which is explained by the procedure having involved vacuum packaging and cold storage (Soladoye et al., 2017). Another factor that may have influenced the non-formation of VOCs along the storage timeline was the conversion of aldehydes to alcohols (propanol and hexanol) before they could accumulate (North, Dalle Zotte, & Hoffman, 2019; Woolf et al., 2009). This explains the decrease in hexanal content during refrigerated storage.

4.3.2. Free amino acid profile

The changes in FAA content (expressed as mg/100 g of dry matter) during storage are shown in Table 2. Arginine, alanine, glutamic acid, leucine and lysine were the most abundant and representative FAAs in each sample analyzed (i.e., for each different time interval). A significant increase ($p < 0.05$) in most FAA occurred after 30 days of storage. In fact, FAA increased from 114.55 to 342.22 mg/100 g of bacon from

the first to the last storage day, confirming our second hypothesis. The increase of FAA with storage time is mainly explained by proteolysis (Garrido et al., 2012; Lorenzo, Cittadini, et al., 2015; Toldrá, 2008). Proteolysis is the break-down of proteins into smaller molecules (peptides, FAA and aldehydes) by the action of endogenous enzymes such as cathepsins/calpains and microorganisms present in raw meat and increased by meat processing and the incorporation of salt ions, among other factors. The main product of the proteolysis, i.e., the FAA, are fundamental for the development of the flavor of smoked bacon (Calkins & Hodgen, 2007; Freiding, Gutsche, Ehrmann, & Vogel, 2011).

The FAA content at the end of the smoked bacon storage was lower than those reported by other authors for Cantonese bacon (Zhang et al., 2017), Chinese traditional bacon (Gan et al., 2019), dry-cured Cecina (Cittadini et al., 2020; Lorenzo, Fonseca, et al., 2015) and dry-cured lacón (Garrido et al., 2012; Lorenzo, Cittadini, et al., 2015). This divergence may have been influenced by several factors, such as free amino acid content in fresh meat, curing time and process, salt content and storage temperature (Zhou et al., 2017).

It is important to note that arginine presented the highest content during the storage period, and its concentration was significantly higher ($p < 0.05$) after 30 days of storage. The presence of this FAA contributes to the bitter taste of the product (Domínguez, Munekata, Agregán, & Lorenzo, 2016). By the end of the storage period, the total content of desirable free amino acids that possess sweet and umami tastes was higher than those possessing bitter and acid tastes, being one of the reasons contributing to increase overall liking of the smoked bacon.

Table 2. Changes in free amino acids (FAA) content of smoked bacon during 60 days at 5 ± 1 °C from a univariate perspective.

FAA	Samples					SEM
	SB0	SB15	SB30	SB45	SB60	
Sweet taste (ST)						
Serine ^{ns}	7.58 ^a	6.18 ^a	7.25 ^a	8.72 ^a	7.21 ^a	0.68
Alanine ^{***}	11.78 ^b	11.96 ^b	32.58 ^a	40.31 ^a	34.30 ^a	2.04
Proline ^{**}	3.84 ^b	4.47 ^b	10.52 ^a	11.94 ^a	10.81 ^a	0.62
Threonine ^{**}	2.59 ^b	2.53 ^b	5.15 ^b	4.17 ^b	9.43 ^a	0.71
Glycine ^{***}	6.77 ^c	5.93 ^c	15.51 ^{ab}	12.10 ^b	16.27 ^a	0.88
Sub-total ST	32.56	31.07	71.01	77.24	78.02	
Bitter taste (BT)						
Leucine [*]	5.67 ^b	7.04 ^{ab}	7.77 ^{ab}	6.76 ^{ab}	8.85 ^a	0.66
Valine [*]	3.80 ^c	4.75 ^{bc}	6.85 ^{ab}	5.25 ^{abc}	7.78 ^a	0.58
Methionine [*]	1.94 ^b	2.48 ^{ab}	3.03 ^a	2.46 ^{ab}	2.94 ^{ab}	0.24
Isoleucine [*]	2.80 ^a	3.42 ^a	4.52 ^a	3.54 ^a	4.80 ^a	0.45
Sub-total BT	18.42	22.89	27.03	22.78	29.67	
Acid taste (AT)						
Phenylalanine ^{ns}	4.21 ^a	5.20 ^a	4.86 ^a	4.77 ^a	5.30 ^a	0.30
Aspartic acid ^{ns}	1.18 ^a	0.63 ^a	0.91 ^a	1.09 ^a	1.12 ^a	0.17
Glutamic acid ^{***}	3.67 ^b	6.46 ^b	13.10 ^a	15.57 ^a	14.40 ^a	0.93
Histidine ^{**}	3.99 ^c	2.84 ^c	13.21 ^a	9.29 ^b	13.90 ^a	0.92
Sub-total AT	8.84	9.93	27.22	25.95	29.42	
Cured flavor (CF)						
Lysine ^{**}	1.40 ^b	1.55 ^b	7.64 ^a	8.55 ^a	6.76 ^a	0.87
Tyrosine [*]	4.06 ^b	4.68 ^{ab}	4.88 ^{ab}	4.77 ^{ab}	5.33 ^a	0.26
Sub-total CF	5.46	6.23	12.52	13.32	12.09	
Others (O)						
Taurine ^{**}	16.69 ^c	22.70 ^{bc}	31.08 ^{ab}	32.10 ^a	32.61 ^a	2.06
Arginine ^{***}	31.90 ^b	30.85 ^b	161.30 ^a	52.30 ^b	158.43 ^a	6.98
Cysteine ^{***}	0.68 ^b	0.65 ^b	2.34 ^a	2.82 ^a	1.98 ^a	0.21
Sub-total O	49.27	54.20	194.72	87.22	193.02	
Total FAA	114.55	124.32	332.50	226.51	342.22	

Average values (mg 100 g⁻¹ of smoked bacon) within a row with different superscripts are significantly different according to Tukey's test at 5% significance (*:p<0.05; **:p<0.01; ***:p<0.001; ns: non significant). SEM: Standard error of the mean; SB0: smoked bacon in storage time 0 day; SB15: smoked bacon in storage time 15 days; SB30: smoked bacon in storage time 30 days; SB45: smoked bacon in storage time 45 days and SB60: smoked bacon in storage time 60 days.

