Winetech Scan
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Netwerk van Kundigheid en Technologie vir die Wynbedryf

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Research News

- INNOYEAST ('Innovation and improvement of European wine industry competitiveness by the research and development of native microencapsulated wine yeasts to produce quality wines') is a 28 month long EU-funded project with a total budget of €1,5 million. All wine producing vineyards currently employ external yeasts with excellent results, however, these yeasts have been isolated and produced in wine-growing regions that have nothing to do with the wines made from time immemorial in their corresponding wine producing areas, the possible result being that the wines lose the character of the region. INNOYEAST aims to isolate, select and microencapsulate oenological autochthonous (literally 'native to the soil' or indigenous) yeasts from several European wine-producing regions (Rioja Alavesa, Bordeaux, Chianti and Vinho verde), in order to allow wineries to control the fermentation process and ensure production of high quality wine while maintaining the typical sensory properties and the aroma profile of each wine area. Wineries and research centres from France, Italy, Portugal and Spain are participating in the project. www.innoyeast.eu

- Epidemiological studies have long reported a greater reduction in cardiovascular risk and greater vascular protection associated with a diet rich in polyphenols, including the moderate consumption of red wine. However, little is known about the mechanism involved or the molecular target responsible for the protective effects red wine has on the cardiovascular system. Now a significant study involving mice has demonstrated that red wine polyphenols lead to epithelium-dependent vascular relaxation in the arteries of wild mice only in the presence of the estrogen receptor alpha (ERa). ERa is a nuclear receptor which is usually activated by the sex hormone estrogen, but this study indicates that polyphenols can play the same role in the activation of ERa. The endothelium is the thin layer of cells that line the interior surface of blood vessels. These cells reduce turbulence of the flow of blood allowing the fluid to be pumped farther. Studies have demonstrated that women have lower cardiovascular risk than men, and this protection progressively disappears after menopause. This is attributed to estrogen in premenopausal women which, in conjunction with Era leads to the production of NO which the endothelium uses to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow. Atheroma is the accumulation in artery walls of cell debris, which contains cholesterol, fatty acids, calcium and a fibrous connective tissue. It is known that estrogen protects against atheroma by means of vasodilation. This study has demonstrated that polyphenols can have same protective effect against atheroma as does estrogen. http://dx.doi.org/10.1371/journal.pone.0008554

- A highly diffusible gaseous bioactive molecule, nitric oxide (NO) is recognised to be an important signalling molecule in plant cell biology. While NO research in plants is not as advanced as it is in animals, it has been shown that NO mediates many developmental and physiological processes, including xylogenesis, programmed cell death, pathogen defence reactions, stomatal closure, and gravitropism. A study into the role of NO production in response to progressive soil drying and its possible role as an intermolecular signalling molecule mediating drought-stress responses in grapevines was carried out in potted grapevine plants (*Vitis vinifera* L. cv. Mavrodafni). The cellular sites of NO production and localisation in stressed plants were monitored by fluorometric techniques. Results indicated that both abscisic acid (ABA) and NO concentrations increased significantly in the leaves of the water-stressed plants. ABA functions in many plant developmental processes, including bud dormancy. The changes in stomatal conductance seemed to be closely related to both ABA and NO increase, providing evidence that NO production might be involved as a signalling molecule in ABA-induced stomatal closure. The results suggested a contribution of hydrogen peroxide to triggering NO production as well as a possible role of NO in both stomatal closure and antioxidant defence in drought-stressed grapevines, thus indicating that NO has a role in the drought-signalling pathway in grapevines. http://dx.doi.org/10.1111/j.1755-0238.2009.00064.x

- The presence of haze or sediment in bottled white wines is a visual defect, negatively impacting on product appeal. Wine proteins are the main cause of this defect. Bentonite, an absorbent aluminium phyllosilicate clay, has been used for more than 70 years as a fining agent, adsorbing proteins so as to stabilise wines. However, bentonite is not a very specific absorbent, removing both desirable and undesirable compounds. Also, bentonite fining results in a significant wine loss and a negative environmental impact because of the use of diatomaceous earth as a filter aid for bentonite removal. A study investigated whether the ion-exchange resin SP Trisacryl M (which selectively adsorbs proteins) could be used instead of bentonite. It was found that a Chilean Chardonnay (2005) could be stabilised by adding at least 0.3 kg m^{-3} of bentonite or 12 kg m^{-3} of trisacryl, removing 95% and 76% of the wine proteins respectively. A sensorial panel could not detect statistically significant differences between the bentonite and trisacryl treatments of wine, but 67% of the judges identified the wine treated by

trisacryl as different in appearance and taste, while 71% of the judges were able to mark trisacryl-treated wine as different in aroma. No difference in colour between the wines could be distinguished. The protein adsorption data for bentonite and trisacryl were fitted using the Freundlich adsorption isotherm and it was found that the wine protein adsorption isotherm on trisacryl was unfavourable. Chardonnay treated continuously by trisacryl was only stabilized during the first 5 bed volumes (BV) of wine. Protein removal from Chardonnay by trisacryl in a packed column at continuous operation was about 50% during the first 70 BV of treated wine and decreased progressively until the end of the treatment (100 BV). http://dx.doi.org/10.1111/j.1365-2621.2008.01720.x

