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Impact of the Smoking Process on Biogenic Amine Levels in Traditional Dry-Cured Chorizo

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Abstract: This study aimed to investigate the influence of various production stages on the quality and spoilage conditions of traditionally dry-cured chorizo. To accomplish this, we employed an experimental design that examined three key production parameters: the batch, the filling stage, and the food smoking process. The study was conducted in collaboration with a local producer who adheres to traditional curing methods utilizing oak wood smoke and heat. Biogenic amine levels were closely monitored throughout the process. This involved their extraction and derivatization through the salting-out technique, followed by identification and quantification using LC-ESI/MS^n and HPLC-DAD, respectively. The findings suggest that both raw materials and the production process are well controlled during the filling stage. However, it became evident that the 14-day oak wood smoking period had a significant impact on biogenic amine formation, whose total mean values increased from 126 to 1385 mg kg^{-1}, particularly with respect to putrescine (PUT), cadaverine (CAD), and tyramine (TYR), although these levels remained below the oral toxicity limit (2000 mg kg^{-1}). Consequently, the concentration of these compounds can influence the quality and safety of traditionally dry-cured chorizos. Therefore, the combined levels of PUT, CAD, and TYR can serve as a valuable quality indicator for these products.

Keywords: sausages; chorizo; food smoking; biogenic amines; salting-out

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1. Introduction

The southern region of Europe is famous for its meat products, which are made through drying, fermentation, and unique culinary techniques, aligning with the Mediterranean dietary pattern. Each country and even specific geographic locations have their own distinct meat products. Portugal, in particular, has a strong tradition of producing these foods, especially in mountainous areas where numerous products showcase their unique technological and sensory qualities [1].

These products, known as chorizos and/or smoked products, are commonly consumed by inhabitants of rural areas in Portugal, representing a long history and a wide variety of traditional products [2]. While the higher consumption in rural areas compared to urban areas is noticeable, it is important to highlight their seasonality. These products are considered to embody tradition as their production methods have prevailed across generations [3] and they are recognized as an integral part of the gastronomic heritage [4].

Chorizo is a flavorful food made by finely mincing or chopping meat, which is then filled into a casing or a flexible tubular covering. It can also be subjected to a food smoking process, where it is exposed to smoke for drying, resulting in an extended shelf life. Although chorizo production is prominent in mountainous regions, it is also widely enjoyed in urban areas, albeit with sporadic availability. Nevertheless, the market for chorizos is experiencing growth [5]. Chorizos are considered safe foods due to their low water activity (aw) and pH, which are acquired during processing and storage, inhibiting microbial growth. Nevertheless, the consumption of these products has been associated with

various gastrointestinal illnesses, attributable not only to microbiological factors but also to chemical components [6].

The smoking process is a preservation method that involves exposing food to the action of smoke. Preservation occurs through surface dehydration and the effect of substances resulting from the incomplete combustion of wood, which possesses antimicrobial and antioxidant properties. In addition to preservation, these substances play a significant role in the organoleptic properties of food [7]. The climate in the northern region of Portugal provides the most relevant conditions for employing this process, including cold and rainy winters—the most favorable time of year for producing smoked foods. Since different wood species produce different types of smoke and there are various techniques for smoke generation and ventilation, the final product will exhibit highly specific characteristics that vary according to these factors [3].

Different food processing methods lead to the formation of chemical compounds that may have a negative impact on health. Some notable substances include dioxins, polycyclic aromatic hydrocarbons (PAH), aromatic heterocyclic amines (AHA), and biogenic amines (BAs) [8]. PAH are known to be present in the smoke used for curing chorizos, and these components accumulate on the surface of the product and gradually migrate into the interior over time. Several factors affect the composition of the smoke and the absorption of PAH by the food [9]. In addition to PAH, AHA can also form during thermal treatments and may vary depending on the temperature to which they are exposed, as well as BAs resulting from enzymatic activity that occurs naturally or is induced. There is also the possibility of the presence of dioxins, such as polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF), which are byproducts of industrial processes such as wood combustion [10].

