APPLICATION OF REAL-TIME PCR FOR DETECTION OF MYCOTOXIN-PRODUCING FUNGI IN THE GRAPE MARC

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Wine production is one of the most important agricultural activities around the world. It is a global billion-dollar industry. As a result of wine-making process, a considerable amount of waste, including grape marc, is generated. Grape marc includes grape skin, pulp, seeds, stems, and is rich in minerals and biologically active substances. Thus, grape marc can have various applications from cosmetics to food industry. However, it has to be confirmed to be biologically safe.

One of potential dangers of plant-derived products is their possible contamination with mycotoxins. Mycotoxins are secondary metabolites of filamentous fungi, and occur naturally in food. In case of wine, the regulated mycotoxin is ochratoxin A, since the fungi capable of producing this mycotoxin can be commonly found on grapes. So, it is reasonable to test the grape marc for the presence of potential producers of *ochratoxin A*, before further using it.

In this work we concentrated on the application of real-time PCR method for detection of possible producers of *ochratoxin A* in the grape marc. We developed PCR primers targeted to the gene involved in the synthesis of the mycotoxin *ochratoxin A* (OTA non-ribosomal peptide synthetase gene), capable of recognizing fungi containing this gene from both *Aspergillus and Penicillium* genera. We used *SYBR Green* real-time PCR protocol. For DNA extraction from the grape marc, we tested various protocols (SDS-based protocol, STAB-based protocol, DNAsol-based protocol), and found that the optimized protocol combining SDS extraction followed by PVPP and ammonium acetate treatment**[1]** resulted in the optimum quality DNA. To confirm that the purified DNA is of PCR quality, and to monitor equal DNA load in the PCR reactions, we developed primers to *Vitis vinifera 26S ribosomal RNA gene*. So, the DNA from each sample was analyzed in parallel by the primers specific to *OTA non-ribosomal peptide synthetase gene* and to *Vitis vinifera 26S ribosomal RNA gene*.

Of all the analyzed samples (eight grape marc samples of four grape varieties collected from different geographical zones), none was positive for OTA non-ribosomal peptide synthetase gene. This indicates that the microorganisms capable of producing *ochratoxin A* are absent or undetectable in those samples, and the grape marcs tested are safe to be used in further applications.

Keywords: *grape marc, mycotoxins, ochratoxin A, PCR primers, SYBR Green.*

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C. S. Kim, C. H. Lee, J. S. Shin, Y. S. Chung, N. I. Hyung, A Simple and Rapid Method for Isolation of High Quality Genomic DNA from Fruit Trees and Conifers Using PVP, *Nucleic Acids Research*, Volume 25, Issue 5, 1 March 1997, Pages 1085–1086, https://doi.org/10.1093/nar/25.5.1085