

# Ochratoxin A in Wines and Evaluation of Consumer Exposure

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**Abstract** The purpose of this work was to assess the incidence of ochratoxin A (OTA) in wines, as well as the risk of human exposure to this toxin. The estimate of OTA content was by means of high-performance liquid chromatography based method. Thirty-eight samples were analyzed, from which 27 red wines, 7 white wines, two rosé wines, and two fortified wines. Samples were grouped into domestic and imported wines. Mathematical equations were used to assess the aggregate exposure, seeking to evaluate the human risk to OTA. The detection limits and OTA estimate were around 0.16 and 0.56  $\mu\text{g}\cdot\text{kg}^{-1}$  respectively. About 36.84% of samples showed OTA contamination, all of them imported. From these samples, about 85.71% showed levels of OTA contamination less than  $2\mu\text{g}\cdot\text{L}^{-1}$ . Results indicate that the consumption of analyzed wines does not imply OTA contamination for the population under study.

**Keywords** Risk evaluation, Contamination, Intoxication, Toxin

## 1. Introduction

The ochratoxin A (OTA) is a secondary metabolite produced by species of filamentous fungi belonging to the *Penicillium* and *Aspergillus* genera (EC, 2006; Brera et al., 2011). The occurrence of this toxin in food has been extensively reported, mainly in cereals and derivatives from cereals, coffee, cocoa, beer, grape, grape juice, dehydrated fruits, medicinal plants, and wine; these being considered the second most important source of OTA for humans in Europe (CAC, 1995; Miraglia and Brera, 2002).

The ochratoxigenic species have been found in temperate and tropical climate regions, and they are found in soil and organic matter, resulting in grapes contamination during the berry growth period. By having a very stable molecule, this mycotoxin is not degraded during processing, and may remain in large amount of food (Murillo Arbizu *et al.*, 2010).

The presence of this toxin in food and beverage is found to be a risk toward population, because OTA has carcinogenic, nephrotoxic, teratogenic and immunosuppressive properties (Solfrizzo et al., 2010), being classified as a possible human carcinogen (group 2B) (IARC, 1993).

After the first report of occurrence of OTA in wine (Zimmerli and Dick, 1996), various studies were performed to evaluate the presence of this mycotoxin in wine and in wine derivative products (Stefanaki et al., 2003; Ratola et al., 2004). Such results had caused considerable concern about potential risk to human health, by the consumption of these products (Tjamos et al., 2004), showing an increasing need to use tools that not only detect the presence of the toxin in the food, as well as assess the exposure risk to this substance. A model that has been increasingly used in many studies is the evaluation of risk, which contributes to take control steps by means of scientific evidences (WHO, 2006).

Considering the importance of risk assessment in the management of food safety, and the existing poor knowledge about this subject in Brazil, as well as the increase in wine intake recorded in the country; this study was performed aiming to assess the human exposure risk to OTA, resulting from the consumption of domestic and imported wines.

## 2. Material and Methods

Thirty-eight wine samples were randomly obtained in different commercial establishments of Lavras, State of Minas Gerais, Brazil. Then, they were analyzed after being grouped in twenty-seven red wines, seven white wines, two rosé wines, and two fortified wines. Samples were grouped

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into domestic (table wines) and imported (fine wines) brought from France, Portugal, Argentina, Chile, Uruguay and South Africa (Table 1). The most part of samples consisted of table wines from Brazil (17), followed by wines from Argentina (8), Portugal (6), Chile (3), France (2), Uruguay (1) and South Africa (1).

The high-performance liquid chromatography (HPLC) method was used to estimate the content of OTA in wine samples, which uses the fluorescence detection system, as outlined in EN 14133/2003 (EN, 2003).