Fermented meat products such as chorizo and fuet, among others, are prone to the accumulation of BAs. Their high protein content, associated with the proteolytic activity of endogenous enzymes and decarboxylase activity of wild microbiota, can support the accumulation of these compounds in fermented chorizos [11], although the final balance depends on the equilibrium between BA formation and degradation [12]. Quantitative studies conducted on various fermented meat products, including some that were smoked, have shown high concentrations of tyramine (TYR) and putrescine (PUT). Significant amounts of cadaverine (CAD) were also detected, along with trace amounts of tryptamine and histamine in some samples [13].

Therefore, considering the process of BA formation, their importance as markers of meat preservation status, and their relevance to consumers' health, research has focused on these chemical compounds. BAs such as PUT, CAD, spermidine, spermine, histamine, TUR, and tryptamine are widely distributed in protein-rich foods due to their propensity to form these compounds owing to their abundance of free amino acids [14]. In general, there are no regulations regarding the content of BAs in food, but it has been known that polyamines, including CAD, spermine, spermidine, and PUT, can react with nitrites to volatile N-nitrosamines, a substrate of N-nitroso compounds exhibiting strong teratogenic, mutagenic, and carcinogenic effects [15]. Furthermore, the presence of high quantities of these chemical substances can indicate poor quality of the raw material, improper storage and preservation, and uncontrolled fermentation [16].

This study was conducted in close collaboration with a local producer of regional chorizo and had two primary objectives. First, it sought to explore the effects of the smoking and curing process on the levels of BAs. Secondly, the study investigated the impact of the filling time, which can extend over several days, on the concentrations of these chemical markers.

2. Materials and Methods

2.1. Chorizo Technology and Sampling Procedures

Two independent batches (30 kg of meat batter each) of chorizos were produced in a local factory, located in the northeastern region of Portugal (Montalegre, Trás-os-

Montes) using commercial pork meat. In order to minimize variability factors, the selected batches were produced using fresh raw materials from the same supplier, and the team of employees responsible for their production remained consistent. Within each batch, there were distinctions between smoked and non-smoked samples. Chorizos were kept in a food smoking chamber with smoke generated from oak wood (*Quercus faginea* L.) for 14 days (Figure 1). After this smoking stage, they were dried under controlled conditions in cure chambers at 9 °C for 5 days. Each type of chorizo (180 g) was collected at two different stages of the filling process (beginning and end). The samples were then frozen (−17 °C) until they were analyzed.

Figure 1. Food smoking chamber, where it is possible to observe the smoke produced as well as the arrangement of the traditional chorizos.

2.2. Physicochemical Analyses

Water activity was assessed with a hygrometer (Hygroskop Rotronic DT, Zurich, Switzerland) equipped with a WA-40 probe at 25 °C. The salt content of end-products was determined following the analytical protocol described in ISO 1841-2 (1996). Benzo(a)pyrene, nitrates, and nitrites were determined in a local laboratory that is accredited/designated for the analytical testing of meat. The results are presented in Table 1.

Table 1. Results of the physicochemical analyses of chorizos.

Parameter	Value
Water activity (a_w)	0.94
Salt content	3.2 g 100 g^{-1}
Benzo(a)pyrene	0.70 μg kg^{-1}
Nitrates	<68 mg $NaNO_3$ kg^{-1}
Nitrites	<11 mg $NaNO_2$ kg^{-1}

2.3. Sample Preparation and BA Extraction

The frozen samples were cut into portions from various parts of the chorizo (ends, center, and random points) and allowed to thaw. They were subsequently ground to homogenize the sample and approximately 4 g was weighed into a 50 mL plastic centrifuge tube. A total of 10 mL of 0.1 mol/L hydrochloric acid (≥37%, Fisher Scientific, Madrid, Spain) and 100 μL of an internal standard (IS, ethylamine, 70% solution in water, Sigma-Aldrich, Steinheim, Germany) at 5 g/L in HCl 0.1 mol/L were added and the samples were

placed in an ultrasonic bath for 15 min, being homogenized every 5 min. The extracts were then centrifuged for 20 min at 6000 rpm at 4 °C. The supernatant was collected into a new 50 mL plastic centrifuge tube and the procedure was repeated (without IS), combining the supernatants (final extract volume of 20 mL).

