

EVALUATION OF THE MICROBIOME ON UNTREATED GRAPES AND FROM THE MUST OBTAINED IN ROMANIAN RURAL HOUSEHOLDS

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Abstract: Grapes have a complex microbiome represented by yeasts, bacteria and molds. This microbial diversity can contribute to the quality, flavor and characteristics of traditional products derived from grapes, which is why microbial monitoring is essential.

The microbial analysis of these microbial groups was carried out on two varieties of Romanian grapes, white and black, which are increasingly rare in Romania. Microbial groups, yeasts, bacteria and filamentous fungi were isolated from diluted suspensions obtained from the surface of white and black grapes and from the must squeezed from the two types of grapes. For the isolation of groups of microorganisms, specific nutrient media were used, such as nutrient agar for bacteria, agarized malt for yeasts and Sabourand for filamentous fungi. After incubation in appropriate conditions, it was observed that yeasts have the highest concentration, followed by bacteria. The smallest number was represented by filamentous fungi. Also, the number of Gram-positive microorganisms dominates. The load of yeasts, bacteria and filamentous fungi varies quantitatively depending on the changes in abiotic factors.

Introduction

The importance of grapes for the table of the consumer in rural and urban areas is vital. The content of grapes in bioactive compounds (phenolic acids, flavonoids, anthocyanins) is high. These are the beneficial compounds for human health. They have antioxidant, antitumor, antimicrobial, anti-inflammatory potential. They reduce cardiovascular risks. They are used in the food, pharmaceutical, cosmetic industry. Information about the composition of grapes, fermentation yeasts, winemaking processes is numerous. Studies focused on the biodiversity of grapes and the importance of epiphyte flora are less. Microorganisms on grapes come from the environment (Zarraonaindia et al., 2015). They can be the natural barrier against pathogens, but they can quantitatively and qualitatively influence the harvest, the must and the wine. During the ripening of the grapes, the microbiome on the grapes is constantly changing. They vary quantitatively and qualitatively on the grape berries. This effect is mainly due to the anthropogenic (chemical treatments) and abiotic (temperature, light, humidity, soil properties) factors. Quantitatively, the microorganisms on the grape berries reach values between 10^{-4} - 10^{-6} CFU/g. Most studies have shown that the most common microorganisms are yeasts. Along with these, groups of lactic and acetic bacteria were found in particular. The non-existent information about the microbiome on the grapes, belonging to some old varieties, from the rural gardens led us to make an evaluation of the microorganisms on the grapes and in the must. Taking into account the fact that some studies mention that soil microorganisms populate the plant as well, we tried to highlight the coliform bacteria on the grains and in the must.

Material and method

Collection of samples to be analyzed

The samples of white and black grapes come from the gardens of two households, from Caras-Severin county. Enriching the soil and protecting the vine stumps at ground level was carried out with cattle manure in the case of white grapes, respectively sheep manure in the case of black grapes. The grape samples were harvested in autumn and transported in appropriate conditions.

Microbiological analysis of grape and must samples

Grape and must samples were diluted with physiological serum, by the dilution method. To enumerate the bacteria, the 10^{-6} dilution was inoculated (1 ml/10 ml medium) by the inclusion method, on nutrient agar medium. The fungi were isolated from the 10^{-3} dilution on Sabouraud nutrient medium. Yeasts were grown on medium with malt. The isolation was carried out in two repetitions. The favorable temperature for growth was established at 28°C. The protection of the vines and the enrichment of the soil in their area was carried out with manure. It is known that edaphic microorganisms reach different parts of the plant. This was a reason why the experiments were set up and for the isolation of coliform bacteria. For their isolation, Lauryl-Sulfate medium was used, in three repetitions. The first three dilutions were used for inoculation. Durham tubes were inserted into the test tubes to capture the gases produced following the fermentation processes. After incubation at 37°C, a probable number of coliforms was established. The bacteria were incubated for 48 hours, the coliforms for 24 hours and the fungi for 5-7 days. Triple sugar iron (TSI), Sim and Citrate Simmons (CS) media were used to confirm the presence of coliforms.

