Isolation of *Saccharomyces cerevisiae* yeast strains from Macedonian "Tikveš" wine-growing region and their impact on the organoleptic characteristics of Vranec and Cabernet Sauvignon wines

Ilieva F.[1,2], Kostadinović Veličkovska S.[2]*, Dimovska V.[2], Mirhosseini H.[3] and Spasov H.[1]
1. Department of Wine and Beer Technology, University of Food Technologies, Marica 26, Plovdiv, BULGARIA
2. Faculty of Agriculture, University "Goce Delčev", Krste Misirkov bb, 2000 Štip, MACEDONIA
3. Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Selangor, MALAYSIA
*sanja.kostadinovik@ugd.edu.mk

Abstract

Isolation of autochthonous yeast strains from 15 microregions from Tikveš winegrowing region of Macedonia was first object of this study. Furthermore, morphological and physiological characterization of yeast strains were performed in order to reveal small species diversity belonging to Saccharomyces cerevisiae genera. Moreover, this study estimated of impact of the isolated yeasts on the organoleptic profile of wines produced from Vranec and Cabernet Sauvignon grape variety.

Our study confirmed improved organoleptic characteristics of wines produced from Vranec and Cabernet Sauvignon grape variety fermented by autochthonous yeast strains from second stage of selection in comparison to commercial yeast strain SiHa. Oeno-chemical parameters obtained from semi-industrial production of Vranec wine fermented by third-stage selected yeast strain can be used as general recommendation for specific regional wines production.

Keywords: *Saccharomyces cerevisiae*, wine-making, organoleptic quality, isolation of yeast strains, Vranec wines, Cabernet Sauvignon wines.

Introduction

A holistic multi-sensory perception of flavor was essential for understanding the body of the wine.[1] Experimental wines produced from few widely available tropical fruits were examined with the objective of comparing the fermentation efficiency (along with progress in fermentation) of two efficient yeast isolates with commercially available strain.[2] The use of indigenous starters, selected among yeast biota of specific environments, was proposed as a tool for production of premium quality wines possessing characteristics correlated to the specific production environment.[3]

Isolation and characterization of yeast strains from twelve traditional sourdough samples which belonged to Black Sea and Aegean regions of Turkey, were performed by 26S rDNA sequencing and FTIR spectroscopy. *Saccharomyces cerevisiae* (50%), *Torulaspora delbrueckii* (40%) and *Kluyveromyces marxianus* (10%) were found in 12 Turkish traditional sourdough samples. *S. cerevisiae* was found to be the most dominant species in Aegean region while *T. delbrueckii* was the most frequently isolated species in Black Sea region.[4]

Investigation of *Saccharomyces* and non-*Saccharomyces* yeast strains from grape/must/wine environments including several yeast genera (e.g. *Saccharomyces*, *Hanseniaspora*, *Pichia*, *Candida*, *Metschnikowia*, *Kluyveromyces*, *Zygosaccharomyces*, *Torulaspora*, *Dekkera* and *Schizosaccharomyces*) and species was evaluated.[5] Nevertheless, an attempt was made to determine the anthocyanin profile in the wines of each vintage according to the varietal fingerprint.[6] Five autochthonous *Saccharomyces cerevisiae* strains (XG1, XG2, XG3, XG4 and XG5) were used for winemaking of Treixadura wines. According to results obtained from trained panelists, significant improvement of chemical and organoleptic profile of Treixadura wine fermented by XG4 yeast was observed.[7]

In the work of Sidari et al[8], the effect of polyphenols on the ability of natural isolates of wine-related *Saccharomyces cerevisiae* strains to form biofilms attaching to plastic surfaces, to grow as mat colonies, to invade media and to display filamentous growth has been studied. Eight *Saccharomyces cerevisiae* isolated from Yuma vineyard in China were used for improving the color and anthocynin profile of Cabernet Sauvignon wines.[9]

Regarding the organoleptic profile of wines, the working group of Avizcuri et al[10], explained that wines with higher total phenolic index had a lower evolution, whereas wines with lower total phenolic index showed a higher evolution and greater variability in behavior. In the work of Varela et al[11], wines fermented by *S. cerevisiae* were compared with those produced with M. pulcherrima. Their results indicated higher concentrations of ethyl acetate, total esters, total higher alcohols and total sulfur compounds, while wines fermented with *S. uvarum* were characterized by the highest total concentration of higher alcohols.