In the subsequent stage, for both identification and quantification purposes, extraction and derivatization of the amines were performed using the salting-out technique [17,18]. From the previously obtained extract, 2 mL was transferred to a 15 mL plastic centrifuge tube. To this, 2 mL of dansyl chloride (DNS, purity ≥ 99%, Sigma-Aldrich) at a concentration of 1.6 g/L in acetonitrile (HPLC gradient grade, Fisher Scientific) and 0.8 mL of 5 mol/L sodium hydroxide (purity ≥ 98%, Panreac, Castellar del Vallès, Spain) were added. The mixture was shaken vigorously and then left in a dark place for 10 min to allow for the derivatization process. Following this, 0.13 g of sodium chloride (purity ≥ 99.8%, Sigma-Aldrich) was added, fully dissolved with vigorous shaking, and then centrifuged for 1 min at 6000 rpm and 4 °C. A portion of 800 µL was retrieved for further analysis and each sample was examined in triplicate.

2.4. Chromatographic Analysis by LC-DAD-ESI/MS^n

Samples were qualitatively analyzed to determine which of the BAs were present in the different samples by comparing them to reference standards using HPLC-DAD-ESI-MS^n. The HPLC system consisted of a low-pressure quaternary pump with an automatic sampler. Separation took place at 30 °C on a Gemini C_{18} column (150 mm × 4.6 mm; particle size 3 µm) and a pre-column (4 mm × 3.0 mm) from Phenomenex (Torrance, CA, USA) using a binary gradient (A, acetonitrile and B, 0.01 mol/L ammonium acetate buffer solution, pH 4.0) at 0.45 mL/min as follows: initial conditions of 55% A and 45% B held linearly up to 10 min, linear increase of A from 55% to 75% between 10 and 30 min, linear increase of A up to 100% between 30 and 35 min with conditions held for 5 min, returning to initial conditions in 5 min and maintained for 10 min before the next injection. The injection volume was 20 µL. DAD was performed by scanning between 200 and 600 nm. A quadrupole mass spectrometer with an ion trap (Finnigan LCQ Deca XP Plus) was equipped with an ESI source. Analysis parameters were optimized prior to HPLC separation by direct injection of derivatized amine compounds directly into the mass spectrometer: capillary temperature, 325 °C; source voltage, 5.0 kV; capillary voltage, 10.0 V; gas current (N2) at 60 arbitrary units and auxiliary gas (N_2) at 23 arbitrary units. Mass detection was carried out in positive ionization mode in the range of 60–1000 m/z. The software used for data collection and processing was XCalibur software Version 2.2 (Thermo Electron Corporation).

2.5. Chromatographic Analysis by HPLC-DAD

The quantitative analysis was carried out using an HPLC instrument (Jasco, Tokyo, Japan) consisting of a low-pressure quaternary pump with an in-line degasser (model PU-2089 Plus), a multi-wavelength detector (model MD-1510), and an automatic sampler (model AS-950). The separation was achieved at room temperature using a Gemini C_{18} column (250 mm × 4.6 mm; particle size 5 µm) and a pre-column (4 mm × 3.0 mm) from Phenomenex (Torrance, CA, USA). A binary gradient was utilized (A, acetonitrile and B, acetate buffer) at a flow rate of 1 mL/min as follows: initial conditions of 55% A and 45% B held linearly up to 10 min, a linear increase of A from 55% to 75% between 10 and 30 min, a linear increase of A up to 100% between 30 and 35 min with conditions held for 5 min, then returning to initial conditions in 5 min and maintained for an additional 10 min before the next injection. The injection volume was 20 µL. The absorption wavelength used was 254 nm. Quantification was carried out based on the internal standard. The following BAs from Sigma-Aldrich (Steinheim, Germany) were used for calibration within a concentration range between 0.1 and 400 mg/L: methylamine (MET, 41% solution in water), phenylethylamine (PHE, 41% solution in water), isopentylamine (ISO-PEN, ≥99%), PUT (≥97%), CAD (≥95%), TYR (≥99%), and dimethylamine (40% solution in water).