**Table 1.** Samples of wine and their respective countries and production years

	Samples of wine	Type of wine	Production year	Production country
1	BRA/VMT/SU1		2011	
2	BRA/VMT/SU2		2011	
8	BRA/VMT/SU3	Soft red table wine	2011	Brazil
9	BRA/VMT/SU4		2011	
10	BRA/VMT/SU5		2011	
11	BRA/VMT/SU6		2011	
3	BRA/VMT/SE1	Dry red table wine	2011	Brazil
7	BRA/VMT/SE3		2011	
6	ARG/VMT/SE2	Dry red table wine	2011	Argentina
4	BRA/VMR/SU1	Soft rosé table wine	2011	Brazil
5	BRA/VMR/SU2		2011	
12	BRA/VMB/SU1	Soft white table wine	2011	Brazil
14	BRA/VMB/SU2		2011	
16	BRA/VMB/SU3		2011	
17	BRA/VMB/SU4		2011	
13	BRA/VMB/SE1	Dry white table wine	2011	Brazil
15	BRA/VMB/SE2		2011	
18	BRA/VMB/SE3		2011	
19	URU/CS/1	Fine red wine	2010	Uruguay
20	ARG/MER/1	Fine red wine	2009	Argentina
23	ARG/MAL/1		2010	
26	ARG/CS/2		2009	
28	ARG/MAL/2		2010	
27	ARG/MAL/3		2010	
30	ARG/MAL/4		2009	
34	ARG/CS/3		2009	
21	PORT/VP/1	Port wine	-	Portugal
36	PORT/VP/2		-	
22	PORT/VMT/SE1		2010	
31	PORT/VMT/SE2	Table wine	2010	Portugal
32	PORT/VMT/SE3		2007	
25	PORT/RL/1	Fine red wine	2010	Portugal
24	FRA/VR/1	Fine red wine	2010	France
37	FRA/JP/2		2011	
29	AFR/CS/1	Half-dry fine red wine	2010	South Africa
33	CHI/MER/1	Fine red wine	2011	Chile
35	CHI/MER/2		2010	
38	CHI/CS/2		2010	

### 2.1. Solution and Reagents

A dilution solution was prepared by dissolving 10 g of Polyethylene Glycol 8000 and 50g of sodium bicarbonate (NaHCO<sub>3</sub>) in 1000mL of purified water. For obtaining the washing solution, 25g of sodium chloride (NaCl) and 5g of sodium bicarbonate were dissolved in 1000mL of purified water. The OTA Sigma stock solution (St. Louis, MO, USA) was prepared in toluene: acetic acid (99:1 v/v). The concentration was estimated according to the Association of Official Analytical Chemists (AOAC, 1997), and was verified in spectrophotometer at 333nm, with  $\epsilon = 5.440 \text{ cm}^{-1}\text{mol}^{-1}\text{L}$ . The working solution was prepared by the appropriate dilution in toluene: acetic acid (99:1 v/v) for the recovery testing and calibration curve. For the mobile phase, the acetonitrile: methanol: aqueous acetic acid (35:35:30) was used, followed by vacuum filtration through regenerated cellulose membranes PTFE about 0.45  $\mu\text{m}$ . The aqueous acetic acid was prepared using glacial acid acetic solution in purified water (1:29 v/v). Solutions were stored in dark, at temperature from -15°C to -20°C.

### 2.2. Preparation of Samples and Purification in Immunoaffinity Column

Firstly, samples were cooled to 4°C. For each sample, 40 mL were added to 40mL of dilution solution and homogenized under mechanical stirring on a shaker at medium speed for 30 minutes. This solution was subjected to vacuum filtration (2mL per minute) in GFA membrane and, 40mL of the filtrate were passed through an immunoaffinity column (Ochraprep, R-Biopharm Rhône Ltd) adapted to the Visiprep TM SPE Vacuum Manifold system. The column was washed using 10mL of washing solution and, then, using 10mL of purified water for the removal of non-specific residues. Subsequently, 2mL of methanol was added to the column for the release of OTA bound to the antibody, with repetition of the procedure for three times. The eluate was evaporated with heating samples (at around 50°C) under nitrogen atmosphere. The dried extract was reconstituted in 250 $\mu\text{L}$  of the mobile phase. Fifty  $\mu\text{L}$  of standard solutions of OTA and of extracts of samples were injected in the liquid chromatograph.

### 2.3. Quantification of Ochratoxin A by Liquid Chromatography

The equipment used was the Shimadzu High-Performance Liquid Chromatograph (HPLC), equipped with two high-pressure pumps, model SPD-M20A; degasser, model DGU 20A3; interface, model CBM-20A; automatic injector, model SIL-10AF; and fluorescence detector RF-10 AXL. The column was the Agilent-Zorbax Eclipse XDB-C18 (4.6  $\times$  250 mm, 5 $\mu\text{m}$ ) connected to a guard column Agilent-Zorbax Eclipse XDB-C18 4-Pack (4.6  $\times$  12.5 mm, 5  $\mu\text{m}$ ).