The non-*Saccharomyces* yeasts used were *Lachancea thermotolerans*, *Metschnikowia pulcherrima* and

Torulaspora delbrueckii in sequential fermentation with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Use of *Lachancea thermotolerans* led to larger amounts of polymeric pigments in sequential fermentation. The species *Metschnikowia pulcherrima* produced higher concentration of esters and total volatile compounds.[12] In addition, the effect of mixed fermentation of non-*Saccharomyces* (Torulaspora delbrueckii Zymaflore Alpha TD n. Sacch and Metschnikowia pulcherrima JS22) and *Saccharomyces cerevisiae* yeasts (D254 and EC1118) on the production of cherry wines, in comparison with commonly used monoculture was studied.[13]

The results from the experiments of Lick et al[14], indicated that label colors have a strong influence on flavor expectations. Quantification of subthreshold concentrations of 2-methylbutyl acetate in wines explained modification on the perception of fruity aromas in the matrices of wines as black, fresh and jammy-fruit notes.[15]

Furthermore, higher levels of three volatiles, in particular ethyl propanoate, ethyl isobutyrate and ethyl dihydrocinnamate, may explain the relation of wine flavour with "red and dry fruits".[16] However, increased abundance of 2-phenyl acetate and isobutyl acetate in wines can be related with "stone fruit" nuances.[17]

The impact of autochthonous yeast strains on the monomeric anthocyanins and total phenolic content of Vranec and Cabernet Sauvignon grape varieties was object of study of Ilieva et al[18,19] The main objectives of this study were isolation, morphological and physiological characterization of *Saccharomyces cerevisiae* yeast strains from 15 microregions of Tikveš wine-growing region of Macedonia as well as their impact of the organoleptic profile of wines produced from Vranec and Cabernet Sauvignon grape variety. Finally, the semi-industrial production of wine fermented by third selected yeast strain F-78 from Vranec grape variety was made in order to initiate specific regional wines production.

Material and Methods

Grapes from Vranec and Cabernet Sauvignon grape variety for spontaneous fermentation: The grapes from Vranec and Cabernet Sauvignon grape variety were harvested at optimal maturity. The sugar content, acidity and pH of crushed grapes for spontaneous fermentation were measured in "Popova Kula" vinery, Demir Kapija (Table 1). The concentration of malic acid was in the range from 0.65 to 0.80 g/L. Titratable acidity was in concentrations between 3.95 and 7.67 g/L while pH range was from 3.32 to 3.63. The percentage of sugar content gave indication that grapes from both varieties are harvested at optimal maturity (21-24%).

Determination of oenochemical parameters of wines obtained by spontaneous fermentation of grapes from Vranec and Cabernet Sauvignon grape variety: Alcohol content, residual sugars, pH, titratable and volatile acids, monomeric anthocyanins and color intensity (IC) of experimental wines obtained from spontaneous fermentation of crushed grapes were determined (Table 2).

Determination of the amount of alcohol was performed ebuliometrically with Dujardin – Salleron ebuliometer and for determination of reducing sugars, the Schoorle's method was used. Determination of titratable and volatile acidity of trial wines was performed by methods of Boulton.[20] Studying the fermentation activity of yeasts from the first stage and second stage of selection was explained in the work of Ilieva et al.[19]

Determination of color characteristics was performed spectrophotometrically by measuring the absorbance of the wines at 420 nm (yellow color), 520 nm (red color) and 620 nm (blue color) with UV spectrophotometer Shimadzu 1800, Shimadzu Corporation, Kyoto, Japan.[21]

Morphological and physiological properties of newly isolated yeast strains: Morphological characterization of the selected yeast strains was performed; the spore formation was studied and fermentation and sugar assimilation were studied.

The sugar fermentation was determined by plating of each examined strain in the mixture of 0.6% yeast extract solution with adding of 0.2% of carbohydrates: glucose, fructose, galactose, maltose, saccharose and 0.4% rafinose. After five days cultivating in thermostat at 25°C, we determined which carbohydrates were assimilated and which were not metabolized by using paper chromatography (Fig. 2).

For determination of the ability of the strains to assimilate carbohydrates, the BioMerieux API 20C AUX kit was used. The kit included the following carbon sources (C): Glu-D-Glucose, Gly-Glycerol, 2KG-Calcium 2 keto-Gluconate, Ara-L-arabinose, Xyl-D-xylose, Ado-Adonitol, XLT-xylitol, Gal-D-Galactose, Ino-inositol, Sor-D-Sorbitol, MDG-Methyl-a-Glucopyranoside, Nag-N-acetyl-Glycosamine, Cel-D-celiobiose, Lac-D-Lactose, Mal-D-Maltoze, Sac-D-Saccharose, Tre-D-Trehalose, MLZ-D-Melezitose and Raf-D-Rafonise.

Biomass from single fresh colony was suspended in saline solution. After first homogenization, 0.1 mL was added to mixture "C", from the kit, which contains nitrogen derivatives, vitamins, growth factors, but no carbohydrate sources. After second homogenization, the mixture was inserted in the gaps of the strip at 25ºC. The mixture was observed on the 24th, the 48th and 72nd hour and the turbidity of the liquid in the gap was evaluated according to the control gap (without "C" source) while turbidity of the liquid in the gaps gives information that the relevant carbon source was assimilated. Based on the summarized data of the results at this point, the partial identification of the strains was made.