2.6. Statistical Analysis

Statistical analyses were performed with the SAS software program (2023; Academic version, SAS Institute Inc., Carry, NC, USA) using the general linear and linear regression models. The statistical model included batch (A vs. B), type (smoked vs. non-smoked), stage of filling (beginning vs. end), and all double interactions as fixed effects and the random residual error. Significance was set as $p < 0.05$ and multiple comparisons of means were carried out using the Tukey test.

3. Results and Discussion

3.1. Biogenic Amine Identification

The initial phase involved the optimization of the analysis conditions for mass spectrometry (MS) and the ionization parameters in positive mode through the direct injection of derivatized BAs into the mass spectrometer. Subsequently, the BA standards were analyzed by MS at a concentration of 90 mg/L. The chromatograms obtained through ultraviolet detection (maximum absorbance peaks) and MS (relative abundance of the total ion current) can be found in the Supplementary Materials (Figures S1 and S2).

Each individual BA possessed unique properties that facilitated its differentiation using data acquired from MS. Given the variable interactions between BAs and DNS, they can either react with a single derivatizing molecule or with two, contingent upon the specific amine. To achieve precise identification, ions $[M+H]^+$ and the predominant secondary fragments were singled out for examination. Additionally, data regarding the wavelength at which absorbance peaked were ascertained. These comprehensive details are presented in Table 2.

Table 2. Retention times, maximum absorbances, and characteristic ion masses of the derivatized amines by HPLC-ESI/MS^n.

Derivatized BA *	Rt (min)	λ_{max} (nm)	$[M + H]^+$	MS^2 (rel.int.)
MET + 1DNS	9.29	239, 332	265.3	250.0 (100%), 171.2 (50%)
Ethylamine + 1DNS	11.61	239, 335	279.2	263.9 (100%), 157.2 (90%)
Dimethylamine + 1DNS	15.05	239, 320	279.2	264.0 (100%), 157.3 (70%)
PHE + 1DNS	23.43	245, 335	355.3	157.2 (100%), 339.9 (70%)
ISO-PEN + 1DNS	24.62	239, 335	321.3	157.2 (100%), 306.0 (30%)
PUT + 2DNS	26.22	221, 251	555.4	304.0 (100%), 220.1 (90%)
CAD + 2DNS	28.48	224, 251, 335	569.4	318.1 (100%), 169.2 (90%)
TYR + 2DNS	38.43	227, 248, 344	604.3	370.0 (100%), 171.1 (40%)

* For the derivatized Bas, the number of DNS moieties present in the derivative are indicated; Rt, retention time; MET, methylamine; PHE, phenylethylamine; ISO-PEN, isopentylamine; PUT, putrescine; CAD, cadaverine; TYR, tyramine.

Based on the information obtained and previously described, the BAs were identified in different samples of chorizo. All the BAs were identified except for ethylamine, which is the reason why ethylamine was chosen as the internal standard (IS).

We conducted a new MS analysis to assess the stability of amine behavior in chorizo samples when using the internal standard. Chromatograms were obtained (Figure 2). While certain amines were not detected in the mass spectrometry chromatograms, they were still detectable through ultraviolet absorbance. We selected the peak of maximum absorbance for each biogenic amine (Table 2), including the internal standard, for quantification.

3.2. Biogenic Amine Quantification by HPLC-DAD

Calibration curves for the BAs were constructed over the concentration range found in the analyzed samples. Extraction was carried out using the procedure described for the samples. The internal standard (ethylamine, IS) was employed to compensate for random and systematic errors and matrix effects. The limit of detection (LoD) was calculated as three times the residual standard deviation and the limit of quantification (LoQ)

was calculated as ten times the residual standard deviation—both from the linear regression of each calibration curve. All the results obtained are presented in Table S1 of the Supplementary Materials.