The wavelength chromatographic conditions were as follows: excitation at 332nm and emission at 476nm. The flow rate throughout the analysis was about 0.8mL.min<sup>-1</sup> and,

the injected volume of samples, as well as of the standard, was 20 $\mu\text{L}$ . The elution was carried out in an isocratic system of 35:35:29:1 (methanol: acetonitrile: water: acetic acid). The OTA quantification in samples was carried out by developing a calibration curve obtained by linear regression ( $y = 1.11756x107x - 2592.1485$ ; where  $y$  = peak area and  $x$  = concentration of OTA), correlating the peak area versus the concentration of the respective standard solution. The coefficient of determination ( $r^2$ ) was 0.9999. The detection limit (DL) and quantification limit (QL) were estimated by means of parameters obtained for the calibration curve, and estimated by means of their mathematical relations:  $DL = 3SD/m$  and  $QL = 10SD/m$  (where  $SD$  = estimate of Standard Deviation of the regression line and  $m$  = angular coefficient of the calibration line) (Harris, 2008). Then, estimates about 0.0004 and 0.0016  $\mu\text{g.g}^{-1}$  respectively were found. All samples were analyzed in duplicate, while standard solutions of OTA were injected in triplicate.

Confirmation of the presence of OTA was determined by the formation of methyl esters, which resulted in the increase of retention time of samples due to OTA derivatization. From the estimate of OTA peak area of samples extracts, and of standard solutions, the content of OTA in samples was quantified. In the analysis conditions, the retention time was about 9.99 $\pm$ 0.56 minutes. The DL and QL were 0.16 and 0.56 $\mu\text{g.L}^{-1}$  respectively.

To ensure calibration quality of results, recovery trials were performed. Wines were fortified in three levels with concentrations equal to 1.0ng.mL<sup>-1</sup>, 2.0ng.mL<sup>-1</sup>, and 4.0ng.mL<sup>-1</sup>; in triplicate. They were extracted using methanol and analyzed according to the previously described methodology.

The following recovery rates were respectively obtained: 82%, 82% and 98%; which proved the excellent reproducibility of the method; in accordance with rules of the CODEX, in the criteria of performance of calibration methods, between 70% and 110% of recovery (CAC, 1995).

To assess the human exposure to OTA found in elements under study, the analysis of the daily mean intake quantity of wine was performed. According to the obtained values, an estimate of exposure was obtained using the following equation:

$$\text{Exposure} = \frac{\text{Substance Concentration} \times \text{Food Intake}}{\text{Corporeal Weight}}$$

The estimate to assess the aggregate exposure was carried out according to deterministic method; on which fixed, punctual, concentration, and intake values were used according to results obtained from analyses of wines.

The estimate of daily intake in mg.kg<sup>-1</sup> corporeal weight per day was carried out considering the sum of wine intake by the population under study, using the following equation:

$$\text{Ingestion} = \sum R_i \times C_i / \text{Corporeal Weight}, \text{ where:}$$

$R$  is the substance concentration in the wine  $i$  in mg.kg<sup>-1</sup> and  $C$  is the daily intake of the beverage by the population or

individual, in liters (WHO, 1997).

Experiments were established in completely randomized design with ten replications per treatment and, for the statistical analysis, the SISVAR statistical package was used. The analysis of variance was carried out, and means were compared by the Scott Knott (1974) test at 5% significance.

An exploratory data analysis, correlating the content of OTA with the origin, type of grape and type of wine, was carried out by means of principal component analysis (PCA). Data were processed in a matrix containing samples of wine arranged in rows, and variables in columns. The qualitative variables considered here were: (i) Brazil, Uruguay, Argentina, Portugal, France, South Africa and Chile - for the origin; (ii) table wine, Cabernet Sauvignon, Merlot, from Port, Malbec and Cabernet-syrah - for type of grape; and (iii) soft red wine, dry red wine, soft rosé wine, soft white wine, dry white wine, fine red wine, and fortified wine - for type of wine. These variables were expressed as binary values: 0 = out of class, and 1 = belongs to the class. Data were autoscaled, and the PCA was run. Results were shown in score and weight charts for the first two principal components. The Chemoface v1.5 software was used for the analysis (Nunes *et al.*, 2012).