As shown in Figure 3, the PCA accounted for 90.01% of the variance of the original data (Dim 1: 70.82%, Dim 2: 19.19%). The first dimension separated the samples based on storage time. As the storage time increased, the samples tended to the right of the PCA, being characterized by a high concentration of FAA. In addition, 2 groups of samples were observed, the first being comprised of samples with less storage time (SB0 and SB15), located in the negative part of the first dimension, while the second group was positioned in the positive part of the same dimension. Non-significant FAA vectors at the univariate level were positioned near the center of the first dimension, contributing in the same way to the first and second group of samples, corroborating similarity.

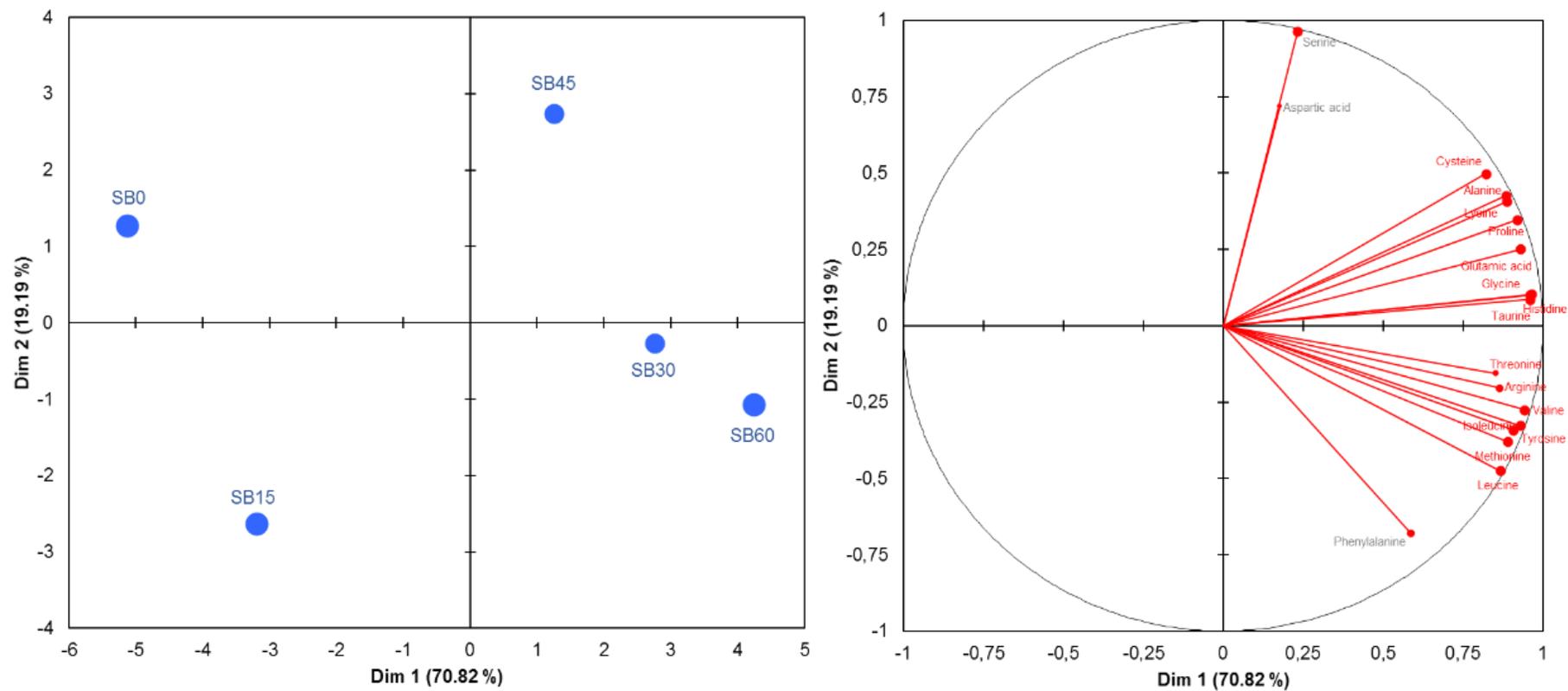


Figure 3. PCA on FFAs mean during refrigerated storage of smoked bacon.

Based on the PCA, we can affirm that as the storage time increases, the amount of FAAs in the smoked bacon increases accordingly. In this regard, a recent study suggests that muscle proteolysis and protein oxidation occur simultaneously (Gan et al., 2019). The protein oxidation generates protein aggregates through the formation of disulfide bonds, which can interfere with muscle proteolysis and, consequently, with the formation of FAA (Berardo, Claeys, Vossen, Leroy, & De Smet, 2015). However, the use of antioxidant compounds (nitrites and ascorbates) and vacuum packaging in meat products prevents the protein oxidation and enables a larger extent of proteolysis, increasing FAA concentration until the end of refrigerated storage (Villaverde, Ventanas, & Estévez, 2014), consistent with the findings of this study.

4.3.3. Overall liking and Rate-all-that-apply

The microbiological analysis performed in the pork belly and smoked bacon are within the limits established by Brazilian legislation (*Salmonella* sp. absence in 25 g of sample and for *Staphylococcus aureus*, a maximum of 5.0×10^2 CFU g⁻¹) (Brasil, 2001). This affirmed that the samples presented satisfactory sanitary quality for the subsequent sensory analysis.

Figure 4 represents the means of overall liking for smoked bacons stored for different lengths of times using an unstructured 9-cm hedonic scale. According to the ANOVA, no significant difference between samples was found. The means found were 6.40; 6.74; 6.65; 6.75 and 6.87 for smoked bacons stored at times 0, 15, 30, 45 and 60 days, respectively. This similarity partially contradicts the third hypothesis, because the overall liking is the same for all samples.

