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MECHANISMS AND FACTORS INFLUENCING MCFA FORMATION BY YEASTS DURING GROWTH AND ALCOHOLIC FERMENTATION AND THEIR IMPORTANCE IN WINEMAKING

Cornel Daniel BANIȚĂ[1], Arina Oana ANTOCE[2], George Adrian COJOCARU[2]

[1]Pietroasa-Istrița Research and Development Center for Viticulture and Pomiculture, Stațiunii Street, 127470, Pietroasele - Buzau, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărași Blvd, District 1, Bucharest, Romania

Corresponding author emails: aantoce@yahoo.com, cojocaru.george@ymail.com

Abstract

The medium chain saturated fatty acids (MCFA) are synthesized by the yeasts and released in fermentation media, where they contribute to fermentation aroma, but are also well known for their inhibitory effect on yeast growth. At low pH and in the presence of ethanol, only few mg/l of MCFA could arrest the AF. One of the mechanisms employed by the yeasts to protect themselves from the MCFA effects implies the conversion of the MCFA in ethyl esters, which are less toxic. In this way, fermentation aroma is even more enhanced, as ethyl esters of MCFA have more appealing sensorial qualities and are perceived at lower thresholds. Understanding MCFA's occurrence in grape must and wine and their inhibitory mechanisms can be useful for a better predictability and control during winemaking process. Certain technological factors were found to dramatically affect the concentrations of MCFA during winemaking, among which are the grapes ripening process, several technological interventions during winemaking and the yeast strains, all influencing style and perceived quality of wines. Furthermore, due to their inhibitory effects on yeasts, the MCFA have drawn the attention of researchers and producers as agents able to reduce the doses of SO_2 necessary for the cessation of alcoholic fermentation (AF) in order to obtain sweet wines. In this review inhibitory mechanisms are discussed, along with possible ways to control the MCFA concentrations during winemaking, also in the view of sweet wine production.

Key words*: MCFA, octanoic acid; decanoic acid; ethyl octanoate, ethyl decanoate, sweet.*

INTRODUCTION

Medium-chain fatty acids (MCFAs) are saturated fatty acids with C_6-C_{12} atoms which are volatile organic compounds produced in wines and other fermented beverages along with other major aroma constituents (Bardi et al., 1999; Zhao et al., 2017). Low to moderate concentrations of medium-chain fatty acids (MCFAs) and their ethyl esters (MCFAEEs) can contribute to positive aroma quality of wines and other fermented beverages (Borrull et al., 2015; Mina & Tsaltas, 2017). Accumulation of MCFAs during AF under certain conditions may inhibit growth of both yeast or lactic bacteria, which may lead to either an incomplete AF or an unachievable onset of MLF (Alexandre et al., 2004; Lonvaud-Funel et al., 1988). For those wines with high concentrations of MCFAs and problems of AF completion or MLF starting, the removal of these compounds must be taken into account (Lonvaud-Funel et al., 1985; Viegas et al., 1989). MCFAs are slightly soluble *in water, the solubility decreasing with the chain length. Their O: C ratio by mass is a simple way to point out the hydrophobicity increase, as the chain of aliphatic carbon increases. As the MCFAs hydrophobicity increases, their solubility in the phospholipids of yeasts membrane increases as well. The insertion of the undissociated acid forms inside membranes leads to an increase in their permeability and* consequently to a loss of their biological function (Viegas et al., 1989). Enhanced permeability of yeast cell membranes due to the presence of MCFAs during AF, promoted yeast cell death, especially under ethanol-induced proton influx, which

determines the decrease of internal pH (Borrull et al., 2015; Leão & Van Uden, 1984; Viegas et al., 1989). Levels of ethanol concentrations to 12% or above, pH values lower than the MCFA's pK_a, as it happens in grape juice (3.0 to 4.0) and promoting those conditions which enable the biosynthesis of MCFAs in the fermenting medium, all lead to a decrease in *Saccharomyces cerevisiae* viability or cell death (Borrull et al., 2015; Viegas & Sá-Correia, 1997). In order to produce ATP through glycolysis, yeast cells need to maintain the internal pH around 6.0 to 6.5 during fermentation, thus, any factors able to lower the internal pH below 6.0 will stop the ATP production and therefore activate the autolysis enzymes, leading to a subsequent degradation of cell components (Alexandre & Guilloux-Benatier, 2006; Torello Pianale et al., 2022). Temperature of the fermenting media influences the inhibitory effect of MCFAs, for instance, a low temperature, as the one used for the white wine production, is able to reduce the loss of viability in *Saccharomyces cerevisiae*, while a higher temperature promotes higher cell death rates (Viegas & Sá-Correia, 1997). These factors and mechanisms are important for wine production, due to their consequences on the quality of the final product. Accumulation of MCFAs in the fermenting media during AF is an undesirable pathway of yeast metabolism, which increase the risk of sluggish or even stopping AF. Relatively recently, medium-chain fatty acids came in to the attention of researchers and producers of natural sweet wines at the international level as a means to reduce the doses of SO_2 used for stopping fermentation to produce sweet wines (Baniţă et al., 2023; Banita & Antoce, 2021; Baroň et al., 2017). The use of MCFAs for AF cessation is still under evaluation as an alternative method to reduce electric energy consumption necessary for cooling and the dose of sulfur dioxide required in the classical method (Baniţă et al., 2023; Baroň et al., 2017; Viegas & Sá-Correia, 1997). Moreover, the addition of MCFAs for AF cessation was found to reduce of the acetaldehyde content compared to the chilling process, as well as to reduce the diacetyl content compared to cross-flow filtration (Licek et al., 2020). Modulation of MCFA concentration during winemaking allows the oenologists to control the kinetics of AF to dryness or to stop the fermentation to produce wines with natural residual sugar.

MCFA PRODUCTION DURING WINEMAKING

MCFA production in wine is dependent on controllable *physical factors*, such as temperature or aeration during AF and less controllable *biological factors,* such as grape cultivar, grape microbiome or must composition, which includes nitrogen compounds, lipids and pH (Lonvaud-Funel et al., 1988; Rizk, 2016).