### 3. Results

#### 3.1. Standardization of the Analytical Methodology

The calibration curve obtained from the linear regression equation, and estimated by the least square method, was linear, and ranged from 0.4 to 10 $\mu\text{g}\cdot\text{L}^{-1}$ .

The mean recovery values and coefficients of variation for wines were 87.5% and 5.3%, respectively (Table 2). These results are within the recommended standards in EC 401/2006 (EC, 2006) for contamination values below 1.0 $\mu\text{g}\cdot\text{L}^{-1}$  and between 1.0 $\mu\text{g}\cdot\text{L}^{-1}$  and 10 $\mu\text{g}\cdot\text{L}^{-1}$ .

**Table 2.** Results of Validation Trials of the Detection Method of OTA

Levels of OTA ( $\mu\text{g}\cdot\text{L}^{-1}$ )	Recovery (%)	Standard Deviation	CV (%)	Detection Limit ( $\mu\text{g}\cdot\text{kg}^{-1}$ )	Quantification Limit ( $\mu\text{g}\cdot\text{kg}^{-1}$ )
0.20	82.3	2.300084	2.79		
0.40	82.3	3.498698	4.25		
0.80	97.9	8.630532	8.82	0.16	0.56
Average	87.5	4.809771	5.3		

#### 3.2. Occurrence and Levels of Ochratoxin A in Wines

The incidence and mean values (minimum and maximum) of OTA found in wine samples are described in the Table 3. As can be seen, the mean levels of OTA of contaminated wines are considered different among the six groups statistically defined (a, b, c, d, e and f) by the Scott Knott test ( $p < 0.05$ ).

The contrast among table red wine from Brazil, white wine, rosé wine, table wine imported, and fine red wine contaminated by OTA was statistically significant. Thus, it is

possible to infer that the mean level of contamination of positive samples of fine red wine was significantly different from the level detected for white, rosé, and table wines.

**Table 3.** Mean Values of OTA in samples under study

Samples of wine	Ochratoxin A ( $\mu\text{g}\cdot\text{L}^{-1}$ )
BRA/VMB/SU3	ND a
BRA/VMB/SU4	ND a
BRA/VMB/SE3	ND a
BRA/VMB/SE1	ND a
BRA/VMB/SU2	ND a
BRA/VMB/SE2	ND a
ARG/CS/3	ND a
ARG/CS/2	ND a
ARG/MAL/2	ND a
CHI/MER/2	ND a
PORT/VP/1	ND a
PORT/VMT/SE1	ND a
BRA/VMR/SU1	ND a
BRA/VMR/SU2	ND a
ARG/VMT/SE2	ND a
BRA/VMT/SU1	ND a
BRA/VMT/SU2	ND a
BRA/VMT/SE1	ND a
BRA/VMT/SU4	ND a
BRA/VMT/SU6	ND a
BRA/VMT/SU3	ND a
BRA/VMB/SU1	ND a
BRA/VMT/SE3	ND a
BRA/VMT/SU5	ND a
CHI/MER/1	0.44 $\pm$ 0,07 b
AFR/CS/1	0.44 $\pm$ 0,07 b
ARG/MAL/4	0.45 $\pm$ 0,07 b
CHI/CS/2	0.50 $\pm$ 0,07 b
URU/CS/1	0.50 $\pm$ 0,07 b
PORT/VP/2	0.56 $\pm$ 0,07 b
ARG/MER/1	0.59 $\pm$ 0,07 b
ARG/MAL/1	0.60 $\pm$ 0,07 b
PORT/VMT/SE2	1.20 $\pm$ 0,07 c
FRA/JP/2	1.39 $\pm$ 0,07 c
PORT/VMT/SE3	1.58 $\pm$ 0,07 d
PORT/RL/1	1.79 $\pm$ 0,07 d
FRA/VR/1	2.46 $\pm$ 0,07 e
ARG/MAL/3	2.77 $\pm$ 0,07 f

\*ND: not detected (Detection Limit: 0.16 $\mu\text{g}\cdot\text{L}^{-1}$ )

Means followed by the same letter in column do not differ one another by the Scott & Knott test at 5% significance.

According to the Table 3, 14 (36.84%) of 38 samples showed contamination by OTA, and values ranged from 0.44 $\mu\text{g}\cdot\text{L}^{-1}$  to 2.77 $\mu\text{g}\cdot\text{L}^{-1}$ . Imported wines, mainly from Argentina and France, were the most contaminated. No samples from Brazil showed the toxin.