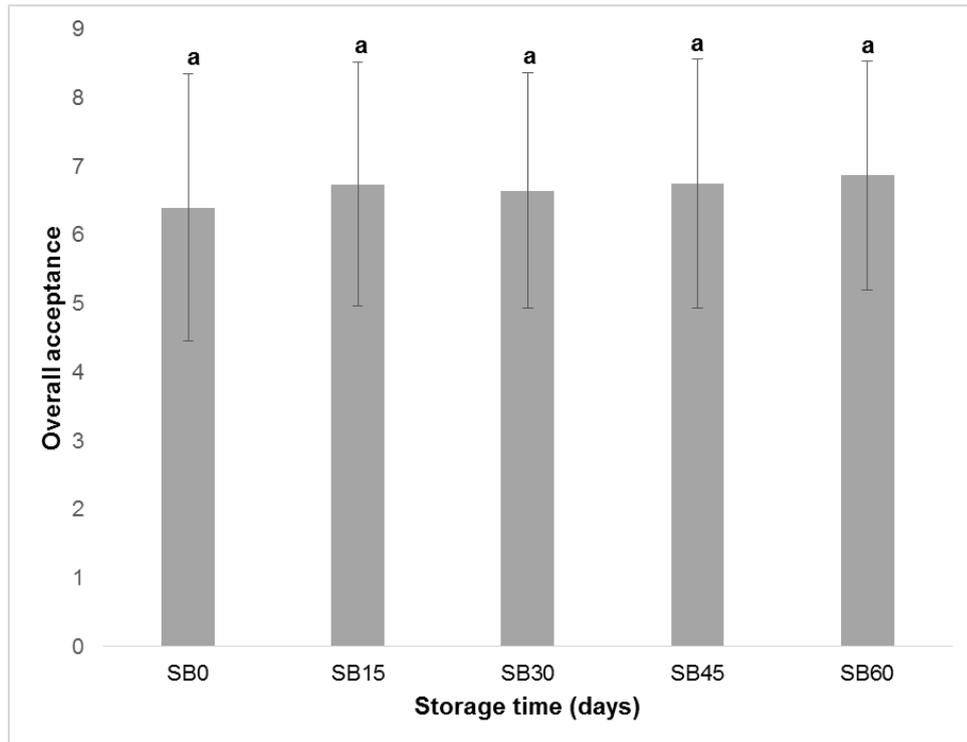


Figure 4. Overall acceptance for smoked bacon at different storage times. Error bars are the standard deviation.

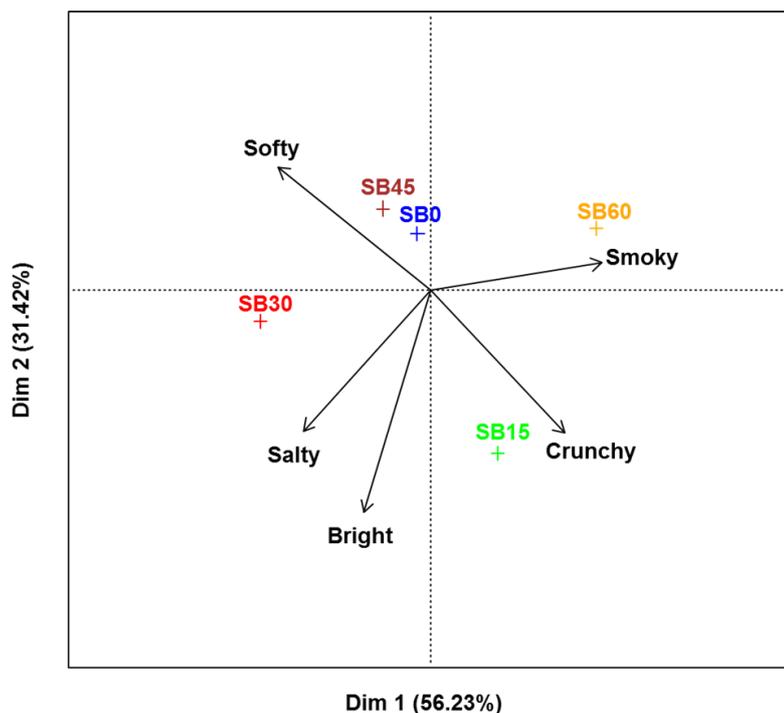
Thus, differences between samples could be demonstrated using the RATA methodology, as shown in Table 3. The high intensity of smoked, crunchy and shiny, salty and soft attributes were noted in the SB60, SB15 and SB30 samples, respectively.

Table 3. Sensory profile of smoked bacon analyzed at different storage times.

Sensory attributes	Samples					SEM
	SB0	SB15	SB30	SB45	SB60	
Smoky*	1.75 ^{ab}	1.92 ^{ab}	1.64 ^a	1.82 ^{ab}	2.19 ^b	0.14
Crunchy*	0.99 ^{ab}	1.27 ^b	0.92 ^{ab}	0.74 ^a	1.23 ^{ab}	0.15
Salty*	0.78 ^{ab}	0.90 ^{ab}	1.12 ^b	0.77 ^{ab}	0.73 ^a	0.11
Softy*	2.24 ^{ab}	1.63 ^a	2.30 ^b	2.08 ^{ab}	1.82 ^{ab}	0.18
Bright*	1.96 ^{ab}	2.37 ^b	2.16 ^{ab}	2.05 ^{ab}	1.77 ^a	0.16
Fatty ^{ns}	1.60 ^a	1.64 ^a	1.93 ^a	1.81 ^a	1.45 ^a	0.16
Hard ^{ns}	0.97 ^a	0.74 ^a	0.61 ^a	0.74 ^a	0.58 ^a	0.14
Dry ^{ns}	1.19 ^a	1.01 ^a	0.74 ^a	1.09 ^a	1.07 ^a	0.15
Rancid ^{ns}	0.23 ^a	0.10 ^a	0.07 ^a	0.12 ^a	0.11 ^a	0.06
Woody ^{ns}	0.64 ^a	0.81 ^a	0.70 ^a	0.55 ^a	0.75 ^a	0.12
Succulent ^{ns}	1.71 ^a	1.81 ^a	1.97 ^a	2.05 ^a	1.75 ^a	0.18
Fibrous ^{ns}	0.97 ^a	0.91 ^a	0.83 ^a	0.79 ^a	0.91 ^a	0.14

Average values within a row with different superscripts are significantly different according to Tukey's test at 5% significance (*:p<0.05; **:p<0.01; ***:p<0.001; ns: non significant). SEM: Standard error of the mean. SB0: smoked bacon in storage time 0 day; SB15: smoked bacon in storage time 15 days; SB30: smoked bacon in storage time 30 days; SB45: smoked bacon in storage time 45 days and SB60: smoked bacon in storage time 60 days.

