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Distribution free methods to model the content of biogenic amines in Spanish wines

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ABSTRACT

Biogenic amines are formed from precursor amino acids by various microorganisms present in wine, and this may happen at any step of production, ageing or storage. The presence of these compounds is important because high amounts of them can lead to health problems. Also, biogenic amines can be potentially applied as indicators of food spoilage and/or authenticity.

Nevertheless, there are complex relationships among biogenic amines in Spanish wines and their distribution is far from a normal distribution. Even though, this structure must be taken into account to provide a frame of reference on the quality of wines and to contribute to the efforts of the entire productive chain to attain consumer safety.

In this work, 684 samples of wines from different Spanish regions have been analyzed in order to determine the content of histamine, tyramine, phenylethylamine, cadaverine and putrescine during 2010, 2014 and 2015. The statistical distribution of histamine has been modelled by using the β-content tolerance intervals. Besides, copulas to obtain the joint multivariate confidence region between histamine and tyramine have been built for the first time in the oenological field. The β-content tolerance intervals for histamine content, in the case of the population of red wines in 2010, lead to decide that only 53.9% of the distribution would be below 10 ppm (with a 95% confidence in this affirmation). This percentage rises from 53.9 to 74.6% in the year 2014 and to 90.2% in 2015. Besides, the conjoint distribution of histamine and tyramine is modeled by a Clayton copula with margins estimated by Gaussian kernel, which allows concluding that this distribution is similar for the three years.

1. Introduction

Biogenic amines (BAs) are low molecular weight organic compounds with biological activities which are frequently found in different fermented foods (e.g. cheese, milk, beer, wine). The formation of these low molecular nitrogen compounds is mainly through four enzymatic reactions: (i) decarboxylation, (ii) transamination, (iii) reductive amination, and (iv) degradation of certain precursor amino compounds [1]. The presence of biogenic amines in wines has been studied extensively since 1980 and particularly over the last 10 years as a consequence of the increasing attention to consumer protection. In order to eliminate biogenic amines in wine, it is necessary to identify the source of these compounds [2]. In wine, several amino acids can be decarboxylated so that histamine, tyramine, putrescine, cadaverine and phenylethylamine are produced, though histamine, tyramine and putrescine are the main biogenic amines observed in wine [3–4]. The amino acid profile, as chemical precursor of BAs, influences their formation, so the presence of biogenic amines in wine can be different according to the variety of grapes [5]. BAs can be already present in "must" or formed by the yeast during alcoholic fermentation. The other alternative for origin of biogenic amines in wine is the action of bacteria involved in malolactic fermentation [6]. Factors influencing the formation of biogenic amines, covering the entire production chain, are critically reviewed in Ref. [7]. Some amines are normally present in grapes depending on the soil type and composition, fertilization of soil, climatic conditions during growth, degree of maturation, elaboration method used for the wine extraction, autolysis and growth of lactic acid bacteria, residual microbial population, clarification process, oenological treatment of the wine as well as the yeast strain used in the fermentation [8]. In several studies, biogenic amines have been suggested as indicators of a lack of hygiene during the winemaking process or associated (in the case of putrescine and cadaverine) with poor sanitary conditions of grapes [2,9]. Fermentation conditions of wine are also important for the biogenic amine production. These are: temperature, pH, access to oxygen, sodium chloride content, "must" treatment, length of

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fermentation, presence of pulp and skin, alcohol content, concentration of sulfurdioxide and added nutrients, quantity and type of finings, and clarification agents used [10].

In Ref. [11] Martuscelli et al. investigate the red, white and rose Abruzzo wines using their content of biogenic amines as an indicator of product and process quality. The goal of this research was to control the achieved levels of toxic amines in relation with some agronomic and oenological factors, to establish the real risks associated with the presence of biogenic amines in these wines and, finally, to provide wine producers the opportunity of new markets and commercial trades.

Trace BAs are usually present in the organism and have physiological functions in biological cells. However, excessive intake of BAs could lead to several health problems, such as headache, blushing, itching, skin irritation, impaired breathing, tachycardia, hypertension, hypotension and vomiting [12].

