RISK OF OCHRATOXIN A EXPOSURE WHEN DAILY CONSUMPTION OF JUICE AND WINE IS FOLLOWED TO PREVENT CARDIOVASCULAR DISEASE

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RESUMO – Os compostos fenólicos presentes no suco de uva e no vinho podem estar associados a redução de doenças cardiovasculares devido às suas propriedades antioxidantes e anti-inflamatórias. Em virtude disso, estudos têm recomendado o consumo diário destas bebidas. Entretanto, a ocratoxina A (OTA) pode estar presente no suco e vinho e esta toxina está associada principalmente a nefrotoxicidade. O objetivo deste estudo foi avaliar o risco de exposição a esta toxina através do consumo de suco e vinho elaborados com uvas naturalmente contaminadas. Os níveis de OTA foram significativamente reduzidos do mosto ao suco produzido através do método de extração a vapor. A exposição estimada a OTA através do suco foi maior do que a ingestão tolerável estabelecida para esta toxina pelo Comitê Conjunto FAO/WHO de Especialistas em Aditivos Alimentares (JECFA). Considerando os níveis de OTA em estudo, o consumo de vinho não representa risco para a saúde. Em relação ao suco, o engarrafamento após 60 minutos de extração pode resultar em níveis de exposição segura à OTA.

ABSTRACT – The phenolic compounds present in grape juice and wine may be associated with reduction of cardiovascular diseases due to their antioxidant and anti-inflammatory properties. In this way, studies have recommended daily consumption of these beverages. However, ochratoxin A (OTA) may be present in juice and wine and this toxin is mainly associated with nephrotoxicity. The objective of this study was to evaluate the risk of exposure to this toxin through the consumption of juice and wine produced with naturally contaminated grapes. The OTA levels were significantly reduced from the must to the juice produced by the steam extraction method. The estimated exposure to OTA through the juice was greater than the tolerable intake established for this toxin by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Considering the OTA levels under study, wine consumption does not pose a health risk. In relation to juice, bottling after 60 minutes of extraction may result in levels of OTA exposure.

PALAVRAS-CHAVE: micotoxinas; avaliação da exposição; compostos tóxicos.

KEYWORDS: mycotoxins; exposure assessment; toxic compounds.

1. INTRODUÇÃO

The phenolic compounds of grape juice and wine are associated with the reduction of the risk of cardiovascular diseases due to their antioxidant and anti-inflammatory properties (Albers et al., 2004; Coimbra et al., 2005; Costa et al., 2017; Joseph et al., 2016). The daily consumption of red wine and grape juice has been recommended in literature (Albers et al., 2004; Boban et al., 2016; Coimbra et al., 2005; Costa et al., 2017; Stein et al., 1999), in addition to the international organization of wine
sector termed “Wine in Moderation, WIM” (WIM, 2018) and the United States Department of Agriculture (USDA, 2017). A red wine cup of 200 or 300 mL is recommended for women or men, respectively, by WIM (2017) and Boban et al. (2016). However, the consumption of 150 mL of (red or white) wine is suggested by the Dietary Guidelines for Americans (USDA, 2017). In relation to the grape juice, daily intake of 400 to 500 mL has recommended in scientific literature (Albers et al., 2004; Coimbra et al., 2005; Stein et al., 1999).

Toxic compounds may be present in these beverages, including ochratoxin A (OTA). This toxin may be present in grape juice and wine due to the contamination of grapes with toxigenic fungi (Welke et al., 2009). OTA occurrence in juice and wine was reviewed and the percentage of samples containing this toxin may be around 70% (Dachery et al., 2016a) with levels up to ten times higher than the legal limit of 2 µg/L established in Brazil (ANVISA, 2011) and European Union (European Commission, 2006). OTA has mainly been associated with nephrotoxic effects, in addition to other toxic effects, including cytotoxicity (OTA induces oxidative stress mechanisms), immunotoxicity, teratogenicity and mutagenicity. The International Agency for Research on Cancer (IARC) classifies this toxin as a possible carcinogenic for humans (IARC, 1993). In 2001, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established 112 ng/kg of body weight as the provisional tolerable weekly intake (PTWI) of OTA (JECFA, 2007). The risk to OTA exposure may exist if the estimated intake exceeds the tolerable intakes established by JECFA. Therefore, the assessment of the risk of human exposure to a substance present in the diet is critical to the development of safe food standards. The goal of this study was to evaluate the risk to OTA exposure when daily consumption of juice and wine is followed to prevent cardiovascular disease.

2. MATERIAL E METHODS

2.1 Samples
Grapes of the cultivars Concord (16 ºBrix), Moscato Italico (17 ºBrix) and Cabernet Sauvignon (18 ºBrix) were used to produce juice, white and red wine, respectively, as described by Peynaud (1997). The three cultivars were chosen because they are among the most used to prepare these beverages around the world (Anderson, 2013; Boulton et al., 2010; Ashurst, 1999). Grapes were harvested in 2014 from the same vineyard located in the region of Serra Gaúcha (29ºS, 51ºW), Rio Grande do Sul, the southernmost state of Brazil.

2.2. Production of grape juice and wine
The production of juice and wine was performed following the steps commonly used in wineries as described below. Each batch was produced using 1.5 kg of grape, and three batches of each beverage were prepared.

Grape juice was produced by steam extraction using Concord grapes. The temperature of the juice extracted ranged between 75 and 80ºC and bottling occurred between 30 to 90 min of extraction.

