INVESTIGATION TECHNIQUES FOR THE DETECTION OF PESTICIDE AND OCHRATOXIN A RESIDUES IN GRAPES AND WINE

PhD Thesis Abstract

The PhD thesis is structured in five chapters along 134 pages as follows:

Chapter 1: Generalities regarding properties, sources, investigation methods and maximum permissible limits for pesticides and ochratoxin A
Chapter 2: The up-to date researches concerning the presence of pesticides and ochratoxin A in grapes and wine
Chapter 3: The aim and the objectives of the research, materials and methods
Chapter 4: Obtained results
Chapter 5: General conclusions
References: – 142 scientific papers published in the last decade regarding this research theme are cited

Research aim

The consumers are interested in the food integrity; thus, the contaminant presence (micotoxins and pesticides) must be treated rigorously.

The Romanian Government had established maximum permissible limits for these residues in agreement with UE legislation. Furthermore, the development of managerial strategies for the minimization of these contaminants in Romanian wine was considered.

Starting from the principle "prevention is better than treatment", the consumer protection against the toxicological effects of these contaminants imposes sustained prevention actions such as a good agricultural practice, appropriate stocking, the treatment schedule fulfillment, etc.

The aim of this study is to reveal the investigation methods for the detection of pesticide and Ochratoxin A in grapes and wine. Their adjustment to the laboratory conditions allows a good recovery of the residues, as well as a good repeatability and reproducibility of the results.

Objectives

Due to the detection of ochratoxin A and pesticides in cereals, milk, coffee, cocoa, human blood, and recently in wine, as well as the nefrotoxic, hepatotoxic, teratogen, imunotoxic, and potently carcinogenic effects, the following objectives were established:

1) identification of ochratoxinogenic moulds on grapes;
2) validation of ochratoxine A quantification methods;
3) investigation and quantification of ochratoxin A in white and red wines;
4) result validation of the pesticide residue determination;
5) determination of the pesticide contamination degree of Oltenia's grapes.

Results

In 2005, the water washing of the grapes take the study were isolated several strains of molds belonging to the species: *Penicillium verucosum*, *Aspergillus niger* and *Aspegillus ochraceus* - species with potential ochratoxigen.

At the beginning, ELISA method and HPLC technique were used for the OTA residue quantification from wines. For the ELISA determination R-biopharm RIDASCREEN® kits and a STAT FAX 2100 reader were used. A Perkin Elmer Series 200 High Performance Liquid Chromatograph with a fluorescence detector was used.

105 wine samples were investigated (44 samples bottled wine from Oltenia, 48 samples spiked with OTA standard, and 8 imported wine samples). Furthermore, 5 samples of molded grapes were analyzed. For these samples, the molds were firstly microbiologically analyzed and thereafter the samples were homogenized, and the extracted juice was than analyzed for the detection of OTA. The PBS buffer was used for the sample extraction. After purification on imunoaffinity columns, OTA was eluate with methanol. Methanol extract was evaporated to dry on a water bath and the residue was dissolved in a sodium bicarbonate for the ELISA technique. For the HPLC method, the methanol extract was used. The obtained results were processed using the corresponding software of each apparatus. Calibration curves were constructed for each sample set. Assessment of recovery, precision, and accuracy of the obtained results was performed. Limits of detection and limits of quantification of the method were calculated. The recovery was in the range of 82 to 95 %, and the repeatability correlation coefficient was 7,2, and the reproducibility was 7,65.

For all the investigated samples over three years, there were no OTA concentrations over the permissible limit. There are several wine samples (11,36%) having detectable OTA concentration in 2005 that could be explained by the unfavorable meteorological conditions. For the remaining samples (88,64%), the OTA concentration was undetectable. These results could be explained by a good vinification practice and by the favorable climatic condition. From oenological point of view, the most important result of this study is that Romanian wines from Oltenia are in accordance with the UE reglementations.

For the pesticide residue analysis by the gas chromatographic method pesticide standards (Riedel de Haën)and Konik HRGC 4000B and Varian CP 3800 (with auto sampler) GC were used.

80 grape samples have been analyzed. 32 samples were obtained from Oltenia's grapes, and 48 samples were spiked with standard substances from Riedel de Haën (β HCH, γHCH, and DDE). The samples were extracted in

acetonitrile, than re-extracted with petroleum ether and purified on a florisil column. After pesticide retention, they have been eluted with petroleum ether – ethylic ether mixture. The ether extract was concentrated by evaporation and injected in GC.

