Tyramine and histamine are the main dietary bioactive amines related to acute adverse health effects. Dry fermented sausages can easily accumulate high levels of these hazards and are frequently consumed in Spain. The present work aims to assess the exposure to tyramine and histamine from the consumption of dry fermented sausages by the Spanish population and to assess the risk to suffer acute health effects from this exposure. A probabilistic estimation of the exposure to these hazards was derived combining probability distributions of these amines in dry fermented sausages \( n = 474 \) and their consumption by the Spanish population. The mean dietary exposure to tyramine and histamine was 6.2 and 1.39 mg/meal, respectively. The risk of suffering hypertensive crisis or histamine intoxication by healthy population due to tyramine or histamine intake, respectively, exclusively from dry fermented sausages, can be considered negligible. For individuals under treatment with MAOI drugs, the probability to surpass the safe threshold dose (6 mg/meal) was estimated as 34%. For patients with histamine intolerance, even the presence of this amine in food is not tolerable and it could be estimated that 7000 individuals per million could be at risk to suffer the related symptoms after consuming dry fermented sausages.

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1. Introduction

Bioactive amines are microbial metabolites, which can be found in nearly all types of foods in a wide and variable range of concentrations, even within the same type of product. Fermented food and beverages, such as dry fermented sausages, constitute one of the food products that can accumulate high amine contents, which are formed by the decarboxylation of precursor amino acids by fermentative microorganisms as well as by spoilage microorganisms [EFSA, 2011; Bover-Cid et al., 2014]. In the last decades, hygienic improvements at all stages of the food chain along with other specific recommendations, such as the use of starter cultures lacking amino acid-decarboxylase potential, might have contributed to reduce the levels of these compounds in fermented meat products. However, data on biogenic amine content in dry fermented sausages on the Spanish market indicate that there are still many products that contain high levels of amines (Latorre-Moratalla et al., 2012a). In fact, different Member States of EU at the EFSA Network Meeting on Microbiological Risk Assessment informed about an increase of bioactive amine content in some fermented foods [EFSA, 2011]. Therefore, the presence of these compounds in fermented foods could be still of concern from the food safety point of view (Leuschner et al., 2013). Dry fermented sausages are the most consumed fermented products in Spain, either as a snack or part of a main dish.

Tyramine and histamine are the main dietary bioactive amines associated with adverse health effects. Adverse health effects can occur both in case of high intake of these amines or when the ability to metabolize them is compromised by different causes (including enzymatic deficiencies due to genetic or physiological circumstances or enzymatic blockage). Under these circumstances they accumulate in plasma and exert bioactive effects (Maintz and Novak, 2007; Ladero et al., 2010; Kovacova-Hanuskova et al., 2015). Tyramine may increase blood pressure, especially if its exposure coincides with the antidepressant monoamine oxidase inhibitors (MAOI) drugs (Paulsen et al., 2012; Vidal-Carou et al., 2014). Histamine intoxication, formerly called scombroid or histamine fish poisoning, is caused by the ingestion of high concentrations of this amine, so that normal metabolic mechanisms are insufficient for...
their detoxification. The symptoms associated with this intoxication are allergy-like, characterized by neurological and gastrointestinal effects such as headaches, nausea, vomiting, diarrhea, by cutaneous pruritus, flush and urticaria, and by rhinorrhea and hypotension. The severity of the disorders related to both tyramine and histamine exposure is variable, but it may be considered mild since only sometimes require medical attention. The frequent misdiagnosis is reported as the main reason accounting for the poor statistics about the incidence of health disorders due to dietary amines (FAO/WHO, 2013).

Furthermore, the onset of symptoms related to histamine accumulation in blood has also been associated with a wide range of foods with relatively low histamine content. This is known as histamine intolerance, which derives from the insufficient activity of histamine detoxification systems, concretely a deficiency of DAO enzyme by genetic, pathological (e.g. inflammatory bowel diseases) or pharmacological blockade caused by the treatment with common drugs with known inhibiting side effects (acetylcysteine, clavulanic acid and metoclopramide, etc.) (Jarisch, 2004; Maintz and Novak, 2007). Although the symptoms of histamine intolerance are similar to those of intoxication, some authors associate DAO deficiency with some diseases of high prevalence in the population, such as migraine, atopic dermatitis, irritable bowel syndrome, cyclic vomiting syndrome, and muscular pain (Guida et al., 2000; Maintz and Novak, 2007; Vidal-Carou et al., 2010; Izquierdo et al., 2012; Rosell-Camps et al., 2013; Toro, 2013; Kovacova-Hanuskova et al., 2015).