Results and discussions

The obtained results show that yeasts are found in the highest number (0.96 - 4.50×10^2 /ml), compared to bacteria (0 - 0.84×10^2 /ml) and molds (0 - $0.37/10^2$ ml) (fig. 1). Bacteria were isolated in large numbers from black grapes. In contrast, the bacteria were not observed on white grapes. Bacteria were highlighted in both types of must. The descending order of the concentration of bacteria in the four samples is BG2 > GM1 > GM2 (fig. 1). Non-pigmented "R type" bacterial colonies dominated in all samples (table 1). Yeasts are naturally found on grape seeds. They are best represented. The yeasts were in greater numbers in the must compared to the bacteria. The highest number of yeasts was observed in the must of white grapes, followed by black grapes. The lowest number of yeasts was highlighted on white grapes. Molds were present in higher numbers on white grapes, followed numerically by must from white grapes. The number of molds was reduced on black grapes. Molds did not develop in the must of black grapes. Yeasts are found naturally on the grape berries. Most studies have observed a high number of yeasts on grapes. The bacteria included Gram-positive and Gram-negative species, bacilli and cocci forms. The highlighted species were part of the genera *Bacillus*, *Micrococcus*, *Leuconostoc*, *Lactobacillus* etc. Among the fungi, the genera with septate hyphae, *Aspergillus* and *Penicillium* were especially present. In accordance with our results are those obtained by other authors (Kántor et al., 2017, Maulani et al., 2012). The number of coliform bacteria varies, the highest number was found in grape must (fig. 2). The presence of coliforms on grapes was also reported by other researchers. This fact is due to environmental factors (Zeman et al., 2023).

