Cider yeasts associated with Hardanger cider during fermentation process

Urban Česnik[1], Mitja Martelanc[1], Branka Mozetič Vodopivec[1], Ingunn Ovsthus[2], Lorena Butinar[1]

[1]Wine Research Centre, University of Nova Gorica, Vipavska cesta 13, 5000 Nova Gorica, Slovenia

[2]NIBIO Ullensvang, 5781 Lofthus, Norway

In the Hardanger area in Western Norway, the production of cider has a long tradition that goes back to the 12th century, when monks introduced apple growing in this area. Nowadays, this is also the main area of fruit production in Norway. Despite the strict regulation of the alcoholic beverage production in Norway, traditional cider is still produced on some farms in this area. Therefore, our aim was to study the ecology and biodiversity of the yeasts associated with the cider production in the Hardanger area during fermentation process; especially of traditional cider, which is produced by a spontaneous fermentation of apple juice, performed by naturally occurring indigenous yeasts that originate from the fruit or the surfaces of the processing equipment.

In our study, samples of fermenting juice/cider were taken during fermentation process from 12 producers, located in 12 different locations in Hardanger region. Classical cultivation methods using WL (Wallerstein Laboratories) agar medium with added chloramphenicol enable us to isolate a total of 530 yeast isolates that were stored in in-house yeast collection at the NIBIO and included also at the Wine Research Centre collection. Based on the sequencing of the D1/D2 domain of the 26S rDNA we managed to identify 357 isolates and distinguished 27 different yeast species as follows: *Aureobasidium pullulans, Candida californica, C. oleophila, C, sake, Hanseniaspora meyeri, H. uvarum, H. valbyensis. Kregervanrija fluxuum, Kregervanrija* sp*., Metschnikowia andauensis, M. chrysoperlae, M. fructicola, M. pulcherrima, Metschnikowia* sp*, Pichia fermentans, P. kluyveri, P. membranifaciens, P. nakasei, Piskurozyma capsuligena, Rhodotorula nothofagi, Saccharomyces bayanus, S. cerevisiae, S. paradoxus, S. pastorianus, Saccharomyces* sp., *S. uvarum* and *Torulaspora delbrueckii.*

Even though we were not able to obtain samples in three different fermentation stages (beginning, middle and at the end of fermentation) from all producers, we could observe yeast succession during fermentation progress. Yeast diversity was higher at the beginning comparing to the middle of fermentation, when mostly different non-*Saccharomyces* yeast species prevailed, while in the middle of fermentation 11 species were detected (*Candida californica, H. uvarum, H. valbyensis, Kregervanrija sp., K. fluxuum, Pichia membranifaciens, Metschnikowia pulcherrima, Saccharomyces sp, S. bayanus, S. uvarum* and *S. cerevisie).* On the other hand, at the end of fermentation mainly *Saccharomyces* species with high ethanol tolerance were present *(Saccharomyces* sp., *S. cerevisiae, bayanus, S. uvarum* and *P. fermentans).*

In samples that were collected from three producers in all three fermentation stages also quality parameters were determined (ethanol, organic acids, sugars, biogenic amines) with in-house developed methods using HPLC-UV/RID. The most important sugars in ciders were fructose and glucose, as expected. Two producers added sugar to increase the level of ethanol in the middle of fermentation, which is a common procedure in the Hardanger area. Ethanol and organic acid analysis indicated that fermentations went in the right direction, since all parameters were within normal limits. Including the acetic acid level, an indicator of low cider quality, was very low (average around 0,06 g/L). The alcohol incised from the beginning to end fermentation in all samples analysed and minimum concentration was 2,71 g/L. In ciders we detected four biogenic amines (putrescin,

cadaverine, histamine and tyramine). The average amount was 32 mg/L and the most abundant was tyramine.

Keywors: indigenous yeasts; biodiversity; spontaneous fermentation; cider-making