The PCA (Figure 5) of the significant attributes explained 87.65% of the original variability in the first two dimensions. The SB0 and SB45 samples were similar, while the other samples were positioned in different parts of the sensory map. For example, the SB15 is noticeably different from the SB45 for the crunchy and soft attributes.

**Figure 5.** PCA on significant sensory attributes which characterize the smoked bacon.

Greater intensity of the crunchy attribute was found in smoked bacon stored for 15 days (SB15). There is not a direct explanation of why this sample was crunchier, but it may be related to moisture loss due to vacuum packaging, which results in drier meat products (Soladoye et al., 2017). In addition, sensory terms that characterize texture are linked to inter-individual differences of each consumer (Saldaña, Saldarriaga, Cabrera, Siche, et al., 2019).

The smoked bacon stored for 30 days (SB30) had the highest intensity of salty taste. This taste is not only related to the amount of salt added to the product, but is also influenced by the amount of intramuscular fat, nucleotide and amino acid compounds (Górska, Nowicka, Jaworska, Przybylski, & Tambor, 2017). It was probably related with the high glutamic acid content in this sample (Table 2). It is well-known that this compound is responsible for imparting a umami taste, which increases the perception of saltiness (Gan et al., 2019). In the same sample, the soft attribute was significantly higher than the rest. This may be related to the proteolysis process, since the proteases action during storage leads to softness of the product and in addition, low concentrations of oxygen prevent protein oxidation and consequently increase proteolytic enzyme activity and myofibrillar fragmentation (Huff Lonergan, Zhang, & Lonergan, 2010; Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007).

Finally, the smoked bacon stored for 60 days presented the smoked attribute most intensely. The smoked flavor is formed by a set of volatile organic compounds (phenols, furans, aldehydes, alcohols and others) originating from the pyrolysis of the wood during the smoking process (Montanari et al., 2018; Saldaña, Saldarriaga, Cabrera, Behrens, et al., 2019). In this study, the bacon was smoked with Eucalyptus reforestation wood, the composition of which presents a high lignin concentration (27.32 g/100 g). During pyrolysis this component is mainly responsible for the formation of phenols, which influence the smoked flavor of the meat product (Saldaña, Soletti, Martins, Menegali, et al., 2019). However, the intensification of the smoked flavor may also be attributed to the proteolysis phenomenon combined with salty ions and microorganisms. This set of factors leads to the formation of precursor substances (e.g. glutamic acid) of the characteristic bacon flavor (Gan et al., 2019; Marcus, 2019), which increases the sensitivity of consumers to identify the smoky attribute.

4.3.4. Relationship between volatile organic compounds, free amino acids and sensory characteristics

MFA explained 73.56% of the variance considering VOCs, FAA and sensory characteristics and overall liking matrices. As shown in the sample positioning showed in Figure 6, it is possible to observe that the samples were very different. In the first dimension, the SB0 sample presented some VOCs (e.g. alcohols and ketones) and bright characteristics, while SB30 in the second dimension had a greater number of VOCs, few FAAs (serine and aspartic acid), being perceived as salty and softy. Samples with longer storage times (SB45 and SB60) were positioned close together, indicating similarity. These samples contained high amounts of FAA, few VOCs, and were identified as possessing the smoky attribute. The SB15 sample had few VOCs and was characterized as crunchy.