Local research results

- In recent years, considerable efforts have been made to improve strains of the wine yeast *Saccharomyces cerevisiae* through the use of modern biotechnological tools. Currently, numerous stable genetically modified wine yeast strains already exist in laboratories, while many others are being constructed. Genetically modified (GM) wine yeast strains have not, as yet, been used in the wine industry, mainly due to concerns relating to the use of GM organisms in food production. A project has focused on several aspects of risk assessment related to the possible use of recombinant wine yeast strains in the wine industry and the potential for fragments of recombinant DNA to spread in a population of yeast through horizontal gene transfer was assessed. The project investigated the following questions: - are large fragments of DNA released during or after alcoholic fermentation by wine yeast strains? - is the released, free DNA stable in wine fermentation conditions, i.e. can DNA fragments persist throughout and after fermentation? - can free DNA be taken up by wine yeast strains in fermentative environments? – and, can DNA be directly transferred between two strains of yeast? The project investigated each of those questions individually and in combination by first constructing marker plasmids and integrative DNA fragments that could easily be followed within a yeast population. These episomal plasmids or integrative DNA fragments were transformed into yeast strains and / or added to fermenting musts. The transformed yeasts were used for fermentations in model grape musts, and DNA fragments and plasmids were added in various concentrations to fermenting non-transformed industrial yeast. At various stages of the fermentations, samples were taken to assess the presence of transforming DNA in the fermentation media or the presence / absence of transformed strains. The data show that free DNA is surprisingly stable in fermentative environments, with DNA added in the beginning of fermentation still being present at the end. However, no release of DNA from transformed strains could be observed, suggesting that DNA is either released at levels too low to be detected, or that DNA is degraded before release. When DNA was added to fermenting yeast, no transformations were usually detected. However, in some cases, the study was able to detect strains that appear to have taken up a fragment of the transforming DNA. The exact molecular nature of the events that led to these transformants is still under investigation. In a second part of the project, the possibility of DNA transfers within a biofilm environment was also assessed. It proved rather difficult to integrate wine yeast strains into laboratory model biofilm systems, and this study is being continued. Finally, no sporulation of the yeast used in our experiments could be detected. However, it was decided that to investigate the occurrence of sporulation in industrial yeasts in the wine industry would require a full-blown ecological study which exceeded the scope of the current project. www.sawislibrary.co.za/dbtextimages/BauerFF4.pdf

- A programme which involved several projects was aimed at generating new wine yeast strains that would give winemakers additional choices in either enhancing or reducing specific characteristics of their wines. Specifically, the projects aimed at (i) adjusting the levels of aroma compounds, in particular esters, that are produced by wine yeast during fermentation, (ii) producing enzymes that will release additional volatile aroma compounds from the grapes and (iii) reducing the amount of alcohol to levels which allow a better perception and release of aroma and flavour compounds. Regarding (i) it was shown that over-expression of the genes ATF1 and ATF2 increased the levels of ethyl-, isoamyl-, and 2-pheylethyl acetate and ethyl caproate in the wine, while IAH1 over-expression decreased the concentrations of ethyl-, isoamyl-, hexyl-, and 2-phenylethyl acetate drastically. Increased production of the other enzymes (TIP1, EHT1) did not lead to significant changes in the aroma profiles of the wines. (ii) In an attempt to optimise the volatile aroma compound levels in wine, the *S. cerevisiae* PAD1 gene (encoding phenyl acrylic acid decarboxilase), the Bacillus subtilis phenolic acid decarboxylase gene and the Lactobacillus plantarum para-coumaric acid decarboxylase gene were cloned and introduced into laboratory yeast strains to determine their activity and efficiency. The endogenous *S. cerevisiae* PAD1 gene was both over-expressed and disrupted to determine the effect this had on the levels of volatile phenols in wine. After analysis of the wines made in microvinifications, it became clear that the presence of the bacterial genes caused an increase in the formation of volatile phenols. Also, a yeast was prepared that was able to increase the monoterpene levels in wine. (iii) So as reduce alcohol levels enzymatically, a glucose oxidase producing yeast was prepared. Together with the other concurrent strategies to overproduce glycerol at the expense of ethanol formation, this might offer a viable way to meet consumers' demands for affordable low-alcohol wine. Micro vinification experiments showed that as much as a 1.8% drop in alcohol level is possible using laboratory yeast strains. www.sawislibrary.co.za/dbtextimages/BauerFF6.pdf