The effect of aeration regime - lipid biosynthesis and wine quality

Generally, yeasts need 4 to 8 mg/l O_2 at the end of growth phase in order to promote lipid biosynthesis, especially unsaturated fatty acids (UFAs) and ergosterol, which are known as "survival factors" (Andreasen & Stier, 1953, 1954; Bell & Henschke, 2005; Boll et al., 1980; Jean-Marie et al., 1996; Lafon-Lafourcade et al., 1979). These growth factors, UFAs and ergosterol, integrated into yeast cell membrane of *Saccharomyces cerevisiae* are produced only in the presence of oxygen and are necessary for yeasts to strengthen their resistance to stress factors such as extreme alcohol, temperatures, osmotic pressure (Costa & Moradas-Ferreira, 2001; Lafon-Lafourcade et al., 1979; Piper, 1995). In beer fermentation, yeast requirements for oxygen to promote sterol biosynthesis are between 0.1 to 0.3 mg O_2/gram of yeast (expressed as dry mass), while for UFA biosynthesis, a dose of 0.35 mg O_2/gram of yeast (expressed as dry mass), is considered sufficient for high gravity brewing fermentation (Kirsop, 1977; Rosenfeld et al., 2003). In the case of wine fermentation a larger dose of 0.5 mg O_2/gram of yeast (expressed as dry mass) is required for sterol biosynthesis, while for UFA synthesis the amount of oxygen is negligible (Salmon et al., 1998).

Overall, a more oxygenated fermenting media may be helpful in the case of nitrogen deficient grape musts, improving the use of proline nitrogen-use by yeasts by activating the responsible enzyme, namely proline oxidase (Bell & Henschke, 2005; Ingledew & Kunkee, 1985). The addition of nitrogen-containing

inorganic nutrients (diammonium phosphate) or even organic nutrients based on amino acids will activate and stimulate the multiplication of yeast cells in the beginning of AF phase, contributing to biomass increase, but without effect on yeast viability at the end phase of AF (Andreasen & Stier, 1953, 1954; Lafon-Lafourcade et al., 1979). The nitrogen is frequently a limiting factor for production of adequate biomass required to complete the fermentation and it is usually adjusted by winemakers, in accordance to the necessities of the selected yeast strain at the desired temperature during AF, following routine grape juice analyses for sugar concentration and yeast assimilable nitrogen (Bell & Henschke, 2005). In order to influence yeast viability towards the end of AF, besides aeration, another solution is adding nutrients rich in "survivor factors" during rehydration of dry yeasts or during fermentation, as their reduced content affects physical characteristics of membrane, reducing the cell resistance to stress factors (Daum et al., 1998). Rehydration nutrients rich in sterols and UFAs will help completing AF by increasing the resistance of yeasts to stress factors (Lafon-Lafourcade *et al.*, 1979). The yeasts are able to directly incorporate into their membranes sterols and UFAs during growth and AF (Chen, 1980). The ergosterol plays a critical role in ethanol resistance in *Saccharomyces cerevisiae* (Ding et al., 2009) allowing for the increase of membrane rigidity, while high concentrations of ethanol can induce plasma membrane fluidity and consequently becoming toxic to membrane proteins, fact that stops cell growth and induce cell death (Jones & Greenfield, 1987). As the modern white and rose winemaking is often based on avoidance of the aeration and stimulation of production of acetate and ethyl esters, many winemakers use nutrients rich in "survivor factors" during rehydration of active dry yeasts, in order to incorporate ergosterol and UFAs inside yeast cell membranes.

Genes involved in acetate esters production, alcohol acetyltransferases *ATF1* and *ATF2*, are repressed by oxygen exposure during AF, while adding UFAs during AF represses only *ATF1*, while *ATF2* is overexpressed (Fujii et al., 1997; Peddie, 1990; Saerens et al., 2010; Yoshimoto et al., 1999). However, depriving the yeast of oxygen or creating hypoxic conditions during AF promote the formation and accumulation of MCFAs in wine, which decreases the viability of yeasts towards the end of FA and leads even to stopping FA (Borrull et al., 2015; Takashi, 1986).

Fatty acids are derived from degradation of storage and complex lipids, *de novo* synthesis and uptake from the environment (Klug & Daum, 2014). *De novo* lipogenesis starts in the cytosol and in mitochondria through acetyl-CoA-carboxylase encoded by cytoplasmic *ACC1* and of mitochondrial *HFA1* genes (Hasslacher et al., 1993; Hoja et al., 2004). After initiation of this process, elongation of the chain is based on the malonyl-CoA, the universal precursor of fatty acids, which serves as a two-carbon building block (Beld et al., 2015). Fatty acid elongation is generated by a FAS system of enzymes (Toke & Martin, 1996). Yeast contains two distinct fatty-acid synthases (FAS), the cytoplasmic FAS (*FAS1*), which is a large multifunctional enzyme producing over >95% of cellular fatty acids and, mitochondrial FAS (*FAS2*), a non-aggregated FAS enzymes similar to that of bacteria (Brody et al., 1997; Harington et al., 1993; Lynen et al., 1980; Schneider et al., 1997).

The MCFAs are by-products of lipid synthesis as a result of overexpression of fatty acid synthetase complex (*FAS1* and *FAS2*) activated by hypoxic conditions during AF and thus, being the main reason for which wines fermented in such conditions have increased concentrations (Restrepo et al., 2019; Saerens et al., 2010; Taylor & Kirsop, 1977). The increase of MCFA concentration in wine is thus a consequence of an active process under anaerobic conditions, as they are not needed for structural lipid synthesis (Bardi et al., 1999). As MCFAs are not necessary for cell function, MCFAs are released into the medium through passive diffusion across the cytoplasmic membrane and are not bound to cell structures (Bardi et al., 1999). The accumulation of MCFAs in fermentation media was found to be a result of the release from FAS complex of acyl-CoA, which is then hydrolysed in order to recycle CoA-SH, thus liberating the MCFA acyl part. Under these conditions, cell growth

stops, as the lipid biosynthesis becomes impossible (Bardi et al., 1999).

To counteract the anaerobic conditions and to improve yeast viability, $C_{16:1}$ fatty acid can be added in the medium, yeast cells showing higher viability and faster fermentation rates at 13°C. These cell membranes incorporated higher concentrations of $C_{16:1}$ and ergosterol, fatty acids with a shorter chain length and had a higher sterol/phospholipid ratio (Redón et al., 2009).

Controlling the oxidation reduction potential (ORP) during wine fermentation and changing it in the range of -100 to + 100 mV, revealed that the maximum concentration of MCFAEEs is produced in wines during AF when ORP is maintained at 0 mV (Xue et al., 2022). Controlled ORP at 0 mV during AF led to a higher flux of citric acid to cytoplasm, a moderate $NADP^+$/NADH ratio and highly expressed genes *ACC1*, *FAS1*, *FAA2* and *EEB1*, thus favouring the production of MCFAEEs, which therefore improve the aroma quality in wines (Xue et al., 2022).

However, overexpression of MCFA-ethyl ester synthases EEB1 and EHT1 is only slightly affected by oxygen exposure, MCFAEE production responding differently to MCFA precursors. Overexpression of *EEB1* gene responds well to the presence of octanoic acid and poor to decanoic acid, while the overexpression of *EHT1* gene responds to both acids, but still more to octanoic acid. Deletion of these genes decreases the MCFAEE production, confirming their involvement in ester production mechanisms. *EHT1* and *EEB1* genes may have both activities of synthesis and hydrolysis, because their overexpression did not enhance MCFAEE content, although are involved in MCFAs detoxification (Saerens *et al.*, 2006). A possible yeast detoxification metabolism involves ethyl esters biosynthesis through *EHT1* and *EEB1* genes and the role of *ATF2*, involved in acetylation of sterols, which is preferentially expressed under anaerobic conditions (Cauet et al., 1999; Saerens et al., 2010).