Sensory analyses of the organoleptic profile of Vranec and Cabernet Sauvignon wines: All the tasting sessions took place in a specific room equipped with individual booths air-conditioned at 20 ºC. Normalized glasses were used. All wines were tasted nine times by 10 trained sensory panelists in ages between 40 and 55 (3 females/7 males). The panelists were selected on the bases of their past experience in winemaking and tasting the flavor of red wines. For purpose of this experiment, they were trained over the period of 45 days to assess wine aroma using AROXATM Uno Wine Flavour Standard kit (supplied by AROXA, Leatherhead Surrey, UK).

The AROXATM Uno Wine Flavour Standard kit was composed of ten different wine flavors (dimethyl sulfide, 4-ethyl phenol, ethyl phenylacetate, hydrogen sulfide, β-ionone, isovaleric acid, 2-isobutyl-3-methoxypyrazine, sotolon, 2,4,6-trichloroanisol and vanillin). The training was carried out twice per week for 30 min. In this study, panelists were asked to describe wine aroma with their own vocabulary.

Statistical analyses: A one – way ANOVA was used to examine the impact of every selected yeast strains on the level of monomeric anthocyanins, total phenolic compounds, reducing sugars, pH, volatile and titratable acids, residual and total SO_2. The level of significance in differences between anthocyanin content and total phenolic content was determined by 5% by a one-way ANOVA using Tukey's test. The results from statistical analyses were classified using letters (different letters means significant differences among results). The letters are a,b,c and d according to the decrease of the result values. SPSS v.16.0 software, IBM corporation, USA was used for the applied statistical treatment.

Results and Discussion

Isolation of yeast strains from wines produced by spontaneous fermentation from Tikveš grape-wine region: For the isolation of yeast strains, we used ten lots of Vranec and five lots of Cabernet Sauvignon grapes, selected from 15 viticulture microregions in the Tikveš region. We considered that the wider range of microregions from which grape samples are taken, will increase the probability of isolation wine yeasts with different properties and that will, in turn, increase the opportunities of selection of specific and varietal local strains. Some of the parameters of the crushed grapes are represented in table 1. We can notice that all the grape lots are in technological ripeness as sugar content varied between 21.0 and 24.0 %. Values of titratable acidity and malic acid vary in relatively narrow ranges depending on the microregion and the condition of the grapes. pH to the crushed grapes varied from 3.32 to 3.62 (Table 1).

Some oenochemical parameters given in table 2 were measured from wines after spontaneous alcoholic fermentation. The lowest level of monomeric anthocyanins was determined in wine V2 produced from Cabernet Sauvignon gape variety spontaneously fermented from "Barovo" microregion (380±42 mg/L). However, this wine had higher color intensity than wine V4 produced from Vranec grape variety from "Cuculka" microregion. Although the level of monomeric anthocyanins in wine V4 was 584±21 mg/L, this wine had the lowest color intensity (13.3±0.13 a.u.). The working group of Kostadinović stated that polymeric colored compounds are more responsible for the color intensity of wine than monomeric anthocyanins.[22]

Furthermore, the highest levels of monomeric anthocyanins were detected in wine V8 produced from Vranec grape variety from "Beli Kamenja" microregion. Consequently, this wine had the highest value for color intensity as wines from "Barovo" and "Gorna Laka" microregions (1.21±0.02 a.u.). This can be related to the fact that Vranec grape variety had higher amount of colored pigments in comparison to Cabernet Sauvignon grape variety.[23]

As we can see from the data presented in table 2, the lots from Vranec and Cabernet Sauvignon fermented similarly. Fermentation process starts, although weakly, around the 24th hour without significant delay. This fact can be due to low doses of sulfur dioxide with the purpose of allowing the faster start of fermentation and avoiding accumulation of hydrogen-sulfide. On the other hand, for samples in which the doses of sulfur dioxide were increased (50-80 mg/kg), the start of the fermentation process was delayed after 48-72 hours. Most of the fermentable sugars were broken between the 2nd and the 5th day which resulted ending of fermentation process by the 9th or 10th day, when dry matter content varied between 6.0 and 7.0 %, typically for dry wine. For the wine V5 "Vranec-Bunishte", we noticed delay in the start and in first stages of fermentation, which we attributed to the higher osmotic pressure.

After the 4th day, however, wine V5 reached the fermentation stages of other lots from other microregions, which gave us reason to suppose the presence of osmotolerant yeasts in this sample. Values of reducing sugars did not exceed 4 g/L, which indicated the end of alcoholic fermentation. Alcohol concentrations varied between 11 and 13 % which corresponded to the grape's initial sugar contents. This amount of alcohol in wines might be due to the good activity of yeasts and high effectiveness of the fermentation process. Values of titratable acidity varied within certain limits and volatile acidity was in normal ranges. The concentrations lower than 0.6 g/L can be indication for clean fermentation without the participation of harmful yeast or bacterial microflora.