The exposure assessment of any specific hazard is a key point on the risk assessment and data quality and treatment could have a significant impact on the risk estimation (FAO/WHO, 2013). Although the quantitative risk assessment is currently recognized as the relevant scientific approach to assess food safety and to provide scientific criteria for decision making in risk management and development of mitigation strategies, only few assessments have been performed regarding bioactive amines in food. Mainly, these assessments dealt with histamine in fish and fishery products (Lehaney & Olley, 2000; Sumner and Ross, 2002; FAO/WHO, 2013), and also tyramine, histamine, putrescine and/or cadaverine in fermented foods based on Austrian data (Rauscher-Gabernig et al., 2009, 2012; Paulsen et al., 2012; Rauscher-Gabernig et al., 2012) and EU data (EFSA, 2011). The deterministic calculations (i.e. point estimates) of the bioactive amine exposure used in most of the above mentioned studies, did not provide exposure level probability. However, a stochastic approach would provide a more representative exposure assessment and hence, a more realistic risk assessment. Therefore, the current work aims to assess the exposure of Spanish consumers to tyramine and histamine from dry fermented sausages by means of a probabilistic estimation, and to quantify until which extend such exposure contributes to reach the maximum tolerable levels generally recognised as safe.

2. Materials and methods

2.1. Data on tyramine and histamine contents in dry fermented sausages from the Spanish market

Data about contents of tyramine and histamine in dry fermented sausages from retail Spanish market were obtained from the database of our own research group (Vidal-Carou et al., 1990; Hernández-Jover et al., 1997; Bover-Cid et al., 1999; Miguelez-Arrizado et al., 2006; Latorre-Moratalla et al., 2008, 2012a). A total of 474 samples of different kinds of dry fermented sausages were considered: Salchichón (n = 357), chorizo (n = 87), salami (n = 18) and sobrasada (n = 12). All these products are manufactured by fermentation and ripening from pork meat and fat with other additional ingredients (e.g. pepper or red pepper, sugar, wine, garlic, preservatives, etc.) with or without the addition of starter cultures. The determination of bioactive amines in all samples was performed by ion-pair HPLC or UHPLC methods coupled to fluorescence detection (Hernández-Jover et al., 1996; Lavizzari et al., 2006; Latorre-Moratalla et al., 2009).

2.2. Data on consumption of dry fermented sausages by Spanish population

Data about consumption of dry fermented sausages was extracted from the Spanish national dietary survey (Encuesta Nacional de Ingesta Dietética) carried out by the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN, 2011). This survey includes a sample of 3000 individuals randomly selected within the Spanish population, 1500 men and 1500 women aged between 18 and 64 years old. Consumption data (i.e. serving size) of the selected food categories (salchichón, chorizo, salami and sobrasada) were expressed in grams. As the toxic effects of tyramine and histamine are of acute nature, the intake was calculated on a meal basis (i.e. grams per meal per person). Only the meals where dry fermented sausages were consumed were considered.

2.3. Exposure assessment

Various probability distributions were fitted to tyramine and histamine contents of Spanish dry fermented sausages and consumption data collected from Spanish population using @Risk 7.0 (Palisade Corporation, NewField, NY). The goodness of fit was evaluated using the Chi-square ($\chi^2$) test. The best-fitting distributions describing tyramine or histamine contents and dry fermented sausage consumption were selected as an input for the assessment of the exposure to these compounds by the probabilistic estimation using the Monte Carlo simulation technique with 10,000 iterations.