During the fermentation of high sugar musts, the accumulation of ethanol can increase excessively the production of reactive oxygen species (ROS), as a consequence of uncoupling electron transport chain from the ATPase, thus, being capable of damaging cellular constituents (Jones & Greenfield, 1987; Landolfo et al., 2008; Moradas-Ferreira *et al.*, 1996). The yeast cells hinder the ROS accumulation through modification of cellular lipid composition, triggering antioxidant defence mechanism such as GSH production. Any redox imbalance can result in oxidative stress (GSH depletion, lipid peroxidation, protein oxidation and, even worse, DNA damage) and can cause yeast cell death (Costa & Moradas-Ferreira, 2001; Piper, 1995). GSH and cysteine depletion under oxidative stress promotes the production of H_2S, a strong reducing agent known to be involved in the protection mechanism used by yeasts to detoxify ROS (Sohn & Kuriyama, 2001). The polyunsaturated fatty acids percentage plays an important role in oxidative stress resistance, which explains why in species better adapted against the oxidative stress, such as *Torulaspora delbrueckii* and *Metschnikowia pulcherrima*, the proportion of these compounds is higher compared with that of *Saccharomyces cerevisiae* (Vázquez et al., 2019).

The effect of temperature regimen during AF

The temperature during AF for white/rosé wine production is generally lower than that for red wines. White and rosé wines need a temperature range between 15 to 18°C in order to develop and preserve the aromatic compounds and to control the yeast biomass during the process, while for red wine production temperatures over 24 to 28°C are more common, to allow for better phenolics extraction during maceration-fermentation phase. Lower temperature regimes may be implemented in case of fruity red wine production. The AF temperature is known to affect the concentrations of MCFAs and MCFAEEs in the final product. The fermentation of several grape white varieties (Feteasca alba, Pinot Gris and Chardonnay) using *Saccharomyces cerevisiae* strain Vinoferm Crio at different temperatures showed the effect of temperature on MCFAs and MCFAEEs production (Csutoras et al., 2022). The controlled temperatures between 15 to 16°C during AF lead to a lower concentration of these compounds, while

elevated fermenting temperatures of 25 to 26°C, lead to a higher concentration of these compounds in the resulted wines (Csutoras et al., 2022).

Compared to white wines, generally, red winemaking requires higher temperatures during AF for the skin maceration and, as a consequence, increased MCFA and MCFAEE concentrations are also produced in the final wines. This happens even more if there is no aeration during pump-over.

Several experimental variants carried out on Carménère grape variety macerated-fermented at different temperatures (24, 28 and 32°C) and inoculated with *Saccharomyces cerevisiae* strain EC1118 revealed that increasing temperature from 24 to 28°C leads to a raise in production of MCFAs, while at 32°C the MCFA level decreased being the lowest among the variants. Also, the biomass resulted at 28°C was the highest while at 32°C was the lowest, which confirms that the temperature is an important factor able to affect yeast growth and metabolism, consequently, altering the final concentration of MCFAs in wines (Restrepo et al., 2019).

However, for white wine fermentation, the results of other authors who experimented with *Saccharomyces cerevisiae* and *Saccharomyces bayanus* revealed opposite results for both yeasts, such as, the production of higher concentrations of MCFAs and MCFAEEs at 13°C as compared to those obtained at 25°C wine samples where the concentrations were lower. In the same study, the fatty acid composition in yeast cells at the end of AF, showed that the yeast membranes contained more MCFAs when they were grown at higher temperatures and more unsaturated fatty acids at lower temperatures, as an adaptive response (Torija et al., 2003a).

Fermentation of several grape juice samples with two yeast strains of *Saccharomyces cerevisiae* (IAM4274 and IAM4268) under controlled temperatures ranging from 10 to 30°C with incremental rises of 5°C showed a decreasing production of MCFAs (expressed as sum of C_6+C_8+C_{10}) in direct correlation with the fermentation temperature increase, while, the C_6 and C_8 showed a decreasing level at elevated temperatures, while C_{10} remained almost constant (Takashi, 1986).

These inconsistencies among results could be attributed to the different levels of oxygen exposure, nutrients or inoculum sizes during experimentation. In one study, using *Saccharomyces cerevisiae* fermenting concentrated grape juice diluted to 200 g/l sugars, it was revealed that the maximum population of yeasts (biomass) resulted at 30°C and the lowest at 35°C, suggesting a higher rate of cell death over 30°C (Torija et al., 2003b). Although, while during AF at temperatures under or equal to 20°C the yeast growth rate after 7 days of fermentation decreased only slightly, at temperatures over 20°C the decrease was more accentuated and at 35°C the decrease was very rapid, indicating a high rate of cell death (Torija et al., 2003b). However, the inhibitory effect of MCFAs was found to be higher at low and intermediate temperatures in the presence of high concentrations of ethanol during AF (Viegas & Sá-Correia, 1997).

The effect of yeast assimilable nitrogen

The consumption of nitrogen is different among different yeast strains and is mostly influenced by the temperature of fermenting media. At higher temperatures the yeasts require more YAN and other nutrients in order to support the growth and produce biomass and as well as volatile compounds (Vilanova et al., 2007). In one study it was shown that *Saccharomyces cerevisiae* grown at 13°C consumed 59 mg/l NH_4^+ and 59 mg/l amino acids, while at 25°C consumed 79 mg/l NH_4^+ and 67 mg/l amino acids (Beltran et al., 2007). The addition of inorganic sources of nitrogen as ammonium cation is strongly influencing MCFAs and MCFAEEs concentrations in the final wines (Vilanova et al., 2007). The timing of ammonium sulphate addition and the dosage influenced mostly the MCFAs production, the additions at the beginning of fermentation (before inoculation) increasing the most the production of MCFAs, while the additions at the halfway point (when 50% of the sugar had been consumed) decreased or only slightly increased the concentration of MCFAs, depending on yeast strain of *Saccharomyces cerevisiae* (Hernandez-Orte et al., 2006). The additions of ammonium sulphate at the beginning of fermentation influenced less the MCFAEE (ethyl hexanoate and ethyl

octanoate) production, the doses of 260 mg/l NH_4^+ tending to only slightly increase the final concentration of MCFAEEs, while doses of 400 mg/l NH_4^+ left the production of fatty acid ethyl esters at about the same level as in the control samples with only 120 mg/l NH_4^+, depending on yeast strain (Hernandez-Orte et al., 2006). The concentrations of MCFAs and MCFAEEs were observed to be increased in Chardonnay wines obtained from musts purposely selected for their low nutrient and sugar content (180 g/l). When fermented with *Saccharomyces cerevisiae* yeast strain Vinoferm Crio, the addition of inorganic or complex nutrients in musts during AF decreased significantly their concentration in a dose-dependent manner (Csutoras et al., 2022).