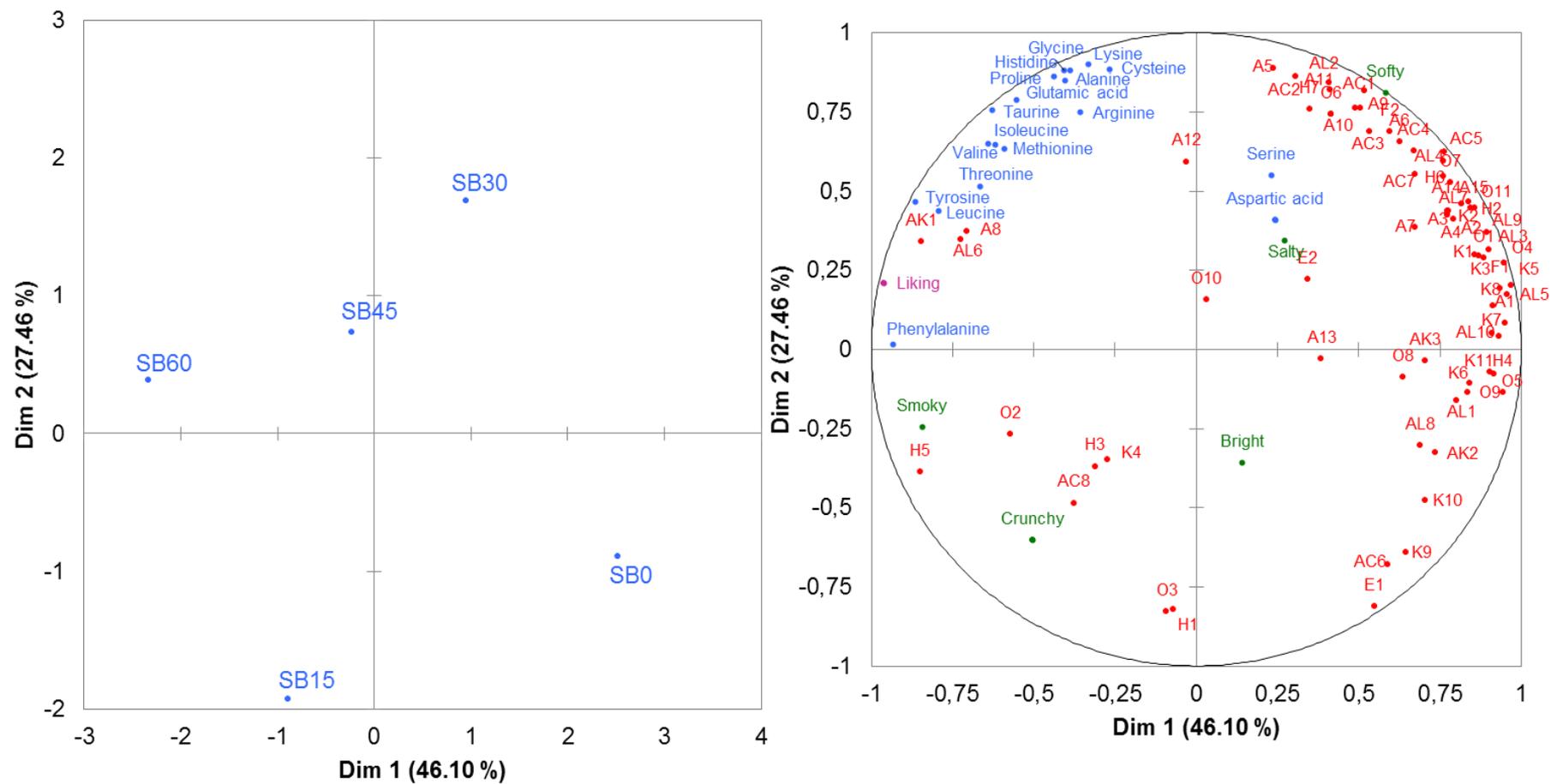


Figure 6. MFA on the matrix of correlation of the chemical and sensory characteristics evaluated of smoked bacon.

On the basis on MFA, two large groups of samples emerged. The first group (SB0 and SB30) was mainly characterized by VOCs originating from smoking and lipid oxidation (thermal process); while in the second group, FAAs were the majority compounds derived from the proteolysis phenomenon.

4.4. Conclusion

Based on the results of this study, it can be stated that the smoked bacon quality, i.e. odor and taste, determines to a large degree the behavior of consumers as well the product's sensory liking. The factors including, VOCs and FAAs play a key role in modulating the flavor of the final product. A multitude of interactions among taste and odor compounds produced during manufacturing and storage time contributed to the intensification of sensory attributes (e.g. smoky) and influenced the high sensory liking. Therefore, this study improved knowledge of the interactions between taste-odor compounds and will enable the understanding of flavor formation and its influence on sensory properties.

Acknowledgments

Authors acknowledge the São Paulo Research Foundation (FAPESP, Brazil) for financing the project 2016/15012-2. Thais C. Merlo received the PhD Scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001. José M. Lorenzo, Rubén Domínguez and Carmen J. Contreras-Castillo are members of the HealthyMeat network, funded by CYTED (ref. 119RT0568). Thanks to GAIN (Axencia Galega de Innovación) for supporting this research (grant number IN607A2019/01).

References

Alfaia, C. P. M., Alves, S. P., Lopes, A. F., Fernandes, M. F. E., Costa, A. S. H., Fontes, C. M. G. A., ... Prates, J. A. M. (2010). Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science*, 84(4), 769-777. <https://doi.org/10.1016/j.meatsci.2009.11.014>.

- Ares, G., Bruzzone, F., Vidal, L., Cadena, R. S., Giménez, A., Pineau, B., ... Jaeger, S. R. (2014). Evaluation of a rating-based variant of Check-All-That-Apply questions: Rate- All-That-Apply (RATA). *Food Quality and Preference*, 36, 87–95. <https://doi.org/10.1016/j.foodqual.2014.03.006>.
- Berardo, A., Claeys, E., Vossen, E., Leroy, F., & De Smet, S. D. (2015). Protein oxidation affects proteolysis in a meat model system. *Meat Science*, 106, 78–84. <https://doi.org/10.1016/j.meatsci.2015.04.002>.
- Brasil, M. da S. (2001). Resolução RDC nº12, de 2 de janeiro de 2001. Regulamento Técnico sobre Padrões microbiológicos para alimentos (pp. 45-53). Brasília, DF: Diário Oficial da República Federativa do Brasil.
- Calkins, C. R., & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat Science*, 77(1), 63–80. <https://doi.org/10.1016/j.meatsci.2007.04.016>.
- Casaburi, A., Piombino, P., Nychas, G. J., Villani, F., & Ercolini, D. (2015). Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology*, 45(PA), 83–102. <https://doi.org/10.1016/j.fm.2014.02.002>.
- Cittadini, A., Domínguez, R., Gómez, B., Pateiro, M., Pérez-Santaescolástica, C., López-Fernández, O., ... Lorenzo, J. M. (2020). Effect of NaCl replacement by other chloride salts on physicochemical parameters, proteolysis and lipolysis of dry-cured foal “cecina”. *Journal of Food Science and Technology*. <https://doi.org/10.1007/s13197-019-04195-6>.
- de Almeida, M. A., Saldaña, E., Pinto, J. S. S., Palacios, J., Contreras-Castillo, C. J., Sentandreu, M. A., & Fadda, S. G. (2018). A peptidomic approach of meat protein degradation in a low-sodium fermented sausage model using autochthonous starter culture. *Food Research International*, 109, 368-379. <https://doi.org/10.1016/j.foodres.2018.04.042>.
- de Carvalho, F. A. L., Munekata, P. E. S., Pateiro, M., Campagnol, P. C. B., Domínguez, R., Trindade, M. A., & Lorenzo, J. M. (2020). Effect of replacing backfat with vegetable oils during the shelf-life of cooked lamb sausages. *LWT*, 122, 109052. <https://doi.org/10.1016/j.lwt.2020.109052>.