Generally speaking, values for color intensity IC and monomer anthocyanins in Vranec wines were expectedly higher than those produced from Cabernet Sauvignon grape variety.[22,23] Organoleptic evaluation of the wines obtained around 20 days after the end of the process, for both grape varieties, did not show reduction in the intensity of the color.

That suggests β-glycosidase activity of isolated yeasts without significant reduction of the wine color.

Using sterile grape juice, we isolated 80 new *S. cerevisiae* yeast strains from the experimental wines by Koch's method.[18] The wines, from which we isolated new *S. cerevisiae* strains, were of bright ruby colors, especially those from Vranec grape variety.[19] According to the investigation of Perrone et al[24] in 35.5% of the combinations of *S. cerevisiae* presented in their study, one of the two strains of *S. cerevisiae* died off or decreased in cellular density so much that it was undetectable in the mixed fermentations. Opposite competition did not take place when the same strains were separated and did not compete for space, even when they shared the same fermenting.

In addition, the method of Morales et al[25], allowed monitoring of 141 compounds throughout the process of fermentation by *Saccharomyces cerevisiae* and *Lachancea thermotolerans* strains. Both strains showed a similar ability to ferment a must with high sugar content. The *S. cerevisiae* strain produced higher amounts of volatile compounds especially esters that constitute fruity aroma than *L.* thermotorelans. We partially agree with this statement since wines produced by autochthonous yeast strains had higher intensity of fruity flavor in comparison with control wine produced by commercial strain SiHa.

Morphological and physiological properties of the second stage selected strains: The morphological characteristics of the studied yeast strains were applied. All cultures have similar morphology with oval shaped cells to slightly elliptical for all strains (Fig. 1). There is a difference in cultures uniformity, for example strains F-8 and F-70 have uniform cells while for F-4 and F-78 we observed more pronounced polymorphism since 20 to 25 % of the cells were smaller than usual size of yeast cells varying between 3-5 and 6-9 µm. Figure 1 (a, b and c) presents microscopic photos of some studied yeast strains.

Studying the sporogenesis, we found out that all strains produce spores during a period of 7-8 days. There is a difference in sporulation degree in different variants. For example, in case of strain F-78 there were 4-5 cells in sporogenesis in one field of view. On the other hand, for strain F-8 we observed one cell producing spores in each 8-10 fields of view. Microscopic photo of cells producing spores is presented in figure 1c and of more active cells in figure 1b.

Studying some physiological characteristics, we determined the ability of strains to ferment sugars. All 10 strains were studied in second stage of selection fermented glucose, fructose, galactose, saccharose, maltose and 1/3 raffinose. Paper chromatogram of few newly isolated yeast strains is presented in fig. 2. Some strains assimilated maltose slowly (F-8, F-39, F-46), other poorly absorb inositol (F-17, F-57, F-78) and F-8, F-20, F-39 and F-42 assimilated cellobiose in small quantities.

Based on the general results of the morphological and some physiological properties of studied strains selected in the second stage of selection, using the descriptions of standard cultures, it was likely to relate all strains to the species *Saccharomyces cerevisiae*.

The results of the working group of Borrull et al[26], indicated that some physiological parameters played a decisive role with respect to reaching the maximum pressure of 6 bars in bottles. These parameters were higher glycogen and trehalose contents, lower ROS accumulation, better vacuolar activity and lower ratios of ergosterol/squalene and oleic acid/stearic acid. Sluggish fermentations were more pronounced when *S. cerevisiae* was inoculated 24 h after the initial stage of fermentation with a NS strain compared to co-inoculation.[27]

Acidity and bitterness, two sensory descriptors, appear to be related to wines produced with pre-fermentative cold soak carried out at 14 ± 1 °C in the study of Maturano et al.[28] This fact could be associated with the increase in non-Saccharomyces during the pre-fermentation stage. Their results emphasized the importance of the temperature as a determinant factor to allow an increase in non-Saccharomyces population during pre-fermentative cold soak and consequently to modify sensorial attributes of wines as well as their sensorial impact.

The impact of newly isolated yeast strains on the organoleptic profile of Vranec and Cabernet Sauvignon wines: Results from color intensity and tonality of wines produced from Vranec and Cabernet grape varieties fermented by ten yeast strains from second stage of selection are presented in table 3. Generally speaking, Vranec wines had the higher values for color intensity than wines produced from Cabernet Sauvignon grape varieties which can be due to the higher level of colored pigments in Vranec than Cabernet Sauvignon grape variety.[22] There is no significant difference among the type of yeasts.