It was also observed that a high YAN concentration in the fermenting media can decrease the production of hexanoic acid and ethyl hexanoate, suggesting a strong influence of the used yeast strains on the production of this compound (Hernandez-Orte et al., 2006; Hernández-Orte et al., 2005).

Supplementation of musts with several amino acids (*β*-alanine, cysteine, arginine and valine) with doses which fall within the normal concentrations found in grapes, or slightly higher than those, was investigated to determine their potential role in CoA biosynthesis pathway in the *Saccharomyces cerevisiae*, after normalizing YAN concentration by addition of ammonium chloride (NH4Cl) (Boss et al., 2015). *β*-alanine, a pantothenate (B5 vitamin) precursor in the pathway of CoA biosynthesis, was the only amino acid shown to increase the production of MCFAs (hexanoic, octanoic and decanoic acids), MCFAEEs (hexanoate, octanoate and decanoate ethyl esters) and acetate esters (ethyl acetate, 2-phenylethyl acetate and isoamyl acetate) in the final wines (Boss et al., 2015). The stimulatory doses for the production of MCFAs and MCFAEEs were found to be around 1 mg/l, but the addition of more *β*-alanine, even up to 100 mg/l, does not significantly increase this production further (Boss et al., 2015).

The effect of pH

Organic acids of wines are generally derived from grapes, corrections made during processing or from microbiological activities occurring before, during or after AF, including in MLF (Chidi et al., 2018). Usually, organic acids determine basic wine parameters, such as titratable acidity and pH, which vary with the grape variety, harvesting time, vintage or terroirs. The pH of grape musts and wines range between 3.0 and 4.0 and frequently requires adjustments, especially in hot climates, due to its importance for wine microbial stability, flavour and aroma (Chidi et al., 2018). Grape musts which underwent pH adjustments in the range of 2.8 to 4.5 revealed an increased in production of MCFAs (expressed as sum of C_6+C_8) at higher pH values and a decline at lower values, while the C_8/C_6 acids ratio showed a decreasing trend with an increase in pH value (Takashi, 1986). Decanoic acid was not detected in pH-adjusted grape musts and therefore no trends are known for this acid (Takashi, 1986). Usually, the MCFAEEs production is correlated with the concentration of MCFAs precursors (Takashi, 1986). These esters are found to be relatively unstable in wines, especially in low pH wines which go through a storage period at elevated temperatures, which is favouring their hydrolysis (Ramey & Ough, 1980). Fermented grape musts with higher pH values tend to contain and preserve more MCFAEEs, due to an increased production of MCFAs during AF (Takashi, 1986) and of CoA, the hydrolysis being especially slowed down when the wine storage is short and done at low controlled temperatures (Ramey & Ough, 1980).

The effect of different yeast strains

The effect of yeast strain on MCFAs and MCFAEEs production is of much importance. A comparison of two yeast strains of *Saccharomyces cerevisiae* popular for white wine production in Australia, AWRI 796 and M05, revealed that the strain AWRI 796 produces 3 to 4 times more MCFAs than the strain M05 in the same conditions of nitrogen adjustments with inorganic nitrogen, when YAN levels ranged from 117 to 500 mg N/l (Vilanova et al., 2007). The production of hexanoic acid is strongly influenced by the *Saccharomyces cerevisiae* yeast strain and YAN levels. Hexanoic acid decreased in samples with high YAN and fermented with

AWRI 796 strain, while this trend was not observed for the strain M05 (Vilanova et al., 2007). In a similar study, some strains (Fermicru AR2 and Stellevin NT116) produced less ethyl hexanoate at high YAN levels, while others (LW LVCB) produced more of this compound (Hernandez-Orte et al., 2006; Hernández-Orte et al., 2005). It was suggested that the MCFAEEs synthesis is dependent on MCFAs substrate concentrations and that an increase in ethyl esters is expected when the MCFAs are added during the AF (Saerens et al., 2006). However, the addition of C_6 or C_8 fatty acids during AF using a wild type strain *BY4741* and constructed strains with overexpression of *BY4741+ pEHT1s*, *BY4741+ pEEB1s* and *BY4741+ pYMR210ws* increased the concentrations of the respective MCFAEEs but, no significant differences were found between the wild type strain and overexpression strains (Saerens et al., 2006).

Several studies conducted on different yeast strains and different levels of adjusted YAN, revealed that production rates of MCFAs and MCFAEEs are also strongly dependent on yeast strains, not only on YAN level (Hernandez-Orte et al., 2006; Hernández-Orte et al., 2005; Vilanova et al., 2007). Other yeasts, such as *Lachancea thermotolerans*, known to be low producers of MCFA and MCFAEEs, are used to avoid strong smells given by fatty acids during red wine production, improving the red wine aroma (Hranilovic et al., 2021; Shekhawat et al., 2017). In Table 1 are presented the ranks of odor activity values (rank 1 being the lowest and rank 6 the highest) for MCFAs and MCFAEEs in wines produced by mixed fermentations, using several non-*Saccharomyces* yeasts and *S. cerevisiae.* Compared to *S. cerevisiae*, the production of MCFAs increases in the case of non-*Saccharomyces* yeasts such as *Metschnikowia pulcherrima*, *Candida stella*, and *Pichia fermentans* (Liu et al., 2016). Opposite results were reported for *Hanseniaspora uvarum* and *Issatchenkia orientalis* in mixed fermentations (Hu et al., 2018; Liu et al., 2016).