The contamination by OTA was relatively low, with the

exception of two samples from France and Argentina, which showed values around 2.47 and 2.77 $\mu\text{g.L}^{-1}$  respectively. From the contaminated samples, 85.71% showed contamination levels by OTA less than 2 $\mu\text{g.L}^{-1}$ . In addition, 100% of domestic wines did not show contamination, 50% of wine from Argentina showed contamination by OTA ranging from 0.45 to 2.77 $\mu\text{g.L}^{-1}$ , and 66.7% of wine from Portugal showed contamination by OTA from 0.56 to 1.79 $\mu\text{g.L}^{-1}$ . Besides, 66% of samples from Chile showed contamination by OTA ranging from 0.44 to 0.50 $\mu\text{g.L}^{-1}$ , while 100% of wine from France showed contamination by OTA around 1.39 and 2.46 $\mu\text{g.L}^{-1}$ . The same occurred for samples from South Africa and Uruguay, on which values were 0.44 and 0.50 $\mu\text{g.L}^{-1}$ , respectively. These results showed that, although fine traditional wines are preferred due to their origin; such France, Portugal and Argentina; they do not fill

the food safety requirement required by consumers, who are currently more demanding and aware of not consuming food that causes any damage to health.

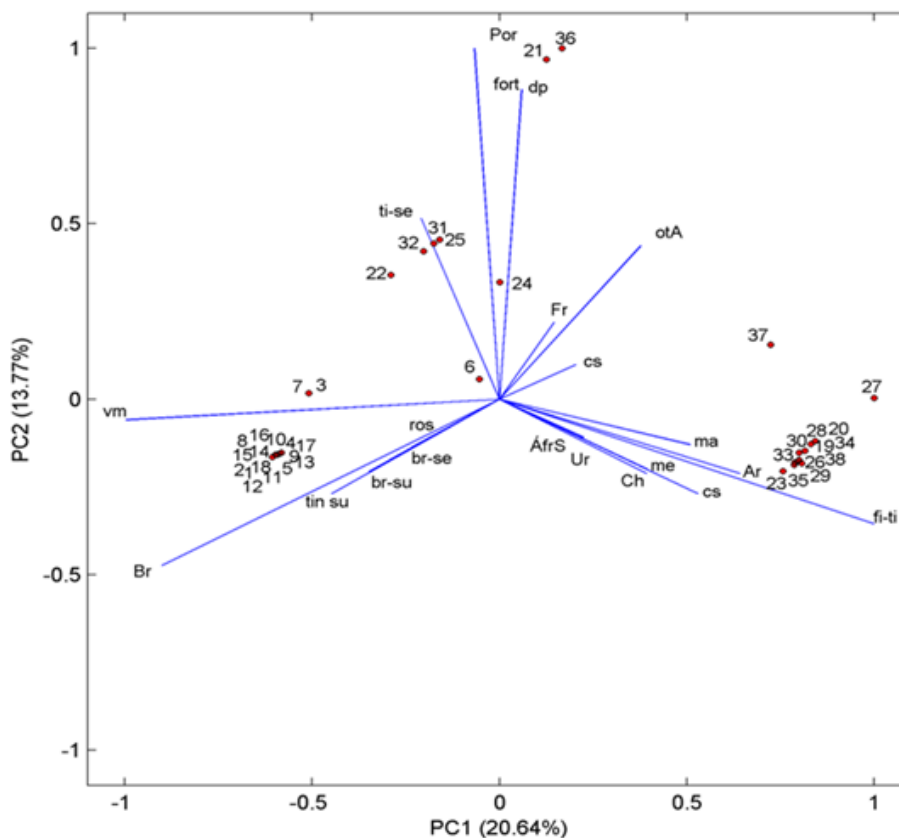
There was not found any OTA in white and rosé wines, what was expected because red wine has showing greater incidence and contamination than white and rosé wines in various studies, due to the winemaking process (Moruno, 2002; Lasram et., 2008). In fact, fine red wines were found to show greater contamination by OTA and, those from Argentina and France showed the greater concentration among them; which was over the maximum limit of 2 $\mu\text{g.L}^{-1}$  established by the European Community and by the Brazilian law.

The contamination levels and the respective countries of origin are detailed in the Table 4.