The percentage of the yellow (A_{420}), red (A_{520}) and blue (A_{620}) colors in the total color intensity (CI) of the wine varies slightly and remains normal for young red wines. The values of yellow color vary between 2.2 and 3.6 a.u., red color from 6.6 to 7.4 a.u. and blue color varied between 0.8 and 1.3 a.u. (Table 3). The lowest values of the yellow color were measured in Vranec wines V1 and V7 fermented by yeast strains F-4 and F-46. These samples also have the highest red color values (7 and 7.4 a.u. respectively) which corresponds to bright ruby-red color. There is no statistical significant values for blue color for almost all samples except for Cabernet Sauvignon wine CS9 produced with yeast F-70 and Vranec wine V5 produced with yeast strain F-39.

However, color of the wine is one of the most important organoleptic characteristics which significantly affects the overall impression for the quality of wine. Working group of Lick et al[14] noticed that red and black color are most likely to create tangy flavour expectations, red and orange are most associated with fruity and flowery flavours. In addition, it appears that frequent buyers have stronger expectations than infrequent buyers with respect to most of the colours analyzed. The wide range of phenotypic expression and variety of grapes observed could have a role in selection of strains suitable for inoculated wine fermentations and may explain the persistence of yeast strains in vineyard and winery environments.[8,29]

The tasting notes of the trial wines were held and the results from the spider diagrams were presented in fig. 3. During the preparation of the trials, spider diagrams showed a color, flavor and mostly mouthfeel characteristics. The data for ten variants Vranec and ten variants Cabernet Sauvignon were average, because they are basically repetition in the center of planned experiment and the differences are negligible.

Regarding organoleptic profile of Cabernet Sauvignon wines produced with ten second stage selected yeast strains, the highest series "average tasting note – ATN" (scale from 0-100) had wine CS10 produced with yeast strain F-78. The wine CS7 fermented by yeast strain F-42 had ATN 82. The lowest evaluation was considered for wine CS6 produced by yeast strain F-46 (ATN 77).

Regarding Vranec wines, the highest ATN was observed for wine V10 fermented by strain F-78 (ATN 84) followed by Vranec wines V2 and V9 produced by yeast strains F-8 and F-70 (ATN 82 and 81 respectively). The lowest ATN was observed for Vranec wines V1 and V3 produced by yeast strains F-4 and F-17.

The tasting notes of the trial wines from Cabernet Sauvignon and Vranec are illustrated in figure 3. In the Cabernet wines, the sample with F-78 has intense and vivid color, pure intensive aroma with dominant fruit notes and nuances of sweet crust, dry mass and tender raisin.[30] The mouthfeel was dense, juicy and spicy with bitter aftertaste. Similarly, high evaluated were also wines CS2 and CS8 fermented by yeast strains F-8 and F-57. The flavor of wine CS7 produced by strain F-46, was weak, even neutral, fermentative and reductive notes were detected. The wine was very dry, with dominate unpleasant acidity, a lack of softness, the aftertaste was crispy and dry. Similar in the organoleptic profile were wines CS1 and CS3. The sample with the commercial strain SiHa stands back in the aroma intensity, the fruit notes were weaker, a pronounced dryness and acidity was observed.

The wines produced from Vranec grape variety fermented by yeast strain V10 F-78 had intensive ruby-red color, intensive pure flavour of red berries, sweet crust and ripe fruit and spice with "stone fruit" nuances which can be related to the increased amount of 2-phenyl acetate and isobutyl acetate.[31] The structure was juicy with medium acidity. The flavor of wine V2 fermented by yeast strain F-8 was similar, with pronounced fruit flavour, the sweet dry and spicy notes stand back. The control wine fermented by commercial yeast strain SiHa had weaker flavor and acidity was greater. The main problem in wine V1 produced by yeast strain F-4, beside the weaker flavour, was sharp acidity, due to the undone malolactic fermentation. The sharp acidity was observed in wines produced from both varieties which gives grounds to conclude that strain F-4 is a strong antagonist versus malolactic bacteria.

To track the sustainability of the organoleptic characteristics and the eventual changes, which may occur, we did a wine tasting 10 months after vinification. It was established, that the wines kept organoleptic profile with phenolic and fruity flavour. This can be explained by the young wines storage in bottles, without oxygen, but with biomass and active reductases available. Most of the samples purify the aroma after a mild aeration, although wines produced by yeast strains F-39 and F-46 keep these notes.[7] For Cabernet Sauvignon wine samples had an intense ruby-red color, with blackberries notes, dry leaves and soft resin nuances. The spider diagrams presented in fig.3. indicated importance of yeast strains on the organoleptic profile of wines.

Although all experimental wines were produced from different *S. cerevisiae* yeast strains, their spider diagrams were significantly different, which lead us to conclusion that type of yeast and region of selection are extremely important for organoleptic profile of wine. Our statement was confirmed by conclusion from experiments of working group of Varela.

