MICROBIOLOGICAL AND CHEMICAL CHARACTERISATION OF SPONTANEOUS FERMENTATION PROCESS OF GRAPES OBTAINED FROM PODKARPACIE AND MAŁOPOLSKA VINEYARDS

The main objective of the experiments was to characterize the microbiological and chemical aspects of spontaneous fermentation process of white and red grapes from vineyards of southern Poland and to determine the oenological properties of isolated microorganisms.

The study included four varieties of white vine: Seyval Blanc, Hibernal, Johanniter and Bianca and two varieties of red vine: Rondo and Regent, grown in the vineyards "Srebrna Góra", "Spotkaniówka", "Zadora" and "Zalipie".

Medium WL was used to the quantitative determination of yeast isolated from grape surface and spontaneously fermenting grape musts. While culture assimilating L-malic acid was isolated on a medium containing Yeast Nitrogen Base (YNB) and L-malate as the sole carbon source. The dominant strains, as well as microorganisms characterized by an ability to assimilate L-malic acid was subjected to identification using conventional biochemical techniques and the analysis of ITS-5.8S rDNA by the PCR-RFLP method. Microorganisms isolated at the first stage of the test were characterized for deacidifying and oenological properties. The strains ability for the utilization of L-malic acid was measured nephelometrically in YNB liquid media with 12 g/dm^3 addition of L-malate as a carbon source. Oenological properties of the selected strains were determined using Zweigelt grape must. Basic parameters of the obtained wines (extract, content of sugars, nitrogenous compounds, alcohol, total acidity) were determined using recommended OIV methods. Yeast resistance to sulfur dioxide and high concentrations of ethyl alcohol were also analyzed. Strains characterized by the best oenological properties were selected to the last stage of research. At this stage, white (Johanniter) and red (Regent) grape musts were fermented using monocultures of selected yeast strains. After completion of the fermentation, obtained wines were analyzed by the aforementioned methods.

Conducting these experiments allow gaining detailed knowledge of yeast cultures biodiversity of the fruits and during their spontaneous fermentation, determining the oenological properties of isolates, as well as analyzing changes in selected components during the process. The study showed a strong quantitative and qualitative differentiation of microbiota composition between grape varieties, growing seasons and vineyards. In the 2012 and 2014 growing periods, higher numbers of yeast were found on the surface of the grapes in comparison to 2013, which directly resulted in their quantitative composition during the

process. The maximum of the microbial population was noted between the fourth and ninth day of spontaneous fermentation. Musts obtained from red grape varieties (Rondo and Regent) were more favorable environment for the growth of microorganisms compared to the white grape musts. Juices obtained from grapes harvested in the Spotkaniówka vineyard showed the highest content of microorganisms, which could be related to the microclimatic conditions in the vineyard. Spontaneously fermenting grape musts analyzed in the 2013 and 2014 growing seasons were characterized by the highest amount of microbes metabolizing L-malic acid. The highest content of deacidifying yeast was determined in samples obtained from fruit from the Zadora vineyard. In the fresh juices prepared from red grape varieties from the Srebrna Góra there were no yeast assimilating L-malic acid present. Lack of these organisms were also found at the end of spontaneous fermentation in samples obtained from Hibernal, Seyval Blanc, Rondo and Regent grapes coming from the Spotkaniówka vineyard. *Kloeckera/Hanseniaspora* cultures accounted for 70 % of total microflora present on the grapes surface while during the spontaneous fermentation reached 18 to 56 % of the yeast total content. The 2014 growing season turned out to be most favorable for the growth of microorganisms on the surface of fruits and during the fermentation process. In 2013, the lowest number of *Kloeckera/Hanseniaspora* cultures were found in the analyzed juices. The presence of these microorganisms in wines at the end of spontaneous fermentation was not observed. They were replaced by a *Saccharomyces* strains.

During three growing seasons, 375 yeast cultures were isolated and 92 strains, as well as 33 species of microorganisms were identified in spontaneously fermented grape musts. During the process there was a succession of the most important groups of microorganisms. At the beginning, there were *Kloeckera/Hanseniaspora* and *Candida* strains, that were displaced by the culture of *Saccharomyces* during spontaneous fermentation. *Metschnikowia* and *Pichia* yeast were identified both at the beginning and at the end of the process. The microorganisms responsible for wines diseases were also identified in grape musts. Among the microorganisms isolated, yeast belonging to the genus *Candida*, *Pichia*, *Zygosaccharomyces* and *Brettanomyces/Dekkera* can negatively affect on the process and the quality of the beverages. It has been proved, that low fermentation properties of yeast strains not belonging to the *Saccharomyces* genus, which were presented during spontaneous fermentation of grape musts obtained from fruit from the vineyards located in Podkarpacie and Małopolska, determine obtaining wines characterised by high concentration of residual sugar and low alcohol content. There were no significant impact of vine varieties on the level of volatile components in wine. The kinetics of the formation of wine aroma components was

probably determined by the quantitative and qualitative profile of grape microbiota and fruit chemical composition. Reduction of the concentration of L-malic acid in spontaneously fermented wines indicates the presence of yeast capable of degrading this compound in grape must. It has been shown that culture of *S.pombe*, *P.anomala*, *D.hansenii*, *Z.bailli* and *C.glabrata* were characterized by the highest ability to assimilation of L-malic. Further studies have shown that strains *C.sorbosa* contribute to the reduction of grape musts acidity. Perhaps mixed culture of this yeast and *S.cerevisiae* species may be used in the wines deacidification process as an alternative for lactic acid bacteria.

The next stage of the study showed that 100 mg SO_2/dm^3 is the minimum dose inhibiting the growth of microorganisms potentially lowering the wine quality. However, *P.anomala*, *C.glabrata*, *Z.bailli*, *C.sorbosa*, *S.pombe* and some strains of *D.hansenii* are able to survive in an environment with sulfur dioxide concentration at 200 mg/dm^3. At the end of the study, fermentation involving microorganisms with the best oenological characteristics was carried out. It has been proved, that native *S.cerevisiae* and *S.bayanus* yeast isolated from spontaneous fermentated grape musts were characterized by desired oenological properties and may be used as starter cultures in the wine industry.

Monika Ciecka