It was pointed out [13] that high levels of histamine might cause food poisoning. Although it is commonly associated with the consumption of scombroid-type fish, other foods such as cheese and wine have also been implicated in outbreaks of histamine poisoning. Excessive intake of tyramine was considered as dangerous because the increasing blood pressure might lead to hypertension. While histamine has received much attention (also tyramine although to a lesser extent) due to higher toxicity, there are numerous reports in the literature concerning other biogenic amines such as putrescine, phenylethylamine and cadaverine; the latter three at least can potentiate the negative effects of the former two (histamine and tyramine). Furthermore, secondary amines such as putrescine and cadaverine were able to react with nitrite producing carcinogenic nitrosamines. As a consequence, monitoring of the BA levels can be a crucial step for decreasing the possibility of health risks and preventing future sanitary problems. The same EFSA, European Food Safety Authority, report showed the percentiles (5, 50 and 95) and mean values found separately in histamine, tyramine, putrescine, cadaverine and phenylethylamine in red and white wines. A wide range of concentrations was observed, starting from not-detected up to 55 mg kg^{-1}. Other authors [14] have found higher values of 130 mg L^{-1}. So the production of wines with a lower content of BAs is a worldwide concern. European project BiamFood refers to this issue [15]. It literally says that "*Control of biogenic amines in the food products will significantly reduce the health risk for the consumer and*, *thereby*, *increasing the competitiveness of the local industries*". Also, the OIV (International Organisation of Vine and Wine) [16] has published the OIV code of good viniculture practices to reduce the BA contents in vine-based products. Even though there are no accurate regulations, several countries are requiring BA analysis. For instance, the recommended upper limit for histamine is 10 mg L^{-1} in Australia and Switzerland, 8 mg L^{-1} in France, 3.5 mg L^{-1} in Netherlands, 6 mg L^{-1} in Belgium and 2 mg L^{-1} in Germany. Such limits imply severe barriers to wine-exporting countries. The only country that established an official maximum limit of 10 mg L^{-1} for the presence of histamine in wines, Switzerland, removed it in imported wines in 2011 [11,17].

To get an accurate description of the BA content in Spanish wines, their structural characteristics of these data should be taken into account, characteristics related to i) the different origin of BAs (grape varieties, viticulture practices, oenological and aging procedures) and ii) the mutual relation between them. The former causes a lack of normality, so the classical approach for tolerance intervals, based on normal distribution, is not adequate. The second causes the nonlinear interrelations between the BAs, due to the multiple natures of their mechanisms of generation.

The β-content unilateral tolerance interval (β-TI), for each biogenic amine, is the interval [0,b] that with a confidence of γ (e.g. $\gamma = 0.95$) contains a percentage 'p' of values (e.g. $p = 0.90$). The procedure to obtain 'b' can be parametric, if the distribution of values follows a known parametric distribution (e.g. normal) or nonparametric. The β-TI is a more robust estimation about the true limits, those that could be expected in the Spanish wines, so it provides more reliable information about the compliance or noncompliance with respect to an established limit. Details about the β-TI, its relation with the confidence interval and technical references can be consulted in Ortiz et al. [18].

Multivariate methods are necessary wherever independence cannot be assumed among the variables under investigation as in the BA content in wines. For example, if simultaneous surveillance of BAs in a quality control framework is needed, univariate methods are inefficient because of the dependence among BAs. Furthermore, classical multivariate statistical methods for this purpose (e.g. the Hotelling T^2 chart for monitoring a vector of means) are based on the multivariate normal distribution [19]. These methods have become so popular that they are often applied without a careful check about whether the multivariate normality can be assumed. Non normality can occur in different ways. First, the marginal distribution of BAs may not be normal. For instance, in the BA analysis we expect that the contents of each amine are skewed and heavy-tailed (many wines have low values but there are a few with very high values) and hence it cannot be adequately modeled by normal distributions. Second, jointly the BAs in wine have a very nonlinear relationship, so the multivariate normality is not valid once again. Nonnormal multivariate distributions built from copulas have proved very useful in recent years in many applications. A copula can be used to couple together different marginals (those of the BA content in wine) and to build new multivariate distributions. This method separates a multivariate distribution into two components, all the marginals and a copula, providing a very flexible framework in multivariate statistical modeling. Comprehensive book references on this subject are [20] and [21]. The β-TI and copulas are used for the first time to more reliably model of the BAs content in wines. Despite its little widespread use in chemometrics, both nonparametric procedures used are of interest to analyze chemical data to globally characterize sets of samples of different origin because, in general, the data are not normally distributed and their internal relations are not lineal.

2. Theory

2.1. β-content tolerance interval (β-TI)

The setting of confidence intervals is most frequently explained for checking whether the parameters of a distribution (e.g. mean or standard deviation of a normal distribution) are into a fixed specification. But the technique of confidence intervals can be used for other problems; for example these intervals can be found for the quantiles of a parent distribution and also for the entire distribution itself, without any assumption on the form of the distribution beyond its continuity. There is another type of problem, commonly met in practical sampling (as in the BA data here) which may be solved by these methods. Suppose that, on the basis of a sample of n independent observations from a distribution (as the histamine content of the Spanish red wines in a year), two limits a and b should be found so that at least a given proportion β of the distribution lies in the interval defined by these a and b. Clearly, such an assertion only has meaning in a probabilistic form, i.e. we assert that, with given probability, at least a proportion β of the distribution lies between a and b. These values a and b are called tolerance

Table 1
Sample distribution per type of wine and year.

Year	Wines	Number of samples
2010	Red	114
	Rose	8
	White	10
2014	Red	221
	Rose	23
	White	44
2015	Red	208
	Rose	18
	White	38