Table 1. Ranks* of odor activity values (OAV) for MCFAs and MCFAEEs in wines produced by mixed fermentations with non-*Saccharomyces* plus *S. cerevisiae* compared with monoculture of *S. cerevisiae* (Liu et al., 2016)

Compounds	*Saccharomyces cerevisiae*	*Hanseniaspora uvarum* + *Saccharomyces cerevisiae*	*Metschnikowia pulcherrima* + *Saccharomyces cerevisiae*	*Candida stella* + *Saccharomyces cerevisiae*	*Pichia fermentans* + *Saccharomyces cerevisiae*	*Issatchenkia orientalis* + *Saccharomyces cerevisiae*
C6-MCFA	6	1	2	4	3	5
C8-MCFA	1	3	4	6	5	1
C10-MCFA	2	3	4	5	6	1
Total MCFA	**3**	**1**	**4**	**6**	**5**	**2**
C6-MCFAEE	4	1	3	5	6	2
C8-MCFAEE	3	1	4	6	5	2
C10-MCFAEE	5	1	4	2	6	3
C12-MCFAEE	4	1	2	6	5	3
Total MCFAEE	**4**	**1**	**3**	**5**	**6**	**2**

*Ranks are ascending, with 1 being the lowest and 6 the highest

The different metabolic pathways activated in yeasts and their effect on the production of MCFAs and MCFAEEs are also important for the red wine production, as these compounds can inhibit the growth of *O. oeni* and impair MLF (Balmaseda et al., 2018). The C_8-C_{14} MCFA can inhibit the growth of *O. oeni* and reduce the consumption of L-malic during MLF (Edwards & Beelman, 1987; Lonvaud-Funel et al., 1988). In mixed fermentations, time of yeast inoculation showed a strong influence on MCFA and MCFAEE production, as revealed in experiments with *H. uvarum* and *S. cerevisiae* (Hu et al., 2018). The increased MCFA concentration is, as expected, directly correlated to an increased MCFAEE production (Hu et al., 2018). Inoculation with *H. uvarum* with 48 h before *S. cerevisiae,* led to increased concentrations of MCFAs and MCFAEEs in wines, while simultaneously inoculation or earlier inoculation (96 h) of *H. uvarum* showed decreased concentrations of these compounds (Hu et al., 2018). The production of C_6-C_{10} saturated fatty acids were significantly different

among various yeasts species and strains tested on sterile filtered grape must, fermented at 20°C in hermetic vessels equipped with fermentation stoppers (Takashi, 1986). The yeast species and strains generally produce different total MCFA concentrations expressed as sum of C_6+C_8+C_{10} of fatty acids, even when they ferment the same media, in the same conditions. Since C_{10} content appears to be affected to a lesser extent, for a better characterization of the MCFA production by yeasts the C_6/C_8 ratio can be used (Takashi, 1986).

PROPERTIES OF MCFAs AND THEIR ETHYL ESTERS

Medium chain fatty acids (MCFAs) and their esters (MCFAEEs) are important volatile compounds, which contribute to sensorial profile of wine. The aroma descriptors of several MCFAs and MCFAEEs are presented in Table 2, along with other properties and their concentration range in wine. The MCFA aroma is generally of fatty type, while MCFAEE aroma is of fruity type (Liu et al., 2016), therefore higher concentrations of MCFAEEs and lower concentrations of MCFAs are desired in wines, but this is only partially obtainable, as the ethyl esters concentration is dependent on the concentration of MCFA, which act as precursors. The MCFAs and MCFAEEs are lipid soluble compounds, which can diffuse through the yeast membrane into the fermenting wine (Nykänen & Nykänen, 1977; Saerens et al., 2010). As compared to acetate esters, for which the excretion occurs rapidly and completely, the transfer of ethyl esters to fermentation media is slower and depends on the length of carbon chain, the bigger the chain the slower the transfer. Generally, the excretion of esters is reported to be 100% for C_6, 54-68% for C_8 and 8-17% for C_{10} (Nykänen & Nykänen, 1977; Saerens et al., 2010).

Table 2. Properties and concentrations of MCFA's and their ethyl esters in wines

Identification of compounds	Chemical properties	Sensorial descriptors	Odor threshold (*model wine), mg/l	Wine concentration range (average), mg/l
Medium-chain fatty acids (MCFAs)				
Hexanoic acid (C_6) (syn. caproic acid) CAS 142-62-1	**Solubility in water (25°C) g/l**[5] = 10.285 **pKa (25°C)**[1] = 4.880 **O:C ratio** = 0.44	goat-like, cheese, sour, fatty, sweat	[29]0.29; [8, 30]0.42; [9, 10, 25]3.00; [26]5.40	White wines: n.d.-25.08 (**3.640**)[12, 13, 14, 15, 16, 17] Red wines: 0.217-3.782 (**1.262**) [8, 21, 22, 23, 24]
Octanoic acid (C_8) (syn. caprylic acid) CAS 124-07-2	**Solubility in water (25°C) g/l**[6,7] = 0.73483 (Calculated from the solubilities at 20 and 30°C) **pKa (25°C)**[2]=4.895 **O:C ratio** = 0.33	cheese, sweat, soapy, waxy	[8, 30]0.50; [10, 25, 29]3.00; [26]5.80	White wines: n.d.-34.7 (**7.230**) [12, 13, 14, 15, 16, 17] Red wines: 0.194-14.536 (**2.181**) [8, 21, 22, 23, 24]
Decanoic acid (C_{10}) (syn. capric acid) CAS 334-48-5	**Solubility in water (25°C) g/l**[7] = 0.06184 **pKa (25°C)**[3] =4.900 **O:C ratio** = 0.27	citrus, sour, fatty, unpleasant, rancid, soapy	[8, 30]1.00; [26]3.50; [10, 25, 29]10.00; [9]15.00	White wines: n.d.-29.62 (**2.570**) [12, 13, 14, 15, 16, 17] Red wines: 0.039-0.857 (**0.290**) [8, 21, 22, 23, 24]
Dodecanoic acid (C_{12}) (syn. lauric acid) CAS 143-07-7	**Solubility in water (25°C) g/l**[7] =0.00324 **pKa (20°C)**[4]=5.300 **O:C ratio** = 0.22	dry, metallic, laurel oil flavour	[11]1.00; [26]10.00	White wines: n.d.-1.24 (**0.240**) [12, 13, 14, 15, 16, 17] Red wines: not found
Medium-chain fatty acid ethyl esters (MCFAEEs)				
Ethyl hexanoate (syn. ethyl caproate) CAS 123-66-0	**Solubility in water (25°C) g/l**[19, 20]= 0.6290 **O:C ratio** = 0.33	green apple, banana, wine, pineapple	[27, 28] 0.001; [9, 30]0.005; [8, 30]0.014; [30]0.023; [10]0.062	White wines: 0.0011-1.636 (**0.502**) [12, 13, 14, 15, 16, 17, 24] Red wines: 0.052-0.974 (**0.342**) [8, 21, 22, 23, 24]
Ethyl octanoate	**Solubility in water (25°C)**	pear,	[9, 30]0.002; [8,	White wines:

Identification of compounds	Chemical properties	Sensorial descriptors	Odor threshold (*model wine), mg/l	Wine concentration range (average), mg/l
(syn. ethyl caprylate) CAS 106-32-1	**g/l**[19, 20]= 0.0701 **O:C ratio** = 0.27	pineapple, floral, apricot	[30]0.005; [30]0.019; [29]0.070	0.0012-2.770 (**1.392**) [12, 13, 14, 15, 16, 17, 24, 33] Red wines: 0.034-0.783 (**0.318**) [8, 21, 22, 23, 24]
Ethyl decanoate (syn. ethyl caprate) CAS 110-38-3	**Solubility in water (25°C) g/l**[19, 20]= 0.0159 **O:C ratio** = 0.22	grape, pear, oily, sweet, waxy, fruity, apple, soapy, winey	[30]0.005; [29]0.122; [8, 30]0.200	White wines: 0.0014-2.800 (**0.557**) [12, 13, 14, 15, 16, 17, 24, 33] Red wines: 0.015-0.470 (**0.078**) [8, 21, 22, 23, 24]
Ethyl dodecanoate (syn. ethyl laurate) CAS 106-33-2	**Solubility in water (25°C) g/l**[19, 20]= 0.0004 **O:C ratio** = 0.19	pear, fruity, floral, leaf	[11]3.500; [32]5.900	White wines: 0.0002-0.202 (**0.042**) [14, 15, 16, 24, 33] Red wines: 0.006-0.037 (**0.018**) [8, 20, 21, 22, 23]

[1](Riddick et al., 1985); [2](Dean, 1987); [3](Barratt, 1996); [4](Serjeant & Dempsey, 1979); [5](Yalkowsky, 2010); [6](O'Neil, 2006); [7](Yalkowsky & Dannenfleser, 1992); [8](Ferreira et al., 2000) - *determined in 11% v/v aqueous ethanol with 7 g/L glycerol, 5 g/l tartaric acid and pH adjusted to 3.4 with 1M NaOH; [9](Guth, 1997) - *determined in 10% w/w aqueous ethanol; [10](Gemert, 1999) - *odour threshold values in water; [11](Sun & Liu, 2004); [12](Csutoras et al., 2022); [13](Li et al., 2008);[14](Kim et al., 2018); [15](Estévez et al., 2004); [16](Vázquez-Pateiro et al., 2020); [17](Vázquez-Pateiro et al., 2022); [18](HMDB, 2023); [19](FooDB, 2023); [20](Slaghenaufi et al., 2021); [21](Geffroy et al., 2020); [22](Zhao et al., 2017); [23](Noguerol-Pato et al., 2014); [24](Philipp et al., 2018); [25](Buttery et al., 1988); [26](Fazzalari, 1978); [27](Takeoka, 1990); [28](Siek et al., 1969); [29](Abilleira et al., 2010); [30](Giri et al., 2010); [31](Francis & Newton, 2005); [32](Lyu et al., 2019); [33](Rodríguez-Bencomo et al., 2002).

TOXICITY OF MCFAs ON WINE MICROORGANISMS

The effect of MCFA chain length and concentration

As already mentioned, the C_6, C_8, C_{10} and C_{12} saturated fatty acids are metabolites produced by yeasts during AF which act as inhibitors for yeasts and bacteria. The MCFA mixture (C_6, C_8 and C_{10}) in the fermentation media exerts a stronger inhibition than the one produced by single acids in the same concentration, meaning that the MCFAs in combinations have synergic inhibitory effect (Lonvaud-Funel et al., 1988).

The length of carbon chain was found to be crucial for yeast toxicity, the longer the chain, the more liposoluble the acid (Table 2). Liposolubility facilitates the entrance of the undissociated acid through membranes, the molecules more liposoluble becoming toxic at lower concentrations (Borrull et al., 2015). The passive diffusion through the cytoplasmic membrane of the MCFAs produced by the yeasts is inversely proportional to fatty acid chain length and thus the equilibrium between the MCFAs inside of a cell and the outside fermentation media is governed by this process (Bardi et al., 1999). The C_6 and C_8 concentrations are practically constant inside cells, while, the concentrations in fermentation media rise to very high concentrations during growing phase. For C_{10} was found to be relatively abundant both in the cell and in the fermentation media, and for C_{12} was found that it is present almost exclusively in the cell, due to the slow diffusion process (Bardi et al., 1999). This process revealed that MCFAs do not replace UFAs in the membrane to maintain its fluidity status (Bardi et al., 1999), but actually tend to remain blocked across the membrane when cell tries to excrete them. The result is an increase in membrane fluidity and an acidification of the cytosol by the organic acids which remain in the cell. This process explains why the inhibitory properties increase with the increase of chain length.

It was established that undissociated MCFAs with longer chains, C_{12} and C_{14}, are the most toxic for *Oenococcus oeni* and therefore may represent a serious problem for red winemaking, where starting MLF is necessary (Guilloux-Benatier et al., 1998). However, the esterified forms (MCFAEEs) of C_{10}, C_{12}, and C_{14} are found to be even more toxic than the free fatty acids for *Oenococcus oeni* (Guilloux-Benatier et al., 1998). The concentrations of octanoic acid up to 16 mg/l and decanoic acid up to 8 mg/l increased the duration of growth latency and decreased exponentially the maximum specific growth rate and the biomass yield in the presence of 12 and 14% v/v ethanol (Viegas & Sá-Correia, 1997). An increased

toxic effect of these tested concentrations was observed with the pH decrease and with the temperature increase (Viegas & Sá-Correia, 1997). The concentrations of decanoic acid between 5 and 10 mg/l inhibited the growth of *Oenococcus oeni,* while doses of 30 mg/l induced rapid bacterial death (Edwards & Beelman, 1987). In another study (Lonvaud-Funel et al., 1988), 4 mg/l decanoic acid or 0.5 mg/l dodecanoic acid were able to inhibit MLF when independently tested. Therefore, it is clear that both the concentration and the carbon chain length of the used MCFA determines the extent of the inhibitory/lethal effect on yeasts and bacteria (Borrull et al., 2015; Edwards & Beelman, 1987; Viegas & Sá-Correia, 1997). Interestingly, for lactic bacteria, decanoic acid up to 12.5 mg/l and dodecanoic acid up to 2.5 mg/l were found to act as growth factors in the presence of low ethanol concentration (4% v/v) in synthetic media with pH=4.5 (higher pH in wine), stimulating their growth activity; however, at higher concentrations the same acids exerted an inhibitory effect (Capucho & San Romão, 1994). Due to the interaction between various stress-inducing factors, such as ethanol concentration and pH of the growth media, the evaluation and prediction of MCFA inhibitory effect is more difficult than expected. Yeasts can tolerate higher concentrations of MCFA. For *Saccharomyces cerevisiae* BY4742 grown in a medium with pH=5.8 and ethanol under 0.5% v/v the non-inhibitory concentrations (NIC) were 35 mg/l for C_8 and 25 mg/l for C_{10}, while the minimum inhibitory concentrations (MIC) were 125 mg/l for C_8 and 80 mg/l for C_{10}, respectively. As expected, C_{10} is more toxic than C_8. Compared to a control sample, the viability of *S. cerevisiae* BY4742 in the presence of MCFA was reduced to 54.9% for C_8 and 42.3% for C_{10} at NIC and to 14.7% for C_8 and 6.5% for C_{10} at MIC. However, for this strain cultivated in YPD media incubated overnight semi-anaerobically at 28°C and pH 5.8, as compared to the acids used independently, the combination of C_8+C_{10} fatty acids led to an increased vitality at NIC and a decreased one at MIC levels, showing that for the combination of C_8+C_{10} the effect is not additive, but synergic (Borrull et al., 2015). Similarly, the inhibitory effect of MCFAs on yeast growth studied through a calorimetric technique was also determined to be correlated with the chain length of MCFA. At pH=5.5 and ethanol concentration of 0.66% v/v the 50% inhibitory concentrations for two anaerobic yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) and two aerobic yeasts (*Candida utilis* and *Kluyveromyces marxianus*) ranged between 62 to 81 mg/l C_8 acid and from 30 to 86 mg/l for C_{10} acid. The determined MIC values ranged between 159 to 239 mg/l for C_8 acid and between 77 to 127 mg/l C_{10} acid (Antoce et al., 1998; Antoce et al., 1997).