Red wine was prepared according Peynaud (1997) through the manually crushing of Cabernet Sauvignon grapes, followed by the potassium metabisulfite and Saccharomyces cerevisiae addition. Maceration occurred for 8 days and reassembly was performed twice a day through the must agitation. Pressing was performed to separate the pomace from the must. Alcoholic fermentation was monitored each day until density reached 0.997. Racking was done to remove lees and/or sediments and finally, the wine was bottled and stored for 120 days (average time used by wineries for shipping wine). These process was also followed to produce white wine with Moscato Italico grapes. However, the maceration and pressing steps were not performed.

2.3 Determination of OTA
OTA extraction was done according to the official method 2001.01 of Association of Analytical Communities (AOAC, 2016) and the quantification was done by high performance liquid
chromatography (HPLC) system (Waters Corporation, Milford, USA) with fluorescence detection (FL). OTA confirmation was done by HPLC (Shimadzu, Kyoto, Japan) connected in a mass spectrometer (MS) with a quadrupole time-off light (Q-TOF) analyzer and electrospray ionization (ESI) source (Bruker Daltonics, model micrOTOF-QIII, Bremen, Germany) in positive mode according to Dachery et al. (2016b). The linear regression coefficient of OTA standard solution curve ($y = 146884x + 212732$) was 0.997 for the concentration ranging from 0.08 to 130 μg/L. The mean recovery of OTA was 95, 96 and 98% for red wine, white wine and juice, respectively. LOD and LOQ were 0.05 and 0.08 μg/L, respectively.

2.3 Assessment of the risk of ochratoxin exposure

OTA exposure was calculated according to WHO (2013): estimated weekly intake (EWI) (mg/kg body weight per week) = [wine or juice consumption expressed in L per week x concentration of OTA in wine or juice expressed in mg/L] ÷ body weight in kg.

The body weight of 30 kg was used to calculate the OTA exposure by the consumption of juice for children. When the exposure is related to adults, the body weight of 66.5 kg was used, that is reported by POF as the median of body weigh of Brazilian adults (IBGE, 2011).

Wine consumption was estimated using two approaches:

(i) when the recommendation of the daily intake of a red wine cup of 200 or 300 mL is followed by women or men, respectively, as recommended by the international organization of wine producers called Wine in Moderation (WIM, 2017) and Boban et al. (2016);

(ii) using the consumption of 150 mL of (red or white) wine as suggested by the Dietary Guidelines for Americans (USDA, 2017).

Daily consumption of juice was estimated taking into consideration:

(i) a cup (200 mL) of grape juice consumed by children, since this beverage has been introduced in meals of public schools in some states of Brazil (Dachery et al., 2016b);

(ii) 400 and 500 mL as recommended in scientific literature to prevent cardiovascular diseases (Albers et al., 2004; Boban et al., 2016; Coimbra et al., 2005; Costa et al., 2017; Stein et al., 1999).

3. RESULTS AND DISCUSSION

Figure 1 shows the OTA levels according in must, juice and wine elaborated with naturally contaminated grapes with this mycotoxin. ANOVA followed by Tukey test at 95% of confidence showed that the longer the juice extraction time (from 30 to 90 minutes), the OTA levels were significantly lower (Figure 1). Regarding winemaking, OTA levels found in wine were statistically lower than those of must.

According to OTA levels found in wines (Figure 1) and the quantities of wine that should be consumed to prevent cardiovascular disease, the estimated exposure to OTA through the consumption of wine showed no risk to consumer health, since the EWI (Figure 2) was lower than the PTWI established for this toxin by JECFA (112 ng/kg body weight) (JECFA, 2007). However, regarding juice, the exposure to OTA could represent risk when the bottling occurs after 30 or 60 min of extraction and the daily consumption was higher than 400 mL as highlighted in bold and italic font in Figure 2. In this situation, the EWI may reach 166% of PTWI of this toxin.
Figure 1. Ochratoxin A levels according to the processing step of juice and wine elaborated with naturally contaminated grapes with this mycotoxin. Sampling of juice was done 30, 60 and 90 after the start of the processing. For each type of beverage, OTA levels in the production stage followed by the same letter are not significantly different (P > 0.05) by Tukey test.

The levels of OTA found in the juice after 30 and 60 min of extraction were higher (1.59 and 1.26 µg/L, respectively) than those verified for red and white wine (0.12 and 0.24 µg/L, respectively), which may explain why EWI was greater than PTWI of this toxin only for juice consumption (and not for wine). Furthermore, the recommended daily consumption of juice (400 to 500 mL) to prevent cardiovascular diseases (Albers et al., 2004; Coimbra et al., 2005; Costa et al., 2017; Stein et al., 1999) is greater than those proposed for wine (200 to 300 mL) (Coimbra et al., 2005; WIM, 2017). In wine, the toxic effect of ethanol, which is considered carcinogenic to humans by IARC (2012), limits the amount of recommended intake to obtain the positive effects of polyphenols.
Figure 2. Estimated weekly intake (EWI) of ochratoxin A (OTA) through consumption of wine and juice elaborated with grapes naturally contaminated with this toxin and considering the daily consumption recommended to decrease the risk of cardiovascular diseases (applies to red beverages due to the presence of polyphenols) as reported in literature. Red line represents the provisional tolerable weekly intake (PTWI) of 112 ng/kg of body weight for OTA (JECFA, 2007).

4. CONCLUSION
OTA levels are significantly reduced from must to wine or juice. Considering the OTA levels under study, the consumption of wine poses no risk to health. About juice production, bottling after 90 min of steam extraction is suggested to possibly further reduce the exposure of consumers to this mycotoxin, since some concern related to juice may occur.

5. REFERÊNCIAS BIBLIOGRÁFICAS