**Table 4.** Frequency range of contamination by OTA in wines under study

OTA ( $\mu\text{g.L}^{-1}$ )	BRA	ARG	URU	POR	FRA	Africa	CH
ND*	17	4	-	2	-	-	1
0.17 – 0.60	-	3	1	1	-	1	2
0.61 – 1.40	-	-	-	1	-	-	-
1.41 – 1.80	-	-	-	2	1	-	-
1.81 – 2.50	-	-	-	-	1	-	-
>2.50	-	1	-	-	-	-	-

\*ND - not detected (Detection Limit: 0.16 $\mu\text{g.L}^{-1}$ )



**Figure 1.** Relation between the presence of ochratoxin A and the analyzed variables. The numbering of samples corresponds to the numbering showed in the Table 1

**Table 5.** Contribution to the exposure to ochratoxin A, considering the consumption of a cup (150ml) of wine per day, by a consumer around 70 kg weight

Average of contamination (ng.mL <sup>-1</sup> )	Country of origin	Daily safety limit (WHO)	Exposure limit	Participation in daily consumption (%)
0.63	ARG	14 ng.kg <sup>-1</sup>	1.30 ng.kg <sup>-1</sup>	9.3%
0.50	URU	14 ng.kg <sup>-1</sup>	0.75 ng.kg <sup>-1</sup>	5.4%
0.86	POR	14 ng.kg <sup>-1</sup>	1.84 ng.kg <sup>-1</sup>	13.1%
1.93	FRA	14 ng.kg <sup>-1</sup>	4.13 ng.kg <sup>-1</sup>	29.5%
0.45	Africa	14 ng.kg <sup>-1</sup>	0.96 ng.kg <sup>-1</sup>	6.86%
0.31	CHI	14 ng.kg <sup>-1</sup>	0.66 ng.kg <sup>-1</sup>	4.71%

OTA was detected in 14 samples (36.84%), with concentrations ranging from 0.1 to 2.77 $\mu$ g.L<sup>-1</sup>. Positive samples were only found for imported red wines. Only two samples of table wines from Portugal were contaminated by OTA. The white and rosé wines showed low OTA concentration with QL<0.16.

The Figure 1 illustrates the relation between the OTA and variables: variety (table win - tw; Cabernet Sauvignon - cs; Merlot - me; Port - dp; Malbec - ma; and Cabernet-sirah - csi); country of origin (Brazil - Br; Argentina - Ar; Uruguay - Ur; South Africa - SAfr; France - Fr; Portugal - Por, and Chile - Ch); and type (soft red wine - srw, dry red wine - drw, soft rosé wine - srw, soft white wine - sww, dry white wine - dww, fine red wine - frw, and fortified wine - fort).

There is a positive relation between OTA, France, Cabernet Sauvignon, Malbec and Argentina.

Regarding the exposure risk to OTA by means of wine intake, an exposure estimate was carried out using values reported in the literature, from which the mean intake of wine in Brazil is around 1.8 L per capita (Brazilian Institute of Wine, 2012) and the mean weight of a Brazilian healthy adult is around 70 kg (IBGE, 2011).

By means of estimates, even for values that exceed the limit of 2 $\mu$ g.L<sup>-1</sup>, the risk exposure is low, both for human exposure and for aggregate exposure. Considering the wine intake in Brazil of 5 mL.day<sup>-1</sup>, the highest levels of OTA found in this study (2.77 $\mu$ g.L<sup>-1</sup> or 2.77ng.mL<sup>-1</sup> and 2.46 $\mu$ g.L<sup>-1</sup> or 2.46ng.mL<sup>-1</sup>) and the mean corporeal weight around 70kg; the estimated daily intake of OTA was 0.20ng.kg<sup>-1</sup> p.c.day<sup>-1</sup> and 0.18 ng.kg<sup>-1</sup> p.c.day<sup>-1</sup>, respectively; what correspond to 1.42% and 1.29% of the maximum tolerated limit (14ng.kg<sup>-1</sup> p.c.day<sup>-1</sup>), and does not represent risk for the population under study.

This may be explained by the low beverage intake in Brazil. In Europe countries such as Italy and France, the mean intake is almost 40L per capita (OIV, 2012). On the other hand, in Brazil bordering countries such as Argentina, Uruguay and Chile, the beverage intake is also high; around 28.3L per capita, 25.9L per capita, and 17.9L per capita, respectively (União Brasileira de Vitivinicultura, 2012).