The effect of ethanol on MCFA inhibitory properties

Ethanol, an important component in wine, has its own antimicrobial properties, but together with other compounds the effect can synergistically increase. A 10 to 80 mg/l of an MCFA mixture (C_8:C_{10}:C_{12} in 2:7:1 ratio dissolved in 70% v/v ethanol) was tested at pH=3.5 on several yeast species (*S. cerevisiae*, *S. uvarum*, *Starmerella bacillaris* and *Zygosaccharomyces bailii*). The study showed that at low ethanol concentrations of about 5% v./v., without added SO_2, the added MCFA mixture, even at the highest dose applied, was not enough to completely inhibit the growth of *S. cerevisiae* and *Zygosaccharomyces bailii* (Horváth et al., 2020). In the same study, an attempt was made to prevent the refermentation of Tokaj Essence (2.32% v/v alcohol; 0/38 mg/l free/total SO_2 and 54% w./w. total soluble solids) by adding 40 mg/l MCFA mixture and 100 mg/l SO_2 and incubating it 15°C. The test also revealed that in the absence of higher amounts of ethanol, the viable yeast cell numbers remained of about 10 CFU/ml, which is considered a non-acceptable risk of refermentation for a bottled wine. The study concluded that the application of MCFAs to control the growth of microorganisms should only be used when significant amounts of ethanol are also present (Horváth et al., 2020). In the presence of increasing levels of ethanol, the inhibitory effect of decanoic acid (Antoce et al., 1998) or of other organic acids is synergistically increased (Antoce et al., 1997). Thus, it was proven that high ethanol concentrations enhance the inhibitory properties of MCFAs (Antoce et al., 1998;

Antoce et al., 1997), while in low ethanol products a reduced toxicity of MCFAs is observed (Horváth et al., 2020).

In the presence of MCFAs, the tolerance of yeast to ethanol is also reduced. A good ethanol tolerance is dependent on the composition of cell membrane, which should contain more lipids with higher degree of unsaturation (UFAs). For this reason, yeasts adapt continuously to the content of ethanol produced during fermentation (Arneborg et al., 1995; Costa & Moradas-Ferreira, 2001; Piper, 1995). If these lipids are not present in the fermentation media, for the synthesis of UFAs and ergosterol, oxygen exposure is required at the end of the exponential phase of growth (Chen, 1980; Ding et al., 2010; Lafon-Lafourcade et al., 1979). Another observation which supports this theory of ethanol tolerance being dependent on the yeast membrane composition is that the presence of high ethanol concentrations during the mid or the end of AF leads to an increase in the fluidity of yeast membranes; meanwhile, the ergosterol antagonizes the effect of ethanol by increasing the rigidity of membranes (Ding et al., 2009).

The effect of pH on MCFA inhibitory properties

The pH of wines range between 2.8 and 4.2 and usually, the growth of *Saccharomyces cerevisiae* is not affected in this range, but lower values of the pH may affect their growth and AF.

The pH becomes harmful to microorganisms at the lower values of the scale (2.8-3.2), especially combined with high ethanol concentrations (Pampulha & Loureiro-Dias, 1989; 1990). The undissociated forms of organic acids are present in higher percentages at low pHs and the influence of ethanol on membrane fluidity increases the toxicity, allowing passive diffusion across the weakened membrane. One of the mechanisms that explains the cell death relys on the cytosol acidification through passing of undissociated MCFA across the cell membranes into the neutral cytoplasm by means of passive diffusion (Viegas & Sá-Correia, 1997). Upon entering the cells, these acids start to dissociate in cytosol, as their pKa is relatively low compared to the cytosol pH, contributing to a decrease of the internal pH (Capucho & San Romão, 1994; Pampulha & Loureiro-Dias, 1989; 1990). As a result, the acidification of the cytosol can lead to sluggish or stuck fermentation. In fermentation media the toxicity of MCFAs was proven to be increased when the pH is decreasing (Antoce et al., 1998). Potassium ions in the fermentation media can counteract the harmful effect of acidic pHs, increasing the tolerance of yeasts (Kudo et al., 1998).

The MCFAs are found undissociated in the musts or wines with low pH, as shown in the Figure 1. Even at higher pH values, of up to 4.2, their undissociated forms represent 80-95%. This mechanism of passive diffusion of the undissociated forms of acidic compounds is often exploited in oenology to produce antimicrobial effects, being valid for various acidic preservatives, including sulphites.

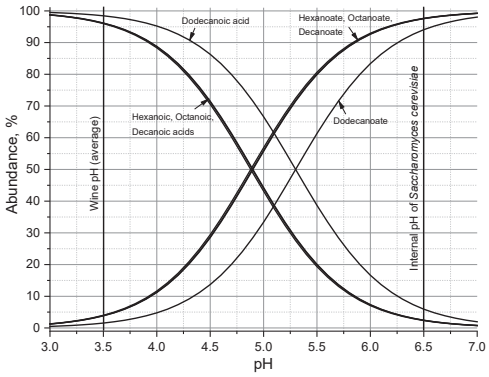

Figure 1. Dissociation of MCFA depending on pH at 25°C

The effect of temperature on MCFA inhibitory properties

Temperature of growth media enhances the inhibitory effect of MCFAs especially during the mid or the end of AF, due to increased ethanol accumulation and increased fluidity of cell membranes (Ding et al., 2009). The lack of oxygen at the end of exponential growth phase of yeasts or absence of ergosterol and UFAs in fermentation media weaken yeast membranes, which are prone to stress factors (Costa & Moradas-Ferreira, 2001; Lafon-Lafourcade et al., 1979; Piper, 1995). During white or rose wine production, where lower temperatures are implemented during AF, the viability for *Saccharomyces cerevisiae* is expected to be not

as affected as in the case of higher temperatures (Viegas & Sá-Correia, 1997), but actually, the overall loss of viability is higher, due to other conditions created in these winemaking processes, such over-clarified musts, anaerobic fermenting media, etc. Some interventions are possible to use to counteract the loss of viability, as mentioned before, stimulating the incorporation in the yeast membrane of ergosterol and UFAs (Chen, 1980) from the nutrients added at rehydration. During red wine production, where AF of must takes place on grape skins, higher temperatures are required to facilitated the extraction of pigments and tannins, and, as a consequence, the viability of *Saccharomyces cerevisiae* is expected to decrease (Viegas & Sá-Correia, 1997), but actually the loss of viability is here counteracted by the other conditions used in red winemaking, such as the presence of UFAs from the contact with the grape fragments, the presence of higher levels of oxygen introduced during pumping-over etc. Thus, the effect of temperature and modulation of yeast cell viability should be considered in connection with the other factors during winemaking.