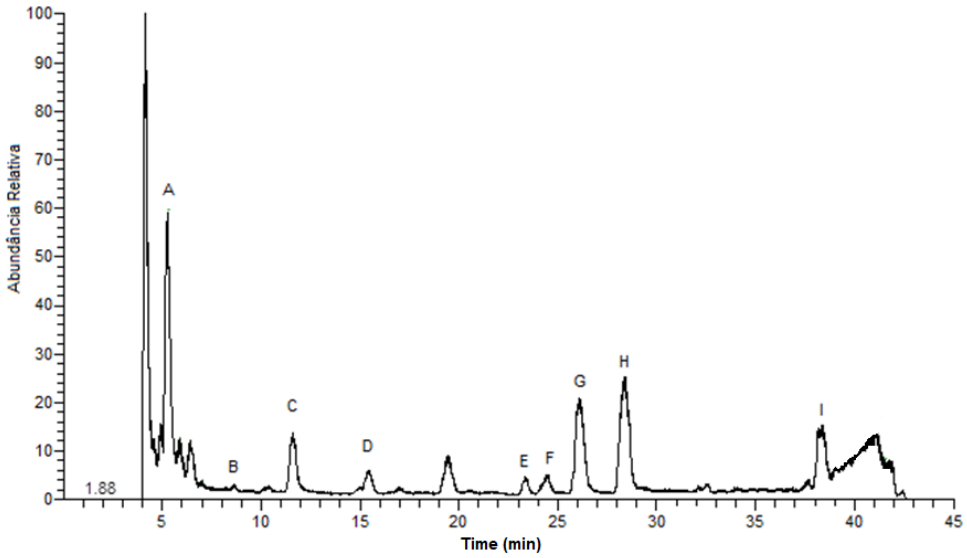

Figure 2. Chromatogram (TIC) obtained by HPLC-ESI/MS of a sample of smoked chorizo containing the internal standard (IS). A—DNS; B—Methylamine (MET); C—Ethylamine (IS); D—Dimethylamine; E—Phenylethylamine (PHE); F—isopentylamine (ISO-PEN); G—Putrescine (PUT); H—Cadaverine (CAD); I—Tyramine (TYR).

Due to the diverse composition of this matrix and its substantial fat content, it was anticipated that the results would exhibit some degree of variability. However, the results exhibited low relative standard deviations, and the method was proven to be suitably applicable to the chorizo matrix. Four aliphatic (MET, ISO-PEN, PUT, and CAD) and two aromatic (PHE and TYR) amines were detected and determined in this study. The most commonly found BAs in the analyzed samples included PUT and CAD. The quantification of BA is a good indicator of the spoilage level of food matrices. In pork, the sum of PUT, TYR, CAD, and histamine, calculated as the biogenic amines index (BAI), is often used to evaluate the freshness and quality of meat [19]. Nonetheless, a recent study states that PUT and CAD are significantly related to the precursor free amino acid content, and their sum value could be used as a novel quality indicator instead of BAI [20]. In the European Union, while there are specific regulations for histamine in certain fish products due to its direct implications on human health, the concentrations of other BAs like PUT and CAD are often considered more as quality indicators rather than direct health hazards. High levels of these amines can indicate spoilage or poor processing practices. But, since PUT and CAD themselves are not as toxic as histamine, they do not have the same strict legal limits. Instead, they are often used in combination with other BAs to evaluate the overall quality of fermented food products [21].

As can be seen in Table 3, MET, PHE, and ISO-PEN were present in substantially lower concentrations compared to the others. The most significant biogenic amines in all the analyzed samples were PUT, CAD, and TYR. In a UPLC-fluorescence method developed to quantify biogenic amines in grilled meat from Beninese markets, TYR and PHE were not detected but MET and PUT were detected at concentrations lower than their limit of quantification [19]. Regarding PUT, it was observed that chorizos subjected to the smoking process exhibited considerably higher levels when compared to those not subjected to smoking (both levels being below the detection limit). Regarding CAD, it was also observed that the smoking process led to an increase in its concentration. TYR, which stands out as the amine with the highest toxicological risks, particularly for individuals who are sensitive or intolerant to TYR [16], followed the same trend as the previously

mentioned amines, albeit less pronounced, exhibiting higher concentrations in the smoked samples compared to the non-smoked ones (Table 3).

Table 3. Content ± standard deviation of biogenic amines (mg kg^{-1} of fresh matter) obtained for the analyzed samples, considering the studied variables: the batch (A and B), the type (smoked and non-smoked), and the stage of filling (beginning and end).