- Domínguez, R., Gómez, M., Fonseca, S., & Lorenzo, J. . (2014). Effect of different cooking methods on lipid oxidation and formation of volatile compounds in foal meat. *Meat Science*, 97(2), 223–230. <https://doi.org/10.1016/j.meatsci.2014.01.023>.
- Domínguez, Ruben, Gómez, M., Fonseca, S., & Lorenzo, J. M. (2014). Influence of thermal treatment on formation of volatile compounds, cooking loss and lipid oxidation in foal meat. *LWT - Food Science and Technology*, 58(2), 439–445. <https://doi.org/10.1016/j.lwt.2014.04.006>
- Domínguez, R., Munekata, P. E., Agregán, R., & Lorenzo, J. M. (2016). Effect of commercial starter cultures on free amino acid, biogenic amine and free fatty acid contents in dry-cured foal sausage. *LWT – Food Science and Technology*, 71, 47-53. <https://doi.org/10.1016/j.lwt.2016.03.016>.
- Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, 8(10), 429. <https://doi.org/10.3390/ANTIOX8100429>.
- Domínguez, R., Purriños, L., Pérez-Santaescolástica, C., Pateiro, M., Barba, F. J., Tomasevic, I., ... Lorenzo, J. M. (2019). Characterization of Volatile Compounds of Dry-Cured Meat Products Using HS-SPME-GC/MS Technique. *Food Analytical Methods*, 12, 1263-1284. <https://doi.org/10.1007/s12161-019-01491-x>.
- Flores, M. (2010). Flavor of Meat Products. In M. L. M. Nollet & F. Toldra (Eds.), *Sensory Analysis of Foods of Animal Origin* (pp. 131–145). CRC Press. <https://doi.org/10.1201/b10822-12>.
- Freiding, S., Gutsche, K. A., Ehrmann, M. A., & Vogel, R. F. (2011). Genetic screening of *Lactobacillus sakei* and *Lactobacillus curvatus* strains for their peptidolytic system and amino acid metabolism, and comparison of their volatilomes in a model system. *Systematic and Applied Microbiology*, 34(5), 311–320. <https://doi.org/10.1016/j.syapm.2010.12.006>.
- Gan, X., Li, H., Wang, Z., Emara, A. M., Zhang, D., & He, Z. (2019). Does protein oxidation affect proteolysis in low sodium Chinese traditional bacon processing?. *Meat Science*, 150, 14-22. <https://doi.org/10.1016/j.meatsci.2018.10.007>.

- García-González, D. L., Tena, N., Aparicio-Ruiz, R., & Morales, M. T. (2008). Relationship between sensory attributes and volatile compounds qualifying dry cured hams. *Meat Science*, 80(2), 315–325, <https://doi.org/10.1016/j.meatsci.2007.12.015>.
- Garrido, R., Domínguez, R., Lorenzo, J. M., Franco, I., & Carballo, J. (2012). Effect of the length of salting time on the proteolytic changes in dry-cured lacón during ripening and on the sensory characteristics of the final product. *Food Control*, 25(2), 789–796. <https://doi.org/10.1016/J.FOODCONT.2011.11.036>.
- Gomez, M., Dominguez, R., Fonseca, S., & Lorenzo, J. M. (2015). Effect of Finishing Diet on Physico-Chemical and Lipolytic Parameters and Volatile Compounds Throughout the Manufacture of Dry-Cured Foal Cecina. *Austin Journal of Nutrition and Food Sciences*, 3(1), 1–9. Retrieved from <http://austinpublishinggroup.com/nutrition-food-sciences/v3-i1.php>.
- Górska, E., Nowicka, K., Jaworska, D., Przybylski, W., & Tambor, K. (2017). Relationship between sensory attributes and volatile compounds of polish dry-cured loin. *Asian-Australasian Journal of Animal Sciences*, 30, 720-727. <https://doi.org/10.5713/ajas.16.0252>.
- Hitzel, A., Pohlmann, M., Schwagele, F., Speer, K., & Jira, W. (2013). Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in meat products smoked with different types of wood and smoking spices. *Food Chemistry*, 139(1-4), 955–962. <https://doi.org/10.1016/j.foodchem.2013.02.011>.
- Hough, G. (2010). *Sensory Shelf Life Estimation of Food Products – Guillermo Hough – Google Books*. Londres, United Kingdom: CRC Press. Taylor & Francis.
- Huff Lonergan, E., Zhang, W., & Lonergan, S. M. Biochemistry of postmortem muscle - Lessons on mechanisms of meat tenderization. *Meat Science*, 86, 184–195. <https://doi.org/10.1016/j.meatsci.2010.05.004>
- Lancette, G. A., & Bennett, R. W. Staphylococcus aureus and Staphylococcal enterotoxins. In K. Ito (Ed.), *Compendium of methods for the Microbiological Examination of Foods* (pp. 387-403). Washington DC, USA: American Public Health Association.

- Leroy, F., Geyzen, A., Janssens, M., De Vuyst, L., & Scholliers, P. (2013). Meat fermentation at the crossroads of innovation and tradition: A historical outlook. *Trends in Food Science & Technology*, 31, 130-137. <https://doi.org/10.1016/j.tifs.2013.03.008>.
- Li, X., Zhu, J., Li, C., Ye, H., Wang, Z., Wu, X., & Xu, B. (2018). Evolution of Volatile Compounds and Spoilage Bacteria in Smoked Bacon during Refrigeration Using an E-Nose and GC-MS Combined with Partial Least Squares Regression. *Molecules*, 23(12), e3286. <https://doi.org/10.3390/molecules23123286>.
- Lorenzo, J. M., & Carballo, J. (2015). Changes in physico-chemical properties and volatile compounds throughout the manufacturing process of dry-cured foal loin. *Meat Science*, 99, 44–51. <https://doi.org/10.1016/J.MEATSCI.2014.08.013>.
- Lorenzo, J. M., Carballo, J., & Franco, D. (2013). Effect of the inclusion of chestnut in the finishing diet on volatile compounds of dry-cured ham from celta pig breed. *Journal of Integrative Agriculture*, 12(11), 2002–2012. [https://doi.org/10.1016/S2095-3119\(13\)60638-3](https://doi.org/10.1016/S2095-3119(13)60638-3).
- Lorenzo, J. M., Cittadini, A., Bermúdez, R., Munekata, P. E., & Domínguez, R. (2015). Influence of partial replacement of NaCl with KCl, CaCl₂ and MgCl₂ on proteolysis, lipolysis and sensory properties during the manufacture of dry-cured lacón. *Food Control*, 55, 90–96. <https://doi.org/10.1016/j.foodcont.2015.02.035>.
- Lorenzo, J. M., & Domínguez, R. (2014). Cooking losses, lipid oxidation and formation of volatile compounds in foal meat as affected by cooking procedure. *Flavour and Fragrance Journal*, 29(4), 240–248. <https://doi.org/10.1002/ffj.3201>.
- Lorenzo, J. M., Fonseca, S., Gómez, M., & Domínguez, R. (2015). Influence of the salting time on physico-chemical parameters, lipolysis and proteolysis of dry-cured foal “cecina.” *LWT - Food Science and Technology*, 60(1), 332–338. <https://doi.org/10.1016/J.LWT.2014.07.023>.
- Lorenzo, J. M., Franco, D., & Carballo, J. (2014). Effect of the inclusion of chestnut in the finishing diet on volatile compounds during the manufacture of dry-cured “Lacón” from Celta pig breed. *Meat Science*, 96(1), 211–223. <https://doi.org/10.1016/j.meatsci.2013.07.007>.