2.4. Hazard characterisation

The maximum tolerable safety levels as an approximation of the no adverse health effect level adopted in the EFSA Scientific Opinion (EFSA, 2011) for different types of population were considered. These threshold levels were obtained from studies published in the literature based on toxicological data of dietary amines, clinical studies with volunteers, clinical cases and other expert opinions about tolerance or intoxication levels. For tyramine the thresholds were: 6 mg/meal/person for patients treated with MAOI drugs, 50 mg/meal/person for patients receiving third generation of MAOI drugs, so called RIMA (reversible inhibitors of MAO-A); and 600 mg/meal/person for healthy individuals. For histamine, the safe threshold considered for healthy population was 25 mg/meal/person as the most conservative level. In the case of patients with histamine intolerance, even small amounts of this amine in food may cause adverse effects, so as the EFSA (2011) stated, “only levels below detectable limits can be considered as safe”.

To quantify the contribution of the exposure to tyramine and histamine from dry fermented sausages to reach the maximum tolerable levels for healthy and sensitive population a ratio between the exposure level and those thresholds was calculated.

3. Results and discussion

3.1. Distribution of tyramine and histamine contents in dry fermented sausages from the Spanish market

Fig. 1 shows the distribution of tyramine contents (mg/kg) in
Retail Spanish dry fermented sausages. Tyramine was the most frequent and abundant bioactive amine found in retail dry fermented sausages (detected in the 99.5% of samples) following a lognormal distribution with the parameter estimates shown in Table 1. The mean value was 139 mg/kg (relative standard deviation, RSD of 86%) and the range was from not detected (<0.01 mg/kg) to 742 mg/kg, though 95% of samples did not exceed 400 mg/kg. No significant differences were found among contents of tyramine depending on the type of sausage (p > 0.05), neither no significant trend was found when assessing tyramine contents as a function of years. Tyramine is extensively reported as the main bioactive amine found in dry fermented sausages, in which is usually produced by fermentative microbial population, mainly lactic acid bacteria (LAB) and more rarely coagulase negative staphylococci (Aymerich et al., 2006; Talon and Leroy, 2011; Latorre-Moratalla et al., 2012b). Latorre-Moratalla et al. (2010) reported that 48% of LAB and 13% of staphylococci isolated from spontaneously dry fermented sausage are able to decarboxylate tyrosine to form tyramine. Likewise some strains of spoilage microbiota have also been described as strongly tyraminogenic (Latorre-Moratalla et al., 2012b).

The occurrence of histamine in retail Spanish dry fermented sausages was less frequent (in the 66% of samples) and usually at lower levels than tyramine. Histamine contents followed a beta general distribution (Table 1). The mean value was 27 mg/kg (RSD of 215%) and the 95-percentil 143 mg/kg (Fig. 2). Among samples containing histamine, 57% did not exceed 10 mg/kg. In some particular samples, high histamine contents were found, reaching 475 mg/kg generally accompanied by high amounts of other bioactive amines, such as tyramine, putrescine and cadaverine (data not shown). Chorizo sausage showed the highest histamine levels, although the mean value was not statically different when compared with the other products (p > 0.05). As in the case of tyramine, histamine contents were not significantly different according to the year of analysis. Some strains of a reduced number of enterobacteria or LAB have the capability to produce histamine in dry fermented sausages. However, the presence of these specific bacteria in this kind of food is not very common unless specific contaminations occur (Roig-Sagués et al., 1996; Bover-Cid et al., 2001). Therefore, high contents of this amine in dry fermented sausages are usually considered as a hygienic indicator of poor

\[
\text{Table 1. Distributions used as inputs for the exposure assessment model and the resulting outputs for dietary exposure of consumers to tyramine and histamine.}
\]

<table>
<thead>
<tr>
<th>Distributional assumption(^a)</th>
<th>Inputs</th>
<th>Outputs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyramine contents (mg/kg)</td>
<td>Log Normal (178.26; 128.74; shift [-37.832])</td>
<td>Pearson5 (2.70; 14.75; Shift [-2.4])</td>
</tr>
<tr>
<td>Histamine contents (mg/kg)</td>
<td>Beta General (0.19; 10.79; 0.01; 1510.4)</td>
<td>Log Normal (6.77; 304.2; Shift [-0.000005])</td>
</tr>
<tr>
<td>Consumption (g/serving size)</td>
<td>Gamma (1.97; 22.1; shift [1.90])</td>
<td></td>
</tr>
<tr>
<td>Exposure of tyramine (mg/meal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure of histamine (mg/meal)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Probability distributions best fitting available data (lowest \(\chi^2\) according to \(\Phi_{\text{risk}}\).