Considering the average of samples of each country, and considering the consumption of a cup (150mL) of wine per day (Brera *et al.*, 2011), the contribution for the exposure to

OTA may be as showed in the Table 5.

Considering the exposure rate of wine from Chile (4.71%), the most consumed in Brazil among imported wines, and considering a bottle containing 750mL of wine, it would be necessary to drink 1,592.35mL of wine, i.e. two bottles of wine and 123mL per day to expose a consumer in around 50% of the daily exposure limit acceptable by the World Health Organization (WHO, 1997). These results show that wines from Chile do not represent an important exposure source. However, regarding wine from France, two cups would be sufficient to reach 59% of the acceptable daily exposure limit.

Knowing that there are several sources of OTA in the diet, it should be considered that the risk of harmful effects caused by imported wines intake is becoming relevant, what would lead to the need for managing this risk. Therefore, other studies addressing about the OTA exposure due to wines intake are necessary. These would allow better comprehension of facts that contribute significantly to the incidence and occurrence of this toxin in the beverage. In addition, they would contribute to minimize the potential risk of presence of this toxin; what would facilitate the maintenance of low levels of contamination, as showed in this study.

## 4. Discussions

In this study, the presence of OTA was not detected in any sample of domestic wine. Thus, considering that the production of OTA by *A. carbonarius* is favored in low temperatures (from 15 to 20°C), the climate conditions of European countries are more suitable for grape contamination by OTA than in tropical climate regions such as Brazil (Lasram *et al.*, 2010). Similar results were found here, once was considered that wines from Brazil are poor source of OTA when compared to wines from other Mercosul countries (Argentina and Chile), and other traditional producing countries of wine (União Brasileira de Vitivinicultura, 2012). Wine analyzed in Brazil showed frequency of contamination by OTA around 31%, while in European countries, such as Spain, the contamination may reach 99% (Welke *et al.*, 2009).

Samples which showed some level of contamination by

OTA were red wines. This finding was expected according to data from other studies. For example, Lasram et al. (2007) assessed the presence of toxin in wines from Europe, and they showed a positive tendency towards contamination of this kind of wine. The highest content of OTA in red wine is probably found due to the characteristics of the winemaking process, where the maceration of grapes contributes significantly to the increase of levels of OTA in this type of wine (Fernandes et al., 2003). During the winemaking process of white wine, after grape maceration, the solid part is separated from the new wine, allowing less contact with peel and seeds. For red and rosé wines, the solid parts are fermented with new wine to potentiate the extraction of color and aroma. During this phase a greater extraction of OTA from grapes occurs, hinting that peel is probably the most contaminated part of the fruit (Lasram et al., 2008).

No sample of white and rosé wine showed OTA contamination. This may be related to various factors such as type of grape, winemaking process, geographical region, climate conditions, and different practices performed during the cropping process of grapes; which influence the incidence of ochratoxigenic fungi and production of toxin.

Great part of samples showed contamination by OTA. However, the majority showed values less than  $2\mu\text{g}\cdot\text{L}^{-1}$ , the maximum limit acceptable established by the law. Terra et al. (2012) performed a study in San Francisco, Northeast Brazil, and they reported the presence of OTA in 75% of wines. However, the contamination level was considered low.

Regarding the exposure risk to OTA due to wine intake, even for values that exceeded the limit ( $2\mu\text{g}\cdot\text{L}^{-1}$ ), the exposure risk is low, both for human exposure and for aggregate exposure, since the consumption of this beverage in Brazil is still less than in other countries. However, when associated with the consumption of other food and beverages, which may contain significant levels of this toxin, its presence becomes relevant. Some authors found levels of OTA in red wines from Brazil from 0.10 to  $1.33\mu\text{g}\cdot\text{L}^{-1}$ . According to them, although the results showed values below the limit, a less but continuous exposure to this mycotoxin may pose risk to human health (Shundo et al., 2006).

## 5. Conclusions

Although the low incidence of OTA in these wines it is necessary to consider the possible exposure to this toxin through the consumption of other products, thus creating a significant risk when contaminated wines are often ingested. Therefore, other studies addressing the OTA exposure due to wines intake are necessary. These would allow better comprehension of facts that significantly contribute to the incidence and occurrence of this toxin in the beverage. In addition, they would contribute to minimize the potential risk of presence of this toxin, which would facilitate the maintenance of low levels of contamination, as showed in this study.

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