Exposure to ochratoxin A through consumption of grape juices produced by steam distillation method and intended for school meals

Bruna Dachery1, Vitor Manfroi2, Juliane Elisa Welke3

ABSTRACT

The grape juice was included in meals of public schools in the states of Rio Grande do Sul and Santa Catarina, Brazil. The juices intended for schools have been produced by small wineries through the steam distillation method. This method presents a particularity in relation to other juice production methods, since the grapes are not macerated to extract the juice. In literature is well established that maceration of grapes is the main critical point associated with the ochratoxin A (OTA) contamination in juices. OTA is a mycotoxin with nephrotoxic and immunosuppressives effects. The aim of this study was to verify the OTA occurrence in juices produced by steam distillation method, as grape juice may be a source of OTA exposure for children. No sample showed OTA levels higher than the detection limit (0.05μg L⁻¹) of method used to determine this toxin. This study demonstrated the adequate quality of juice produced by this extraction method. Furthermore, data about OTA occurrence are important to support risk assessment studies related to the exposure to toxic compounds through diet.

Key words: mycotoxins, toxic compounds, ochratoxin A.

Ochratoxin A (OTA) is a mycotoxin produced by filamentous fungi, such as Aspergillus niger and A. carbonarius. Exposure to OTA through food consumption poses a health risk, since this mycotoxin has been related to nephrotoxicity (DAI et al., 2014). The International Agency for Research on Cancer (IARC) classified OTA as possible carcinogen to humans (IARC, 1993). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake of 112ng kg⁻¹ body weight (bw), which corresponds to approximately 16ng kg⁻¹ bw per day (JECFA, 2007).

The occurrence of OTA in grapes and juice has been associated to the climate conditions of grape cultivar, maturation degree and physical damage on grapes (WELKE et al, 2009). The concern related to the OTA exposure through daily consumption of grape juice by children is due to the inclusion of this beverage in meals of public schools of Rio Grande do Sul and Santa Catarina states, Brazil. This approach was adopted via legislation and aims: (i) the offering of healthy products for children and
samples) and Niágara Rosada (9 samples). These were analyzed: Bordô (15 samples), Concord (17 samples) and Niágara Rosada (9 samples). These samples harvested in the years 2010, 2011 and 2012 in Serra Gaúcha region were used to obtain juice by steam distillation method.

OTA was extracted following the developed and validated method by WELKE et al. (2010) and samples were analyzed using high performance liquid chromatography (HPLC) with fluorescence detector (Agilent series 1100, USA) that was equipped with an online degasser, a quaternary pump, and an automatic injector (Figure 1A-C). For fluorescence detection of OTA the excitation wavelength was 333nm, and the emission wavelength was 443nm. A C18 column (XBridgeTM Shield RP 18; 5μm; 4.6x150mm, Waters, Irleand) and the following mobile phase (acetonitrile: water: acetic acid, 99:99:2, v/v/v) were used in HPLC. The analysis was performed under isocratic conditions at a flow rate of 1mL min\(^{-1}\). The same conditions were used in high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS, micrOTOF-Q III, USA) for confirmation of OTA (Figure 1D).

Recuperation of the method was 96% and 98% for the levels of 1 and 2ug L\(^{-1}\) with a standard deviation of 6.1 and 5.4%, respectively. The LOD and LOQ were 0.05 and 0.08μg L\(^{-1}\) respectively. The validation results confirm the efficiency of the method, which is sensitive enough to be used in the OTA quantification.

No sample showed OTA levels higher than 0.05μg L\(^{-1}\), which is the limit of detection of the HPLC method. The juices evaluated in this study (produced using steam distillation) presented a particularity in relation to the other methods that may be used to obtain grape juice. In steam distillation, the grapes were not macerated to extract juice, as occurs in welch and Flanzy methods (MARZAROTTO, 2010). Maceration of grapes has been considered a critical control point of OTA contamination in juice, since higher levels of OTA are found in grape skin compared to pulp. Thus, maceration step allows the migration of OTA present in skin to the must used to juice production (DACHERY et al., 2015).

OTA was first reported in grape juice in 1996 and this mycotoxin was found in 8 samples produced in Switzerland with median concentrations amounting to 0.235μg L\(^{-1}\) (ZIMMERLI & DICK, 1996). CAO et al. (2013) found OTA in 30% of Chinese grape juice samples in levels ranging from 0.26 to 0.54μg L\(^{-1}\). A greater number of juice samples containing OTA was reported by LEE et al. (2012). The levels found in 91% of samples marketed in Malaysia were of 0.02 to 1.05μg L\(^{-1}\). In evaluations of juice produced in Brazil, no sample presented
Exposure to ochratoxin A through consumption of grape juices produced by steam distillation method and intended for school.

OTA levels above the maximum limit established by Brazilian legislation (2 μg L⁻¹) (ROSA et al., 2004; SHUNDO et al., 2006). ROSA et al. (2004) analyzed 64 grape juice samples marketed in Rio de Janeiro city, Brazil. OTA was found in 25% of samples and the maximum level was 0.1 μg L⁻¹. SHUNDO et al. (2006) evaluated 38 grape juices produced in Brazil and OTA was not found in samples. Other data on occurrence of OTA may be checked in recently review published by DACHERY et al. (2015), in which the exposure levels related to consumption of grape juice containing OTA were reported.

This study demonstrated the adequate quality of juice samples produced by the method of steam distillation. The no detectable levels of OTA confirmed the safety of these juices intended for meals of several schools of Brasil and the low exposure to this mycotoxin through grape juice. OTA occurrence data are important to support risk assessment studies related to the exposure to toxic compounds through diet. Furthermore, a study to evaluate the effect of steam distillation method on OTA levels of juices produced using naturally contaminated grapes with OTA will conducted.

ACKNOWLEDGMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and the Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for financial support and scholarships.

REFERENCES


Ciência Rural, v.46, n.10, out, 2016.


Ciência Rural, v.46, n.10, out, 2016.