\(^b\) Mean.

\(^c\) Standard deviation.

\(^d\) Minimum.

\(^e\) Maximum.

\(^f\) Shape \(\alpha\).

\(^g\) Scale \(\beta\).

Fig. 1. Distribution of tyramine contents (mg/kg) in Spanish dry fermented sausages.
The large variability of tyramine and histamine contents observed in retail Spanish dry fermented sausages could be explained by multiple factors such as the microbiological quality of raw materials, which varies in each production batch; ingredients and additives (sugar, curing agents, spices, etc.). Additionally, diameter of sausage and technological ripening conditions (temperature and relative humidity) can also influence the phenomena associated with aminogenesis, including microbial growth, acidification, proteolysis and activity of decarboxylases (Suzzi and Gardini, 2003; Spano et al., 2010; Singh et al., 2012). Therefore, the probabilistic exposure assessment from data about biogenic amine in retail products becomes the most suitable approach to capture and deal with the actual variability of the occurrence of these hazards.

3.2. Distribution of the consumption of dry fermented sausages

According to the last extensive national dietary survey performed in Spain (AECOSAN, 2011) a wide variability in the amount of sausage consumption was recorded, ranging from 2 to more than 250 g/meal. The mean consumption was 45 g/meal (RSD of 73%) and the 95-percentile 100 g/meal (Fig. 3). Consumption data described a gamma distribution (Table 1). According to this survey, the 19% of meals contain some kind of dry fermented sausages as starter, main dish or snack, although the serving size changed in each case. Concretely, the product most often consumed is chorizo.
(about once a week) with a mean value of 42 g/meal/person. **Salchichón** sausages type is consumed less frequently (about once every 3 weeks) but in higher amounts (mean of 52 g/meal). **Sobrasada** and **salami** are more sporadically consumed but in similar amounts to **salchichón**. No statistically significant differences were detected in consumption according to age or gender of consumers.

### 3.3. Exposure assessment and hazard characterization of tyramine

The outputs of the probabilistic exposure assessment for tyramine performed by combining probability distributions of tyramine contents and sausages consumption are shown in Fig. 4. The exposure to tyramine from dry fermented sausages followed a Pearson5 distribution (Table 1). The mean value for tyramine exposure was 6.2 mg/meal (RSD of 124%). In the 95% of meals involving dry fermented sausages, the exposure to tyramine was below 21 mg, whereas the maximum level was 93 mg/meal.

In the assessment carried out by EFSA, tyramine exposure data on dry fermented sausages from Spain is not available and only a total European 95-percentile was reported, ranging from 17.2 to 99.3 mg/day, depending on the country (EFSA, 2011). Also considering the high level exposure (95-percentile) to tyramine of the Spanish population (21 mg/meal), this data is placed in the lower European range determined by the EFSA. This lower intake of tyramine per meal could be attributed to lower serving size of these products by the Spanish population in comparison with other EU countries, as the occurrence of tyramine in Spanish dry fermented sausages is similar in both assessments.

Table 2 shows the contribution of the exposure to tyramine from Spanish dry fermented sausages to achieve the maximum tolerable levels for healthy population and for vulnerable population under treatment with RIMA (reversible inhibitor of monoamine oxidase A) or MAOI (monoamine oxidase inhibitor) drugs. Although this contribution is apparently low, it must be taken into account that meals may also contain other food with variable bioactive amine levels (e.g. cheese, wine, etc.). The assessment of the overall exposure to tyramine by the multiple sources of this bioactive amine remains unknown.