Yeast adaptation and resistance to MCFA toxicity

Generally, yeasts adaptation and improvement in resistance to inhibitors mainly involves cell detoxification through transmembrane transporters, aiming to excrete the toxic compounds out of the cell, but other mechanisms are not excluded (Balzi & Goffeau, 1995; Goffeau et al., 1997). The cells detoxification is rather a complex process, including many, even overlapping, pathways.

The detoxification mechanisms for MCFA, could include plasma membrane H^+-ATPase induction, MCFAEEs production, activation of a membrane transporters for the MCFA corresponding anions or activation of beta-oxidation pathway in peroxisomes (Borrull et al., 2015; Cabral et al., 2001; Legras et al., 2010).

Weak acids could induce plasma membrane H^+-ATPase transporters (proton pump). Through this, under mild stress, *Saccharomyces cerevisiae* regulates the cytosol pH. The exposure of yeast cells to C_8 fatty acid or to ethanol was found to induce this transporter to translocate protons out of the cytosol, but an octanoate active transporter from the cytosol to the surrounding medium has not been identified (Cabral et al., 2001). However, in case of a rapid exposure to C_8 fatty acid, the unadapted cells of *Saccharomyces cerevisiae* are able to activate *de novo* protein synthesis and produce a plasma membrane transporter, which mediates the active efflux of octanoate out of the cell (Cabral et al., 2001).

For octanoic acid another transporter was also found in yeasts. The induction of Pdr12p transporter confers yeast resistance to several weak acids, including sorbic and benzoic acids, allowing the expulsion of the anions from the cytosol (Holyoak et al., 1999; Piper et al., 1998). This transporter was initially found to work on monocarboxylic acids with an aliphatic chain shorter than C_7 (Holyoak et al., 1999), but later was discovered that Pdr12p transporter may be induced for C_8 acid expulsion as well (Legras et al., 2010), because the length of C_8 acid is relatively close to the one of short-chain organic acids and induces a similar response.

Esterification of MCFAs to MCFAEEs could be considered a possible yeast detoxification metabolism, which requires the expression of *EHT1* and *EEB1* genes, but also combined with *ATF2*, which is expressed under anaerobic conditions and is known for its role in the acetylation of sterols (Cauet et al., 1999; Saerens et al., 2010). *EEB1* gene expression responds differently to the supplementation of C_8 or C_{10} fatty acids during AF. An increased expression of *EEB1* gene was observed when C_8 acid supplemented into media, while this was not observed for C_{10} acid (Legras et al., 2010). This could explain why in wines C_8 ethyl ester is found in higher concentrations as compared with C_{10} ethyl ester (as also shown in Table 1). This metabolic route could contribute more for the C_8 detoxification, than to C_{10} or other longer chain fatty acids.

A major role for resistance to C_{10} fatty acid, which contributes also to C_8 resistance, was attributed to Tpo1p carrier protein, known to protect cells against various inhibitory molecules. Due to this mechanism, it was observed that a high level of C_{10} acid resistance in yeasts is always associated with a high level of C_8 acid resistance (Legras et al., 2010).

Another possible response to improve the yeast resistance against MCFAs could be the membrane adaptation through the induction of Pdr16p transporter (Legras et al., 2010). However, the expulsion of C_8 and C_{10} fatty acids, along with that of many other inhibitors, is mainly determined by the two transporters discussed previously, Tpo1p and Pdr12p. These two transporters represent the main elements of the yeasts resistance to MCFAs (Legras et al., 2010).

Finally, the adaptation of yeasts to MCFAs could involve an oxidative stress response, similar to that observed for other inhibitors, which implies the activation of β-oxidation pathway in peroxisomes. The MCFAs are broken down to other non-inhibitory molecules with the formation of hydrogen peroxide, which subsequently is converted by catalase into water and oxygen, to protect the cells from reactive oxygen species (Borrull et al., 2015; Legras et al., 2010).

CONCLUSIONS

The production of MCFAs is triggered by an anaerobic environment and is generally enhanced at higher temperatures during AF. Higher concentrations of MCFAs in fermentation media, in the composition of yeast membranes or in cytosol can become toxic during the middle or the end phase of AF. The toxicity is especially manifest in the case of white and rose winemaking, where an anaerobic environment and low pH are technologically preferred, which coupled with the increasing concentration of ethanol, can lead to slow or stuck fermentative processes. The red winemaking is generally less problematic as far as the MCFA toxicity is concerned, because it is partially countered by the contact of fermenting must with the grape solids containing UFAs and by the higher dissolved oxygen introduced during cap management, especially by means of pump over. To support this, the studies showed that the fermentation on skins of Cabernet Sauvignon produced less MCFA then the clarified must of the same grapes, under the same conditions (Guilloux-Benatier et al., 1998; Takashi, 1986). Under certain circumstances, such as those for red fruity wine production, where the maceration is shorter and oxygen level is lower during the fermentation, MCFA concentrations are generally higher and may inhibit the activity of lactic acid bacteria and, consequently, the onset of MLF. The yeast strains differ very much in their abilities to produce MCFAs and MCFAEEs, their response being variable depending not only on their genetic characteristics, but also on the intrinsic and extrinsic factors affecting the fermentation environment and fermentative performance. The strains proposed for red wine production are generally selected in order to produce as few as possible MCFAs during AF, so that they will not impair later on the development of lactic bacteria and onset of MLF. In white wines, yeasts which produce moderate MCFAs and MCFAEEs can be interesting for the final fruity aroma of wines. In the case of high levels of MCFAs in the must, the esterification of MCFAs during AF can be considered a defence mechanism developed by yeast to protect themselves against the toxicity of medium-chain saturated fatty acids, but other overlapping mechanisms, such as induction of several membrane transporters to excrete the acids or the protons from the cells or β-oxidation pathway for MCFA degradation can also be involved for cell adaptation and resistance.

Knowing all these complex mechanisms and depending on what kind of effects the winemaker wants to trigger in relation to the MCFA production or toxicity, various types of yeast metabolism modulation are, within limits, possible.

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