- Lund, M. N., Lametsch, R., Hviid, M., Jensen, O. N., & Skibsted, L. H. (2007). High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine *longissimus dorsi* during chill storage. *Meat Science*, 77(3), 295-303. <https://doi.org/10.1016/j.meatsci.2007.03.016>.
- Lustre, A. O., & Issenberg, P. (1970). Phenolic components of smoked meat products. *Journal of Agricultural and Food Chemistry*, 18, 1056–1060. <https://doi.org/10.1021/jf60172a009>.
- Marcus, J. B. (2019). Flavor Enhancement Ingredients. *Aging, Nutrition and Taste*, 173–206. <https://doi.org/10.1016/b978-0-12-813527-3.00006-5>.
- Marušić, N., Petrović, M., Vidaček, S., Petrak, T., Medić, H. (2011). Characterization of traditional Istrian dry-cured ham by means of physical and chemical analyses and volatile compounds. *Meat Science*, 88(4), 786-790. <https://doi.org/10.1016/j.meatsci.2011.02.033>.
- Merlo, T. C., Antonio, J. C., Savian, T. V., Villegas, C., Dargelio, M. D. B., Pinto, J. S. S., Contreras-Castillo, C. J. (2020). Effect of the smoking process using Brazilian reforestation woods on volatile compounds, lipid oxidation and microbiological quality of bacons during shelf life. *Meat Science*, 164, 108110. <https://doi.org/10.1016/j.meatsci.2020.108110>.
- Meyners, M., Jaeger, S. R., & Ares, G. (2016). On the analysis of Rate-All-That-Apply (RATA) data. *Food Quality and Preference*, 49, 1-10. <https://doi.org/10.1016/j.foodqual.2015.11.003>.
- Montanari, C., Gatto, V., Torriani, S., Barbieri, F., Bargossi, E., Lanciotti, R., ... Gardini, F. (2018). Effects of the diameter on physico-chemical, microbiological and volatile profile in dry fermented sausages produced with two different starter cultures. *Food Bioscience*, 22, 9–18. <https://doi.org/10.1016/j.fbio.2017.12.013>.
- Nachtigall, F. M., Vidal, V. A. S., Pyarasani, R. D., Domínguez, R., Lorenzo, J. M., Pollonio, M. A. R., & Santos, L. S. (2019). Substitution effects of NaCl by KCl and CaCl₂ on lipolysis of salted meat. *Foods*, 8(12), 595. <https://doi.org/10.3390/foods8120595>.

- Narváez-Rivas, M., Gallardo, E., León-Camacho, M. (2012). Analysis of volatile compounds from Iberian hams: a review. *Grasas y Aceites*, 63, 432–452. <https://doi.org/10.3989/gya.070112>.
- North, M. K., Zotte, A. D., & Hoffman, L. C. (2019). The effects of dietary quercetin supplementation on the meat quality and volatile profile of rabbit meat during chilled storage. *Meat Science*, 158, 107905. <https://doi.org/10.1016/j.meatsci.2019.107905>.
- Novelli, E., Gandemer, G., Meynier, A., Zanardi, E., & Chizzolini, R. (1995). Composizione dell'aroma di due prodotti di salumeria: mortadela e salame milano. Parma: Atti del Congresso Grassi e Qualita' della Carni, 133–153.
- Parker, J. K., Elmore, J. S., & Methven, J. Flavour Development, Analysis and Perception in Food and Beverages; Woodhead Publishing: Cambridge, U.K., 2015.
- Peryam, D., & Pilgrim, F. (1957). Hedonic scale method of measuring food preferences. *Food Technology*, 11, 9–14.
- R Development Core Team. (2017). R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing Vienna Austria, 0*, {ISBN} 3-900051-07-0
- Resconi, V. C., Escudero, A., & Campo, M. M. (2013). The development of aromas in ruminant meat. *Molecules*, 18, 6748–6781. <https://doi.org/10.3390/molecules18066748>.
- Rios-Mera, J. D., Saldaña, E., Cruzado-Bravo, M. L. M., Martins, M. M., Patinho, I., Selani, M. M., ... Contreras-Castillo, C. J. (2020). Impact of the content and size of NaCl on dynamic sensory profile and instrumental texture of beef burgers. *Meat Science*, 161, 107992. <https://doi.org/10.1016/j.meatsci.2019.107992>.
- Saldaña, E., Castillo, L. S., Sánchez, J. C., Siche, R., Almeida, M. A., Behrens, J. H., ... Contreras-Castillo, C. J. (2018). Descriptive analysis of bacon smoked with Brazilian woods from reforestation: methodological aspects, statistical analysis, and study of sensory characteristics. *Meat Science*, v. 140, p. 44–50, 2018. <https://doi.org/10.1016/j.meatsci.2018.02.014>.