According to findings, sensorially, *M. pulcherrima* wines received relatively high scores for sensory descriptors such as "red fruit" and "fruity" flavour and overall exhibited a sensory profile similar to that of wine made with *S. cerevisiae*, whereas the main sensory descriptors associated with wines fermented with *S. uvarum* were barnyard and meat.[11] In addition, the sensory analysis of wines produced by *non-Saccharomyces* yeasts pointed out differences in fruitiness and aroma quality which were linked with metabolites from non-*Saccharomyces* yeasts which may contribute to form stable polymeric pigments while also influencing wine complexity.[12]

Moreover, the results from the volatile components indicated *S. cerevisiae*/MJS22 couple significantly boost the production of most detected compounds, more particularly in higher alcohols, esters, acids and terpenes; while the characteristic of *S. cerevisiae*/Alpha pair is an increase in fruity esters, higher alcohols and decrease in acid production. Sensory evaluation revealed that *S. cerevisiae*/MJS22 pair reinforced sweet, green and fatty notes to the cherry wines and *S. cerevisiae*/Alpha trial enhanced the fruity odour and reduced green note.[13] Regarding flor yeast growing a short time under velum conditions, decreases the ethanol and volatile acidity contents, has a favorable effect on the colour and astringency and significantly changes the wine content in 1-propanol, isobutanol, acetaldehyde, 1,1-diethoxiethane and ethyl lactate.[32]

According to the results presented in this study, we can conclude that autochthonous yeast produced wines of higher organoleptic quality. Our statement completely agrees with statement of working group of Callejon et al[33], since they confirmed that autochthonous yeast produced wines gave highest value for the general impression attribute. Sensory analysis of wines showed enhanced 'mature fruit' nuances and a chemical profile characterized by higher content of ethyl propanoate, ethyl isobutyrate and ethyl dihydrocinnamate.[16]

Semi-industrial trial of Vranec wine fermented by third-stage selected yeast strain "F-78": The values for organoleptic characteristics obtained from Vranec and Cabernet Sauvignon wines produced by ten second-stage selected yeast strains, gave us general indications regarding the grape variety and selected yeast strain. Our results favoured Vranec grape variety and yeast F-78 as variable for production of wine with the best organoleptic characteristic. For this purpose, we produced semi-industrial Vranec wine fermented by third stage selected yeast strain F-78 from 1000 kg of Vranec grapes.

The oeno-chemical composition of the semi-industrial trial of Vranec wine is presented in table 3. Compering the results for this wine from industrial-scale and experimental wine V10 we noticed some significant differences. The levels of monomeric anthocyanins and total phenolic compounds were 30% higher in semi-industrial Vranec wine in comparison to experimental wine fermented by the same yeast strain and produced from Vranec grapes from the same vintage year.[17] Furthermore, this wine had lower percentage of alcohol (13.5%) and almost half of the quantity of residual sugar in comparison to experimental wine V10.

The results lead us to conclusion that appreciate amount of monomeric anthocyanins and phenolic content, acceptable color and tonality as well as good amount of alcohol and residual sugar are proof for satisfying fermentation which can be excellent possibility for semi-industrial production of high quality regional wine.[34] The coefficient of sugar transformation was 0.589, which we estimated as high efficient in industrial conditions. The low value of volatile acidity, even after malolactic fermentation, confirmed the suitable metabolism of the selected yeast strain.

We took into account the noticeable difference between the quantity of the anthocyanins and total phenols between the experimental wines and the wine from the planned semi-industrial experiment. This might be due to the bigger volume of the lot, significantly more intensive extraction through recirculation of the fermentation tank.

Despite that, we observed great ratio between the color compounds with distinguishable domination of the red color (6.1 a.u.) These values along with the low value of the tonality (0.45) indicated intense ruby-red, vivid color. A great part of the anthocyanins was in flavylium form and had high coloring ability.

Table 1
Crushed grapes from Vranec and Cabernet Sauvignon grape variety for spontaneous fermentation

