METHODS FOR PRODUCING LOW-ALCOHOL WINE II. FERMENTATION AND POST-FERMENTATION STRATEGIES

METODE DE PRODUCERE A VINURILOR CU GRAD ALCOOLIC SCĂZUT. II. STRATEGII FERMENTATIVE ŞI POST-FERMENTATIVE

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Abstract. *According to current legislation, wine is defined as the alcoholic beverage resulted from fermentation of grapes or grape must, with an ethanol concentration of minimum 8.5% (v/v). Climate change, modern viticultural practices and the use of selected yeast strains gradually lead to wines with increased alcohol concentrations. Moreover, consumer demand is apparent for wines with lower ethanol levels, perceived as healthier. Since viticultural and pre-fermentation practices not always lead to high quality products, the aim of this paper was to provide technological data on the currently available microbiological and post-fermentation practices to produce low alcohol wine from well-ripened grapes. However, the most efficient strategies involves a compromise between ethanol removal, energy consumption and potential impact on wine composition.*

Key words: alcoholic fermentation, grape must, membrane processes, wine yeasts

Rezumat. *Conform legislației actuale, vinul este produsul alimentar obținut exclusiv prin fermentarea strugurilor sau a mustului de struguri, cu o concentrație alcoolică de minimum 8,5% (v/v). Modificările climatice, aplicarea unor tehnologii viticole moderne şi utilizarea tulpinilor de levuri selecționate au condus treptat la obținerea unor vinuri cu concentrații alcoolice tot mai ridicate. In mod contrar, este tot mai evidentă preferința consumatorilor pentru vinuri cu un conținut mai scăzut de etanol, percepute, în general, ca fiind mai sănătoase. Întrucât practicile viticole şi cele pre-fermentative de reducere a concentrațiilor de zaharuri din struguri şi must nu conduc întotdeauna la obținerea unor vinuri conforme calitativ, scopul acestui studiu a fost prezentarea unor practici microbiologice (fermentative) şi post-fermentative de reducere a titrului alcoolic natural al vinului. Cele mai eficiente strategii implică identificarea unui echilibru între eliminarea etanolului, consumul de energie şi impactul potențial asupra compoziției vinului.*

Cuvinte cheie: levuri selecționate, fermentație alcoolică, must de struguri, procese membranare

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INTRODUCTION

Throughout time the alcoholic concentration of wines has increased gradually, a trend attributed to climate change, advanced viticultural practices, improved plant material and modern vinification techniques, all leading to obtaining elevated sugar levels in grapes and implicitly to an increase in alcohol content of wine. In warmer regions, the average alcohol content has risen by approximately 2% (v/v) over the past 30 years (Varela *et al.*, 2015). As an alternative to high alcohol wines currently marketed worldwide, dealcoholised or low-alcohol wines (LAW) may offer a number of potential social and health benefits for consumers: improved productivity, lower risk of driving accidents, a better social acceptance and important health advantages (reduced calorie intake, decreased risk from alcohol-related illness) (Pickering, 2000). Moreover, consumer demand is apparent for wines with lower ethanol levels, perceived as healthier, products that ensure food security, nutritional richness and participation in quality of life. Since viticultural strategies and pre-fermentation practices for limiting sugar accumulation in grapes or lowering sugar concentration in must not always lead to high quality products, a series of fermentation processes (changing the sugars consumed/alcohol produced ratio, early interruption of fermentation, use of low-alcohol producing yeast) and post-fermentation practices (physical removal of alcohol) to produce LAW from well-ripened grapes need to be considered.

This work presents currently known possibilities to produce low or reduced alcohol wines, providing a brief overview for producers and consumers concerning the technological aspects and factors that influence the quality and acceptance of LAW.

MATERIAL AND METHOD

The study summarizes recent data from international literature on microbiological and post-fermentation practices for producing dealcoholised, low or reduced alcohol wines, for a better understanding of the concept and easier implementation of the technologies by winemakers.