- Saldaña, E., Saldarriaga, L., Cabrera, J., Siche, R., Behrens, J. H., Selani, M. M., ... Contreras-Castillo, C. J. (2019a). Relationship between volatile compounds and consumer-based sensory characteristics of bacon smoked with different Brazilian woods. *Food Research International*, 119, 839-849. <https://doi.org/10.1016/j.foodres.2018.10.067>.
- Saldaña, E., Saldarriaga, L., Cabrera, J., Behrens, J. H., Selani, M. M., Rios-Mera, J., Contreras-Castillo, C. J. (2019b). Descriptive and hedonic sensory perception of Brazilian consumers for smoked bacon. *Meat Science*, 147, 60-69. <https://doi.org/10.1016/j.meatsci.2018.08.023>.
- Saldaña, E., Soletti, I., Martins, M. M., Menegali, B. S., Merlo, T. C., Selani, M. M., ... Contreras-Castillo, C. J. (2019c). Understanding consumers' dynamic sensory perception for bacon smoked with different Brazilian woods. *Meat Science*, 154, 46-53. <https://doi.org/10.1016/j.meatsci.2019.04.006>.
- Soladoye, O. P., Shand, P., Dugan, M. E. R., Gariépy, C., Aalhus, J. L., Estévez, M., & Juárez, M. (2017). Influence of cooking methods and storage time on lipid and protein oxidation and heterocyclic aromatic amines production in bacon. *Food Research International*, 99, 660–669. <https://doi.org/10.1016/j.foodres.2017.06.029>.
- Sun, W., Zhao, Q., Zhao, H., Zhao, M., & Yang, B. (2010). Volatile compounds of cantonese sausage released at diferent stages of processing and storage. *Food Chemistry*, 121, 319–325. <https://doi.org/10.1016/j.foodchem.2009.12.031>.
- Toldrá, F. (2008). Biotechnology of flavor generation in fermented meats. In F. Toldrá (Ed.), *Meat biotechnology* (pp. 199–215). New York, NY, USA: Springer.
- Toldrá, F., & Flores, M. (1998). The role of muscle protease and lipases in flavour development during the processing of dry-cured ham. *Critical Reviews in Food Science and Nutrition*, 38(4), 331–352. <https://doi.org/10.1080/10408699891274237>.
- Varela, P., & Ares, G. (2014). Novel techniques in sensory characterization and consumer profiling. In P. Varela & G. Ares (Ed.). New York: CRC Press.

- Ventanas, S., González-Mohino, A., Estévez, M., & Carvalho, L. (2020). Innovation in sensory assessment of meat and meat products. *Meat Quality Analysis*, 393-418. <https://doi.org/10.1016/B978-0-12-819233-7.00021-5>.
- Villaverde, A., Ventanas, J., & Estévez M. (2014). Nitrite promotes protein carbonylation and Strecker aldehyde formation in experimental fermented sausages: are both events connected?. *Meat Science*, 98(4), 665–72. <https://doi.org/10.1016/j.meatsci.2014.06.017>.
- Williams, E. J. (1949). Experimental designs balanced for the estimation of residual effects of treatments. *Australian Journal of Scientific Research, Series A: Physical Sciences*, 2, 149–168.
- Woolf, A., Wong, M., Eyres, L., McGhie, T., Lund, C., Olsson, S., ... Requejo-Jackman, C. Avocado Oil. In R. Moreau & A. Kaml-Eldin (Eds.), *Gourmet and Health-Promoting Specialty Oils* (pp. 73-125). <https://doi.org/10.1016/B978-1-893997-97-4.50008-5>.
- Yang, Y., Zhang, X., Wang, Y., Pan, D., Sun, Y., & Cao, J. (2017). Study on the volatile compounds generated from lipid oxidation of Chinese bacon (unsmoked) during processing. *European Journal of Lipid Science and Technology*, 119, 1600512. <https://doi.org/10.1002/ejlt.201600512>.
- Zhang, J., & Zhao, X. (2017). Changes in protein hydrolysates during processing of Chinese traditional dry-cured bacon (Laròu) production. *Journal Food Biochemistry*, 41(2), e12304. <https://doi.org/10.1111/jfbc.12304>.
- Zhang, S., Zhang, C., Qiao, Y., Xing, L., Kang, D., Khan, I. A., ... Zhou, G. (2017). Effect of Flavourzyme on proteolysis, antioxidant activity and sensory qualities of Cantonese bacon. *Food Chemistry*, 237, 779-785. <http://dx.doi.org/10.1016/j.foodchem.2017.06.026>.
- Zhao, C. J., Schieber, A., & Ganzle, M. G. (2016). Formation of taste active amino acids, amino acid derivatives and peptides in food fermentation – A review. *Food Research International*, 89, 39-47. <http://dx.doi.org/10.1016/j.foodres.2016.08.042>.

Zhou, C-Y., Wang, Y., Pan, D-D., Cao, J-X., Chen, Y-J., Liu, Y., ... Ou, C-R. (2017). The changes in the proteolysis activity and the accumulation of free amino acids during chinese traditional dry-cured loins processing. *Food Science and Technology*, 26(3), 679-687. <https://doi.org/10.1007/s10068-017-0089-z>.

5. GENERAL CONCLUSION

At the present time, the global food challenge is ensuring access to safe, healthy and nutritious meat products for world population. In this context, studies about carcinogenic compounds can assist to elucidate issues related to formation and analytical techniques. However, in Brazil, there are no regulatory limits for PAHs in meat products and there are few studies regarding the concentration of these compounds in this type of product. The experimental results obtained enabled the development of a reliable method for the analysis of PAHs and the use of reforestation woods for smoking is safe. Therefore, obtaining data was very important to help meet the demand for information on the occurrence of PAHs in smoked meat products in the Brazilian scenario.

Although bacon is a very popular product, innovation is important to attend consumer needs and requirements made by legislation. Traditional smoking involves the exposure of meat products to smoke and is recommended by Brazilian legislation for designated smoked products. In this way, the results of this research recommend the use of *Eucalyptus citriodora* wood for smoking. This wood improved the profile of volatile organic compounds, inhibited lipid oxidation and increased sensory acceptance during storage.

Finally, understanding the formation and interaction between compounds that will influence product acceptance is very important. From the results obtained, the appreciation of smoked bacon is related to the interaction of a wide diversity of compounds that provide a complex flavor impression. The formation of volatile and non-volatile compounds allowed us to understand the flavor formation in the final product and its influence on sensory characteristics. Thereby, this research was crucial to contribute to the Brazilian industry in development of quality smoked bacon, attractive to the consumers and environmentally friendly.