	Grapes from microregions from Tikveš grape-wine region	Dry matter (%)	pH	Titratable acidity (g/L)	Malic acid (g/L)
1.	Vranec-Belgrade	22.3±0.04^{c}	3.37±0.03^{b}	6.02±0.05^{b}	1.27±0.00^{b}
2.	Vranec-Barovo	24.8±0.06^{a}	3.62±0.02^{a}	3.95±0.01^{d}	1.17±0.02^{c}
3.	Vranec-Gornichkite	23.9±0.02^{b}	3.42±0.01^{a}	6.35±0.03^{b}	1.51±0.01^{a}
4.	Vranec-Cuculka	23.6±0.05^{b}	3.51±0.00^{a}	6.20±0.02^{b}	1.52±0.02^{a}
5.	Vranec-Bunishte	24.2±0.01^{a}	3.48±0.04^{b}	7.20±0.01^{a}	1.59±0.06^{a}
6.	Vranec-Krushi	24.4±0.02^{a}	3.49±0.01^{b}	5.00±0.02^{c}	0.96±0.01^{d}
7.	Vranec-Pod ridot	23.5±0.09^{b}	3.40±0.03^{b}	6.56±0.00^{b}	1.31±0.01^{c}
8.	Vranec-Beli kamenja	23.6±0.00^{b}	3.55±0.02^{a}	5.54±0.01^{c}	0.82±0.02^{d}
9.	Vranec-Gorna Laka	22.3±0.03^{c}	3.35±0.02^{b}	7.67±0.01^{a}	0.65±0.03^{d}
10.	Vranec-Roviti Kamen	23.2±0.01^{b}	3.43±0.00^{b}	6.48±0.07^{b}	1.33±0.06^{c}
11.	Cabernet Sauvignon-Barovo	21.0±0.03^{c}	3.32±0.01^{b}	6.35±0.01^{b}	1.68±0.01^{a}
12.	Cabernet Sauvignon-Kavadarci	22.4±0.07^{c}	3.32±0.01^{b}	6.12±0.02^{b}	1.80±0.01^{a}
13.	Cabernet Sauvignon-Negotino	22.8±0.01^{c}	3.38±0.03^{b}	5.85±0.01^{c}	1.55±0.09^{a}
14.	Cabernet Sauvignon-Ljubash	23.1±0.02^{b}	3.42±0.02^{a}	5.60±0.01^{c}	1.40±0.01^{b}
15.	Cabernet Sauvignon-Suva Gora	24.0±0.01^{a}	3.40±0.02^{a}	5.25±0.01^{c}	1.45±0.00^{b}

The results were expressed in mg/L±SD of two replicates. The different letters means significant differences among results. The letters are a, b, c and d according to the decrease of the result values.

Table 2
Color intensity and tonality of wines from Vranec and Cabernet Sauvignon grape variety produced by ten second-stage selected yeast strains

	Strain	Color intensity ($A_{420}+A_{520}+A_{620}$)	A_{420}	A_{520}	A_{620}	Tonality (A_{420}/A_{520})
CS1	F-4	11.0±0.01[a]	3.2±0.00[b]	6.9±0.06[a]	0.9±0.01[b]	0.46±0.01[b]
CS2	F-8	11.0±0.03[a]	3.3±0.01[b]	6.6±0.04[b]	1.1±0.03[b]	0.50±0.03[a]
CS3	F-17	11.2±0.01[a]	3.6±0.04[a]	6.4±0.03[b]	1.2±0.06[b]	0.56±0.02[a]
CS4	F-20	11.2±0.04[a]	3.4±0.02[a]	6.8±0.01[a]	1.0±0.01[b]	0.50±0.08[a]
CS5	F-39	10.9±0.01[a]	3.2±0.01[b]	6.6±0.02[b]	1.1±0.03[b]	0.48±0.01[b]
CS6	F-42	11.0±0.02[a]	3.2±0.00[b]	6.6±0.01[b]	1.2±0.08[b]	0.48±0.00[b]
CS7	F-46	10.9±0.02[a]	3.1±0.00[b]	6.8±0.02[a]	1.0±0.02[b]	0.45±0.02[b]
CS8	F-57	11.1±0.04[a]	3.2±0.04[b]	6.7±0.03[b]	1.2±0.01[b]	0.47±0.02[b]
CS9	F-70	11.2±0.03[a]	3.1±0.07[b]	6.8±0.02[a]	1.3±0.04[a]	0.45±0.06[b]
CS10	F-78	10.9±0.01[a]	3.1±0.05[b]	6.6±0.01[b]	1.2±0.07[b]	0.47±0.01[b]
CS11	SiHa	11.0±0.01[a]	3.3±0.01[b]	6.7±0.00[b]	1.0±0.03[b]	0.49±0.03[b]
	Strain	**Color intensity ($A_{420}+A_{520}+A_{620}$)**	**A_{420}**	**A_{520}**	**A_{620}**	**Tonality (A_{420}/A_{520})**
V1	F-4	10.3±0.01[b]	2.2±0.02[b]	7.0±0.02[a]	1.1±0.02[a]	0.31±0.01[c]
V2	F-8	11.2±0.00[a]	3.0±0.02[a]	7.3±0.01[a]	0.9±0.01[b]	0.41±0.00[b]
V3	F-17	11.0±0.03[a]	3.3±0.01[a]	6.8±0.03[b]	0.9±0.04[b]	0.48±0.09[b]
V4	F-20	11.2±0.02[a]	3.2±0.00[a]	6.9±0.05[b]	1.1±0.03[a]	0.46±0.01[b]
V5	F-39	11.3±0.09[a]	3.1±0.02[a]	7.0±0.01[a]	1.2±0.08[a]	0.44±0.01[b]
V6	F-42	11.1±0.01[a]	3.3±0.04[a]	6.8±0.00[b]	1.0±0.02[a]	0.48±0.03[b]
V7	F-46	11.2±0.02[a]	2.9±0.04[a]	7.4±0.03[a]	0.9±0.03[b]	0.39±0.05[c]
V8	F-57	10.9±0.02[a]	3.0±0.06[a]	7.1±0.01[a]	0.8±0.02[b]	0.42±0.07[b]
V9	F-70	11.4±0.04[a]	3.1±0.05[a]	7.2±0.00[a]	1.1±0.05[a]	0.43±0.03[b]
V10	F-78	11.1±0.05[a]	3.1±0.01[a]	7.1±0.01[a]	0.9±0.05[b]	0.44±0.00[b]
V11	SiHa	11.2±0.01[a]	3.4±0.01[a]	6.6±0.03[b]	1.2±0.01[a]	0.51±0.01[a]