RESULTS AND DISCUSSIONS

a. Microbiological practices

1. Low-alcohol producing yeast

Saccharomyces cerevisiae is the principal yeast selected for wine fermentation. Recent studies have shown that the use of different *S. cerevisiae* strains induced small differences (0.4% v/v) in ethanol yielded in wine following fermentation (Bucher *et al.*, 2019). Selection of yeasts used in winemaking was made until recent years in order to obtain a high alcohol concentration. Currently, along with the ability to amplify the aromatic profile, it is necessary to isolate yeasts that consume more sugar and produce less ethanol. Also, several non-conventional yeasts were used to obtain lower levels of alcohol in wines. *S.*

uvarum and *S. cerevisiae* × *S. uvarum* hybrids produced 0.30% (v/v) less ethanol than *S. cerevisiae* yeasts (Tilloy *et al.*, 2013). Moreover, some experiments confirmed the low volatile acidity and glycerol production of *Torulaspora delbrueckii* with lower ethanol production (Herraiz *et al.*, 1990). *Candida zemplinina* strains possessed a poor alcohol/sugar yield, with 12% less compared to *S. cerevisiae* strains. No significant differences in ethanol production were observed when *C. zemplinina* and *S. cerevisiae* co-cultures were used. In contrast, sequential fermentations (*S. cerevisiae* added after 24 h) lead to a lower ethanol production up to 0.90% (v/v) (Tilloy *et al.*, 2013).

Worldwide investigations are focused on generating by genetic engineering new *S. cerevisiae* and non-*Saccharomyces* strains to produce less ethanol by diverting sugar metabolism to other end-points (*e.g.* glycerol), while maintaining wine quality (Varela *et al.*, 2015). In normally functioning yeast, most of the NADH produced during glycolysis is subsequently oxidized during ethanol formation, but yeast can utilize other pathways for oxidising NADH, including cytosolic production of glycerol (Kutyna *et al.*, 2010). These yeasts usually show a higher content of fermentation by-products. Moreover, the use of genetically modified organisms is often seen as a negative aspect by most consumers. Transgenic incorporation of cytosolic oxygen-dependant NADH-oxidase enzymes showed promising results in lowering ethanol production (Schmidtke *et al.*, 2012). Also, expression in wine yeast of lactate dehydrogenase gene (LDH) from *Lactobacillus casei* has resulted in reduced ethanol concentration (0.25% v/v) by diverting carbon metabolism to lactic acid production (Dequin *et al.* 1999).

2. Other microbiological approaches

A tested alternative to producing LAW was to exploit the *oxidative metabolism of yeasts*. In the fermentation process there is first a considerable increase in yeast biomass through aerobic metabolism (cca. 2% of total sugar amount). Then, due to the lack of oxygen, the anaerobic metabolism is triggered. Future studies will have to find solutions to prolong the aerobic phase of yeast growth, without altering the organoleptic characteristics of obtained wines (Varela *et al.*, 2015). Strong *aeration* for the first 48 h before the anaerobic process enabled the production of LAW by *Metschnikowia pulcherrima* (Morales *et al.,* 2015).

Several *metabolites* can influence yeast metabolism by altering redox balance, like: furfural, vanillin, glycolaldehyde, organic acids (cinnamic, benzoic, formic and propionic acids), sodium or potassium chloride, sulphur dioxide and sodium carbonate (Kutyna *et al.*, 2010). Some of these metabolites can be added to the fermenting must, but might affect wine sensory profile.

Increasing *fermentation temperature*, can also alter yeast metabolism and divert carbon away from ethanol production, but such conditions may affect dramatically wine sensory profile. Also, *early arrest of fermentation* is often restrictive in terms of nutritional and microbiological stability of the sweet wine obtained (high residual sugar content) (Pickering, 2000).

b. Post-fermentation practices

1. Wine blending

In order to reduce excessive alcohol concentration of wines a strategy can be represented by blending high alcohol wines with wines containing less alcohol. Wine obtained from early harvested grapes contains a lower level of alcohol, but does not have the aromatic or phenolic complexity of the ripe grape wine and may present excessive acidity or bitterness (Varela *et al*., 2015).

2. Reverse osmosis

Reverse osmosis is the most used procedure for lowering wine alcohol (Gil *et al*., 2013). Varying the pore size and pressure applied on the membrane result in a series of membrane filtration processes (reverse osmosis, nanofiltration, ultrafiltration and microfiltration). Reverse osmosis membranes can be made of cellulose acetate, regenerated cellulose, synthetic polymers and ceramics (Schmidtke *et al*., 2012). Water and ethanol, being small molecules, pass through the membrane into the permeate, which has to be reduced in alcohol in a second step, usually by osmotic distillation. This alcohol reduced fraction is finally blended with the concentrate (Schmitt and Christmann, 2019). Wine can be aslo restored to the original water content by the *addition of low sugar grape must* or by water (*diafiltration)*, but in the most countries the water dilution is not legally allowed.