In the case of people under treatment with RIMA drugs, the 95-percentile of exposure of tyramine (21 mg/meal) meant the 42% of the maximum tolerable level. The probability to reach an exposure of 50 mg of tyramine in a meal was very low (0.01%) and only this threshold limit was exceeded with the maximum value of tyramine exposure (92.5 mg/meal). The situation is different in those individuals treated with MAOI drugs, in which the mean exposure to tyramine by the consumption of sausages already exceeded the safe dose (6 mg/meal). According to the tyramine exposure output, the probability to surpass this tolerable exposure level per meal was estimated to be 34%.

According to the last report on antidepressant drug consumption by the Spanish population published by the Spanish Agency on Medicinal Products, the only MAOI and RIMA drugs used for the treatment of depression are tranylcypromine and moclobemide. Nowadays, up to 0.001% of the Spanish population is receiving a daily dose of these drugs, being used only as an alternative therapy in depressive patients resistant to more frequently used antidepressants (AEMPS, 2015). In view of this data and taking into account the occurrence of tyramine in Spanish dry fermented sausages, it is evident that the contribution of this bioactive amine to the overall exposure to tyramine by the multiple sources remains unknown.

![Fig. 4. Results of tyramine exposure (mg/meal) from dry fermented sausages by the Spanish population.](image)

**Table 2**

<table>
<thead>
<tr>
<th>Tyramine exposure (mg/meal)</th>
<th>Contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy population</strong></td>
<td><strong>RIMA patients</strong></td>
</tr>
<tr>
<td>Mean</td>
<td>6.2</td>
</tr>
<tr>
<td>95-Percentile</td>
<td>21</td>
</tr>
<tr>
<td>Maximum</td>
<td>92.5</td>
</tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

* 600 mg/meal for healthy population, 50 mg/meal for patients treated with RIMA drugs and 6 mg/meal for patients treated with MAOI drugs.
account that approximately 46 million people in Spain are potential consumers of dry fermented sausages (i.e. total Spanish population excluding the 1.5% reported as not consumers of meat products according to ENIDE survey), 3 individuals per million (i.e. 156 individuals) would be daily exposed to a level of tyramine above the maximum tolerable threshold when taking MAOI drugs. However patients prescribed with MAOI and RIMA are usually advised to limit or avoid the consumption of dry fermented sausages. Therefore, as MAOI treated individuals could have consumption-patterns different from the data used in the present assessment (from mostly non MAOI treated people), the actual exposure to tyramine of this vulnerable population will be most probably lower than the above estimation.

3.4. Exposure assessment and risk characterization of histamine

The outputs of the probabilistic exposure assessment for histamine performed by combining probability distributions of histamine contents and sausages consumption are shown in Fig. 5. Levels of exposure to histamine from the consumption of dry fermented sausages followed a lognormal distribution (Table 1) with a mean value of 1.39 mg/meal (RSD of 228%). Only 5% of meals resulted in histamine intake levels higher than 6.8 mg. As in the case of tyramine, in the European exposure assessment histamine exposure data specifically for Spain is not available. The total European 95-percentile reported for histamine exposure in these type of products was from 6.4 to 37.1 mg/day, depending on the country (EFSA, 2011). Spanish exposure levels obtained in the present assessment are clearly placed within the lower interval, which could be explained, as in the case of tyramine, by the lower consumption of these products by the Spanish population in comparison with those reported for the European population.

Table 3 shows the contribution (percentage) of the exposure to histamine from dry fermented sausages to achieve the maximum tolerable level adopted for preventing histamine intoxication (25 mg/meal). Considering the mean exposure, only the 6% of the critical threshold was reached, and even considering 95-percentile of the exposure less than the 30% of this limit was reached. Based on the present results, the risk to suffer acute effects related to histamine by the consumption of Spanish dry fermented sausages is really low, since only in very rare occasions (maximum exposure value) the threshold was nearly doubled.

An additional concern associated with the occurrence of histamine in food is for people suffering from histamine intolerance, in which even the presence of this amine at very low levels is considered to be capable of triggering adverse effects. This intolerance related to a deficiency in the metabolism of histamine due to the lack of specific enzymes, which produces its accumulation in plasma and the appearance of multi-faced clinical symptoms. For this reason, for individuals with histamine intolerance only food with levels of this amine below detectable limit could be considered as safe (EFSA, 2011). Moreover, it should be taken into account that the occurrence of dietary potentiators, such as other bioactive amines or alcohol and its metabolite acetaldehyde, could also increase or enhance the susceptibility to histamine and, accordingly, the risk of adverse reactions could rise (Maintz and Novak, 2007). In the dry fermented sausages from the Spanish market considered in this study, the 66% contained histamine, and very frequently accompanied with other potentiate bioactive amines such as putrescine and cadaverine.