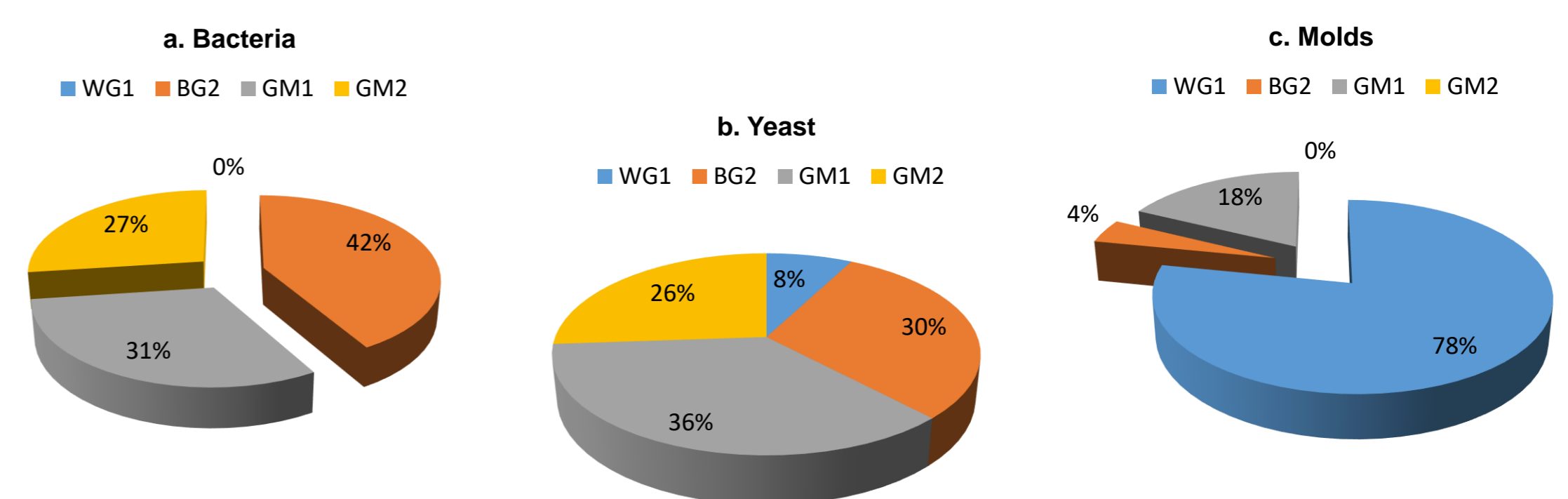


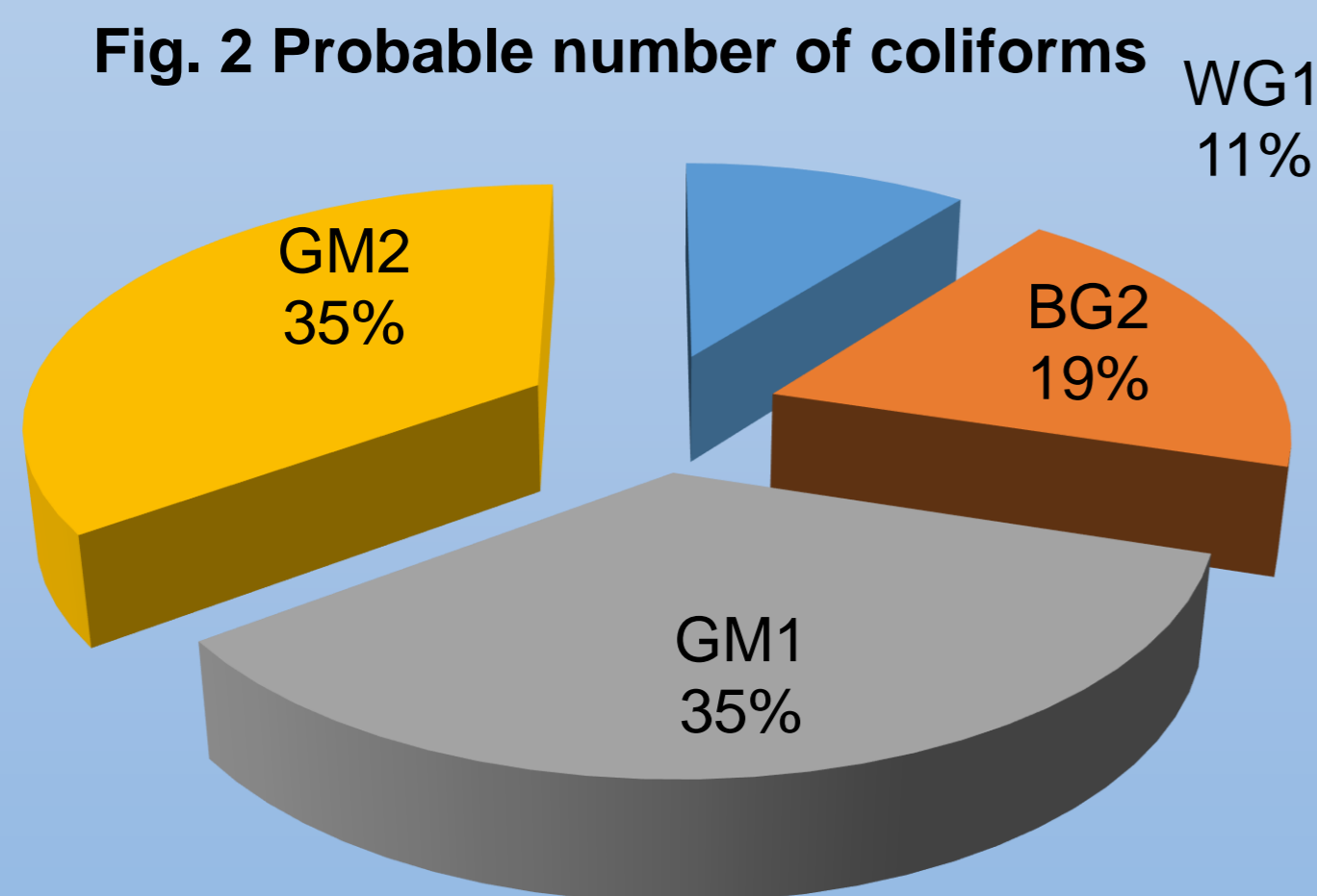
Fig. 1 Estimation of the number of microorganisms (a-c) on white and black grapes (CFU/ml)

Table 1 Characteristics of colonies grown on nutrient media

Samples	Colony characteristics	Character Gram
WG1	-	-
BG2	regular, non-pigmented, small, convex, glossy, smooth colonies dominate; 4 large, irregular, translucent, matte, non-pigmented, umbonate colonies	bacillary forms and cocci Gram positive and Gram negative
GM1	regular, non-pigmented, small, convex, glossy, smooth colonies	Most of them are cocci, arranged in piles Gram positive
GM2	regular, non-pigmented, small, convex, glossy, smooth colonies dominate; 36 of the total are large, flat, translucent colonies with a wrinkled surface	Cocci and bacillary forms Gram positive WG1-white grapes; BG1-black grapes; GM1- white grape must; GM2- black grapes

Legend: WG1-white grapes; BG1-black grapes; GM1- white grape must; GM2- black grapes

Fig. 2 Probable number of coliforms



Conclusions: The presence of useful bacteria and yeasts is a benefit for the grapes, the consumer and the fermentation processes. The presence of coliforms in small quantities below the level imposed by the regulation in force does not raise problems, but shows the need to wash the grapes before consumption. The mushrooms are reduced in number. Some species in these groups are toxin producers and must be monitored if they are in high concentrations.