3. Vacuum distillation

The boiling point of pure ethanol is around 78 °C, this temperature being too high for performing a wine treatment. The vacuum distillation achieves lower boiling points by applying a vacuum in the column. This process is performed at reduced pressure (0.07 to 1 bar) and a lower temperature (30 to 60 °C), in order to keep aromatic losses as low as possible. At the end of the treatment, the alcohol content of the wine can achieve 0.5% (v/v) (Varela *et al*., 2015). Aguera *et al*. (2010) separated 135 L of pure ethanol from 1000 L wine with 14% (v/v) alcohol. However, lowering alcohol by 2% (v/v) has been found to reduce the concentration of higher alcohols by 25% and esters by 45%. For lower volumes of wine a rotary evaporator can be used for vacuum distillation. In industrial *vacuum rectification* the condensate is passed to an aroma leaching and some of the flavours are returned to the final LAW (Schmitt and Christmann, 2019).

4. Osmotic distillation and pervaporation

Osmotic distillation (evaporative perstraction or membrane distillation) and pervaporation share similar processes. Ethanol removal occurs as a process of evaporation at the membrane interface, diffusion of the vapor across the membrane and condensation into a stripping phase (Schmidtke *et al*., 2012). Most processing can be conducted at low temperatures (cca. 30°C), without applying high pressure (Saha *et al*., 2013). In pervaporation, the wine is separated by partial vaporization through a nonporous selectively permeable membrane. Pervaporation uses an inert gas or vacuum to remove the permeate. Ethanol migrates through the membrane in a gaseous phase and recondenses as permeate. Unfortunately, significant aroma losses may arise when pervaporation is applied.

At low rates of ethanol removal (<2 % v/v), osmotic distillation is reported to have a modest impact on aliphatic acids, monoterpenes and some wine alcohols. However, ethyl esters were substantially reduced in wine (Saha *et al.*, 2013).

5. Spinning cone

Spinning cone column (SCC) is a gas-liquid contacting device consisting of a vertical counter-current flow system that contains a succession of rotating and stationary metal cones (Pickering, 2000). The liquid flows down on the stationary cones under the influence of gravity and moves up in a thin film by the action of applied centrifugal force. Vapour flows up the column, traversing the spaces between the successive fixed and rotating cones. Minerals and other non-volatile components in the original wine are preserved. In the first stage, delicate aroma components are removed at moderate vacuum (0.04 atm) and low temperature (26-28 °C) (1% of wine). The second stage is conducted at higher temperature (38 °C) and results in an alcohol concentrate above 50% (v/v) (Saha *et al.*, 2013).

6. Other approaches

In *freeze concentration* method water of wine can be removed by freezing and the alcohol in the residual liquid can be removed by vacuum distillation. LAW can be adjusted to various alcohol concentrations with the alcohol fraction.

In *dialysis*, water is used to provide the concentration gradient, allowing the movement of ethanol out of the wine into the water. As the concentration gradient exists only for alcohol, previous dealcoholised wine can be used instead of water.

Alcohol and aroma-containing condensate resulting from wine evaporation can be extracted with *organic solvents* such as pentane and hexane (Pickering, 2000). Disadvantages of solvent extraction of wine include thermal damage and the potential presence of solvent residuals in the extract.

CO_2 is one of the most commonly solvent used for *liquid-liquid extraction*. Alcohol may be extracted from wine using liquid CO_2, which, under specific pressure and temperature conditions, has properties similar to those of solvents. Carnacini *et al.* (1989) concluded that extraction with supercritical CO_2 is a promising process. Unlike organic solvents, supercritical CO_2 is not toxic.

Alcohol can be also *adsorbed* onto porous resins such as styrol/ divinylbenzol copolymers or silica gels. These processes are more suitable at laboratory level rather than large-scale production.

CONCLUSIONS

1. The use of *Saccharomyces* and non-*Saccharomyces* yeast strains in co-cultures or sequential cultures, has proven to be an effective strategy for reducing ethanol production, especially by redirecting yeast carbon metabolism to the production of other compounds like glycerol or lactic acid by genetic engineering.

2. Reduction of ethanol concentration following alcoholic fermentation of mature-grape must remains the main approach currently used, reverse osmosis-vacuum distilation and spinning cone columns best preserving the sensory features of low-alcohol wines.

3. For reasons associated with health, economics and high quality, currently exists a need for the development of effective, fast and less expensive technologies to reduce the alcoholic concentration of wines. However, the most efficient strategies involves a compromise between ethanol removal, energy consumption and potential impact on wine composition and sensory attributes.

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