Batch	Type of Production	Stage of the Filling Process	MET	PHE	ISO-PEN	PUT	CAD	TYR	Total
A	Non-smoked	Beginning	0.213 ± 0.066	1.14 ± 0.12	ND	26.3 ± 9.7	261 ± 28	88.0 ± 8.2	377 ± 45
		End	0.241 ± 0.057	ND	ND	ND	52.2 ± 1.7	17.7 ± 1.7	70.1 ± 0.5
	Smoked	Beginning	0.489 ± 0.026	14.9 ± 1.4	1.91 ± 0.07	372 ± 85	570 ± 83	281 ± 29	1239 ± 192
		End	0.488 ± 0.169	0.689 ± 0.255	1.62 ± 0.07	181 ± 27	263 ± 43	230 ± 50	677 ± 116
B	Non-smoked	Beginning	0.274 ± 0.053	0.209 ± 0.116	ND	ND	ND	ND	0.482 ± 0.159
		End	0.182 ± 0.057	ND	ND	ND	36.9 ± 10.1	19.3 ± 3.0	56.4 ± 12.9
	Smoked	Beginning	0.524 ± 0291	36.4 ± 2.9	1.89 ± 0.53	1372 ± 70	261 ± 68	199 ± 64	1871 ± 147
		End	0.698 ± 0.101	33.9 ± 1.4	2.20 ± 0.20	837 ± 113	701 ± 159	175 ± 41	1749 ± 307

In general, it is herein observed that the smoking process increases the biogenic amine content in traditional dry-cured chorizos. This is a result of multiple factors associated with this preservation method, such as microbial activity, pH modulation, reduction of water activity, and the enhanced proteolytic breakdown of proteins. While food smoking provides several preservation benefits, such as imparting flavor and extending shelf-life, its influence on biogenic amine formation will depend on specific conditions like temperature, duration, and the specific bacterial communities present. This observation is not undisputed, as other studies conducted on ham, sausage, and cheese demonstrated the opposite results. In non-smoked dry cured hams, higher concentrations of BAs were observed compared to smoked ones [22]. Research has demonstrated that smoked cooked sausages typically exhibit much lower levels of BAs in comparison to ordinary fermented sausages [23]. Finally, the content of single biogenic amines, as well as their total amount, was found to be very low in examined smoked cheeses [24]. The raised hypothesis is that the smoking process can yield products with aseptic characteristics, thus decreasing the level of BAs by inhibiting the growth of amine-decarboxylating bacteria [25].

The presence of amines such as PUT and CAD at nearly trace levels during the initial stage of the filling process may suggest a low degree of raw material degradation as these amines also serve as indicators of food freshness and deterioration. Although the concentrations of both amines were elevated after the smoking process, they remained below the levels that induce oral toxicity (2000 mg kg^{-1}) [26]. It is worth noting that none of the products had values exceeding toxicity limits; thus, the chorizos placed on the market are safe from the perspective of the contaminants analyzed in this study.

3.3. Statistical Analysis

In order to determine whether there is sufficient evidence to conclude that the batch, filling phase, or type of production significantly influences the content of each of the BAs, a one-way ANOVA was applied. The results, as shown in Table 4, reveal that only the type of production (smoking vs. non-smoking) influences all the analyzed BAs. On the other hand, the batch only influenced the PHE and PUT contents, while there were no statistically significant differences, with a confidence level of 95%, between filling the samples at the beginning or end of the production.

The results for the individual BAs show that the production batch does not significantly influence the levels of individual BAs in the analyzed traditional dry-cured chorizos, except for PHE and PUT. It has been argued that the presence of PHE usually results from thermal processing rather than the microbiological decarboxylation of phenylalanine. Moreover, concentrations of up to 180 mg kg^{-1} have been found in fermented sausages [27]. However,

the values found here are much below these, and the fact that their concentration is not considered statistically different during the filling stage proves that their origin is not due to microbiological contamination. The results showed herein also indicate that not enough time elapses for a significant level of raw material deterioration to occur during the filling process. In contrast, the curing process (food smoking) exerts a significant influence not only on the total BA content but also on each of the individual BAs. Therefore, a statistical test was applied to investigate the impact of the curing process on the individual levels of BAs, as well as the double interactions with the filling phase. The results can be found in Table 5.