The method validation has been performed using grape samples which have no pesticide residue. The samples were spiked with standard solutions at the following levels: 0,05; 0,1 and 0,15 mg/kg. The recovery level was in the range 78-89%, with a variation coefficient (Cv %) of 2,7 - 8,5%, and a quantification limit (LOQ) ranging from 0,01 to 0,02 mg/kg. The measurement uncertainty depends on each component and is lower than 15%.

The methods implied in the investigation of pesticide residues from grapes gives accurate and reproducible results. The absence of pesticide residues in investigated samples indicates a good practice for the phytosanitary treatments, the compliance with the treatment schedule, and the pesticide usage under the specialized supervision. Although the mobility of pesticides from grapes to wine is quite reduced due to the winery process (especially the alcoholic fermentation drastically diminishes the pesticide level) there is always a hazard for the wine contamination, being a toxic source for the consumers.

The rational usage of pesticides minimizes the OTA contamination risk.

The **conclusions** of the research are:

► The aim of UE legislation concerning the maximum levels of toxines in grapes and wine was to find a solution for the minimization of consumer exposure to these toxines.

► The greatest amount of OTA could be reduced during the grape pressing stage and continues during the solid-liquid separation stage. By the treatment with pectolitic enzymes for the unfermented white wine, bentonite for white juice finishing, static or rotative fermentation of unfermented red wine, and the juice recovery by centrifugation, this reduction process is continued.

► The OTA removement during vinification process is performed rather by adsorption on solids and proteins than by degradation. Particularly, the OTA bounding to the yeast cells during the fermentation seems to play an important role in the OTA reduction. This is not the case in the grape juice production, indicated by higher contamination level compared with the wine. Bejaoui şi al. 2004 proposed the capitalization of the yeast adsorptive capacity for the OTA bounding from synthetic or commercial juices.

► During the post-fermentation finishing, the adsorption of OTA from red wines on yeast is possible. A new decontamination technique using the adsorptive properties of the active carbon was considered to remove the wine dyes.

► Since the first reported presence of OTA in wine, in the last years many progresses have been registered for the identification of contamination sources, the conditions associated with the *Aspergillus* mould growth and toxin production, and the OTA bounding to the solids during vinification. The involved strategies for wine and vineyards currently deals with the reduction of *Aspergillus* toxine occurence, the delinitation of the areas with high OTA contamination risk for grapes, and the mould controll by fungicide usage.

► Nowadays many methods are available for the OTA determination from wine before its comercialization. Among them, two methods have been developed in this research study: imunoenzymatic and HPLC techniques.

► Regarding the quality of worlwide wine production, it was proved that the OTA concentrations seldom exceeds 2 μg/kg. This limit was imposed by UE in 2005, limiting the international wine trade.

► Nowadays, over 300 comercial products are known, based on 50 active products used for the phytopatogen agent control in vineyard. There are many fungicide products for viticulture on the market. Their diversity and efficiency allow a good prevention and control of the main vineyard deseasses.

► The currently used insecticides for vineyards are classified in three main catagories: contact, ingestion, and systemic insecticides, they belonging to the

following chemical groups: organophosphoric, carbamates, synthetic piretroides, biological, antropode metamorphosis inhibitors, mixtures.

► The significance of profilactic use of pesticides is the minimization of grape damages. Nevertheless, the risk of pesticide residue on grapes have to be considered in the case of wrong treatment procedure.

► When the pesticide residues are present in wine, there are a lot of negative consequences such as: the quality of wine diminishes, the fermentation slow down, and throubles with the malolactiv fermentation.

The growing interest for the study of pesticide presence in grapes is justified by oenologic point of view by the possible interferences with the fermentative microflora used for the wine prodution. Even if the pesticide mobility from grapes to wine is generaly reduced due to the winery process (especialy the alcoholic fermentation drasticaly reduce the pesticide level) there is always a risk for wine contamination and, therefore a toxic source for the consumers.

► The main conclusion of this research study is that the viticol areas from Oltenia are not susceptible to a high risk for ochratoxin A contamination. The favorable geographycal possition and climateric conditions, as well as the proper phytosanitare treatments and schedule assure a good quality for Oltenia's grapes and wine, safely for the consumers. An increase vigilance is still necesary to minimizade the contamination risk for grapes and wine with OTA or pesticides by periodic soil, grape, and wine analysis.