Some studies estimate that histamine intolerance may affect 1% of the general population (Maintz and Novak, 2007; Kovacova-Hanuskova et al., 2015). However, the increasingly widespread occurrence of histamine in food has increased the awareness of this issue, and more studies are needed to better understand the prevalence and clinical implications of histamine intolerance. Furthermore, the occurrence of potential dietary potentiators, such as other bioactive amines, should also be considered when assessing the risk to suffer adverse effects related to histamine.
diagnosis of histamine intolerance by DAO deficiency could augment this population percentage. In fact, it has been published recently more evidence about the relationship between a reduced capacity to metabolize dietary histamine by a deficit of DAO and health problems such as migraine, gastrointestinal disorders, skin reactions and muscle pain, among others (Guida et al., 2000; Vidal-Carou et al., 2010; Izquierdo et al., 2012; Rosell-Camps et al., 2013; Tormo, 2013). Some recent interventional studies have shown a high incidence rate of DAO deficiency (up to 80%) among this type of susceptible population (Izquierdo et al., 2012; Rosell-Camps et al., 2013). In addition, it should be considered that histamine intolerance can be a consequence of inhibition of DAO activity as a side effect caused by a number of commonly used drugs, including acetylsalicylate, clavulanic acid, metoclopramide, verapamil, isoniazid, etc. (Maitz and Novak, 2007; Kovacova-Hanuskova et al., 2015). Indeed, it was estimated that approximately 20% of the European population could be taking some of the DAO inhibitor drugs (EFSA, 2011).

Considering that 46 million people in Spain are potential consumers of dry fermented sausages and that the histamine intolerance could affect at least the 1% of the population and taking into account the estimation that the 66% of retail dry fermented sausages contained histamine (percentage obtained in the present study), it could be expected that the 0.7% of Spanish population (303,600 individuals) may suffer some of the related symptomatology after the consumption of dry fermented sausages. Moreover, it should be taken into account the existence of other potential sources of histamine, such as cheese, fish or fish products, fermented beverages and some vegetables, that are fairly widespread making the risk of suffering this intolerance higher.

4. Conclusions
A reliable estimation of exposure has been achieved through the probabilistic assessment from representative Spanish data of amino contents and consumption of dry fermented sausages, in which the actual distribution of the content of the hazard and the mathematical function describing the probability of occurrence of every value is described. According to this probabilistic estimation, tyramine and histamine intake would hardly contribute to reach their maximum tolerable threshold for healthy individuals. However, sensitive people treated with MAOI drugs or with histamine intolerance by deficit of DAO would be at risk of suffering adverse effects due to the consumption of dry fermented sausages. Concretely, the maximum tolerable limit established for patients under MAOI treatment (6mg/meal) was exceeded in the 34% of meals containing dry fermented sausages. Therefore, it could be estimated that 3 habitants per million of the Spanish population could suffer adverse health effects due to the exposure to tyramine from dry fermented sausages and the concomitant consumption of MAOI drugs. This estimated risk is very low compared with that found for individuals with histamine intolerance, which rises up to practically 7000 cases per million habitants.
Due to the estimated risk of suffering adverse effects attributed to histamine in food, it seems important that food industry accepts the challenge to proceed with measures focussed on the minimization of the occurrence of histamine in food. This implies to control the potentially aminogenic microorganisms, its growth and its amino acid-decarboxylase activity during the whole production and merchandising chain. Moreover, the statement of tyramine and histamine content in food labelling would be another additional measure to prevent the occurrence of the related adverse effects of those bioactive amines in sensitive individuals, especially for histamine intolerant individuals. Additional studies are needed to estimate the maximum level of histamine and eventually other amines in food to provide histamine intolerant patients with clearly labelled products.

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Transparency document
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