Table 4. Results from one-way ANOVA for the statistical analysis of the effects of batch, food smoking, and stage of the filling process on the content of BAs in chorizo samples.

	MET	PHE	ISO-PEN	PUT	CAD	TYR	Total
p-values							
Batch	0.481	<0.05 *	0.704	<0.05 *	0.582	0.720	0.289
Type	<0.05 *	<0.05 *	<0.05 *	<0.05 *	<0.05 *	<0.05 *	<0.05 *
Stage of filling	0.760	0.464	0.958	0.387	0.981	0.524	0.454

"*" denotes statistically significant differences ($p < 0.05$).

Table 5. Results obtained for the statistical model which included type (Non smoked vs. Smoked) and stage of filling process (Beginning vs. End), as well as all double interactions as fixed effects and the random residual error.

		MET	PHE	ISO-PEN	PUT	CAD	TYR	Total
p-values								
Type		<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.001 *
Stage of filling		0.589	0.260	0.814	0.238	0.974	0.297	0.108
Type × Stage of filling		0.239	0.362	0.814	0.278	0.472	0.955	0.446
RSD		0.133	10.9	0.255	354	194	68.2	379
r^2		0.647	0.547	0.945	0.430	0.382	0.598	0.775
Adjusted r2		0.594	0.479	0.937	0.345	0.289	0.538	0.741
Mean values for the main effects								
Type	Non-smoked	0.228	0.451	nd	6.59	64.3	31.3	126
	Smoked	0.550	21.5	1.93	525	337	181	1384
Stage of filling	Beginning	0.375	13.3	0.952	345	202	119	872
	End	0.402	8.65	0.975	187	199	93	638

"*" denotes statistically significant differences ($p < 0.05$); RSD, residual standard deviation; r^2, coefficient of determination.

Upon first inspection, the filling stage does not have any significant effect on the individual content of BAs. Even in combination with the type of curing, the influence of the filling stage can be dismissed. Despite several days passing, the filling process is sufficiently controlled to prevent the formation of BAs through microbiological decarboxylation. Conversely, the curing process through smoking affected the content of all biogenic amines analyzed in this study. As seen in Table 5, the probability of incorrectly rejecting a true null hypothesis is less than 0.001, or less than 0.1%, for all the assessed compounds. Among these, three were detected at relatively low concentrations, ranging from 0.55 to 21.5 mg kg^{-1}, even after the smoking process (MET, PHE, ISO-PEN). Conversely, three compounds were found at significantly higher concentrations (PUT, CAD, TYR). Particularly, ISO-PEN was exclusively identified in the smoked chorizos (Figure 3). The PHE content exhibited a substantial increase during smoking, confirming its formation through thermal processing. Prior research by Li et al. reported that Chinese smoked-cured bacon, whether of artisanal or industrial origin, also exhibited elevated phenethylamine levels (>30 mg kg^{-1}) [28]. PUT, initially in limited quantities in the unsmoked chorizos, saw

a significant rise during the curing process. The cumulative content of BAs, which increased by approximately an order of magnitude (Figure 3), can be considered a reliable indicator of protein degradation. However, according to our investigation, the levels of PUT, CAD, and TYR appear to be the most dependable indicators of microbiological contamination or thermal processing.

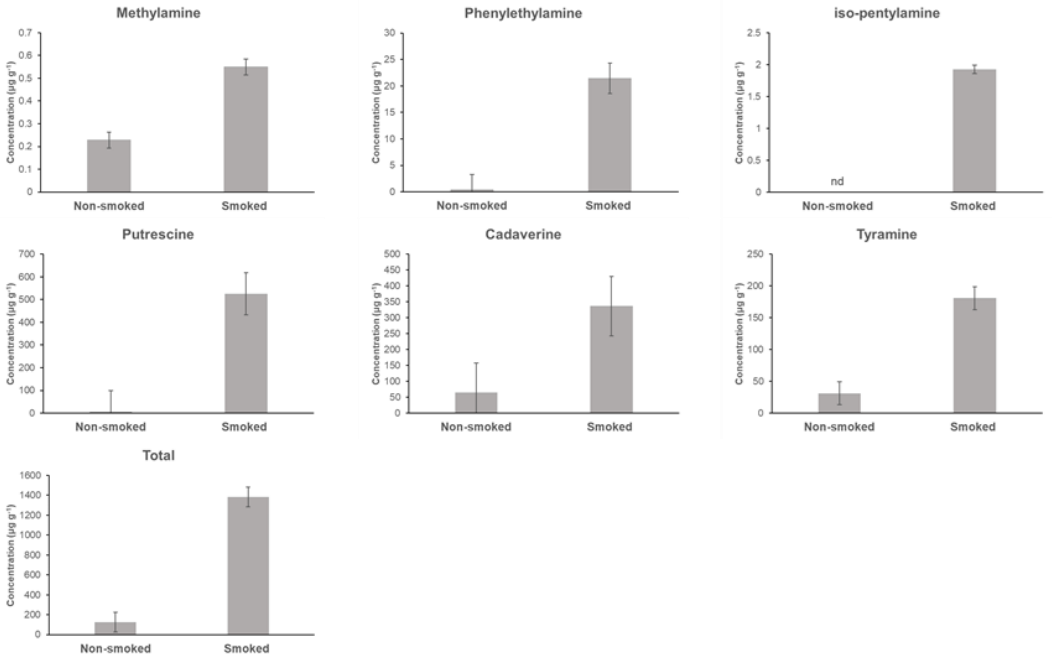

Figure 3. Results of statistically significant differences for the individual BAs obtained by the statistical model, which included type (Non smoked vs. Smoked), reported in Table 5.

The smoke produced during the burning of oak wood contains various chemicals, some of which have antimicrobial properties, further extending the preservation period. However, the temperatures reached are not sufficient to degrade the biogenic amines formed during the process as they are highly temperature-stable. On the contrary, the humidity and temperature conditions, which do not exceed 40 °C, provide a favorable environment for bacterial growth and subsequent biogenic amine production. Indeed, the optimal temperatures for decarboxylase activity fall within the range of 20 to 50 °C [16], which coincides with the smoking process temperature interval.

4. Conclusions

Food smoking, as a method of food preservation, can influence the levels of BAs in various food products, such as sausage, fish, or cheese. The impact of smoking on BA levels is contingent upon several factors, including the type of food, specific smoking process conditions, and the presence of certain bacteria. Smoking treatments can either induce the formation or enhance the content of specific BAs through thermal processing or bacterial activity. Our study reveals that non-smoked traditional chorizos exhibit minimal signs of spoilage, indicating the high quality of raw ingredients and hygienic production practices. While some prior research had suggested that food smoking might reduce BA levels, possibly via the reduction of free amino acids or the inhibition of amine-decarboxylating bacteria growth, our study contradicts this. It clearly demonstrates that the smoking process significantly elevates the levels of PUT, CAD, and TYR. The quantities of these compounds can therefore impact the quality and safety of the food product; thus, the sum of PUT, CAD, and TYR levels can serve as an index for assessing the quality of these products. Acknowledging histamine as a significant amine with toxicological implications, regulations should extend beyond histamine to include other amines like tyramine, considering their toxic effects.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/separations10120585/s1: Figure S1: Chromatogram obtained at 254 nm in the analysis of a solution containing standards of the studied BAs (90 mg/L). A—DNS; B—Methylamine; C—Ethylamine; D—Dimethylamine; E—Phenylethylamine; F—Isopentylamine; G—Putrescine; H—Cadaverine; I—Tyramine. Figure S2: Chromatogram (TIC) obtained by HPLC-ESI/MS of the solution containing standards of BAs (90 mg/L). A—DNS; B—Methylamine; C—Ethylamine; D—Dimethylamine; E—Phenylethylamine; F—Isopentylamine; G—Putrescine; H—Cadaverine; I—Tyramine. Table S1: Equations of the calibration curves, determination coefficients, linearity ranges, and limits of detection and quantification for the determined BAs.

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