The results were expressed in mg/L±SD of two replicates. The different letters means significant differences among results. The letters are a, b, c and d according to the decrease of the result values.

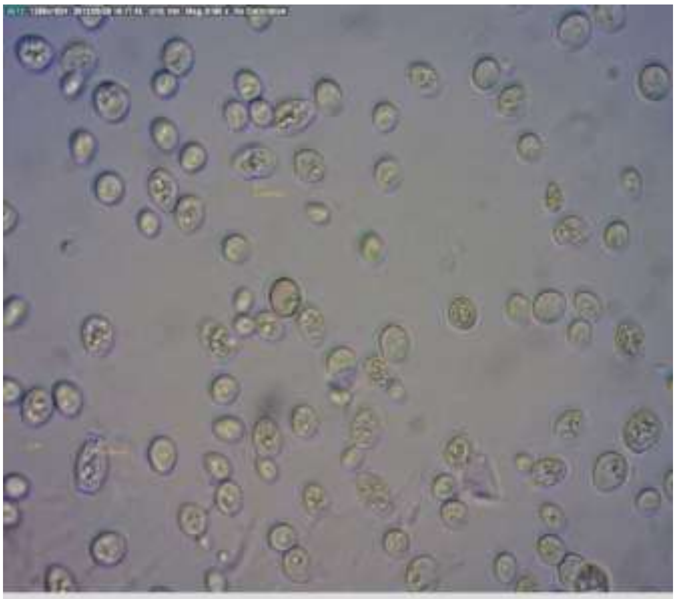
Table 3
Oeno-chemical composition of the Vranec wine for semi-industrial application fermented by third stage selected yeast strain F-7

Oeno-chemical composition of Vranec wine for semi-industrial application fermented by third stage selected yeast strain F-78	Value
Alcohol (%vol.)	13.5±0.29[b]
Sugars (mg/L)	1.76 ±0.05[b]
Titratable acidity (g/L)	6.29±0.11[a]
Volatile acidity (g/L)	0.49±0.02[a]
Monomeric antocyanines (mg/L)	708±32[a]
Total phenolic compounds (mg/L)	3574±49[a]
IC (a.u.)	10.0±0.09[b]
Tonality	0.45±0.02[a]
A_{420}	2.8±0.07[a]
A_{520}	6.1±0.09[a]
A_{620}	1.1±0.05[a]

The results were expressed (absorbance units a.u.) in mg/L±SD of two replicates. The different letters means significant differences among results. The letters are a, b and c according to the decrease of the result values.

a

b



c

Fig. 1: Morphology of yeast strain F78 (a), sporogenesis of yeast strain F-8 (b) and sporogenesis of yeast strain F-78

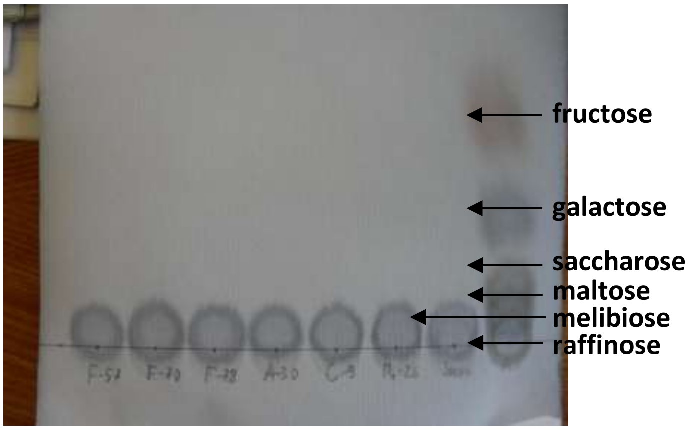


Fig. 2: Paper chromatogram of carbohydrates

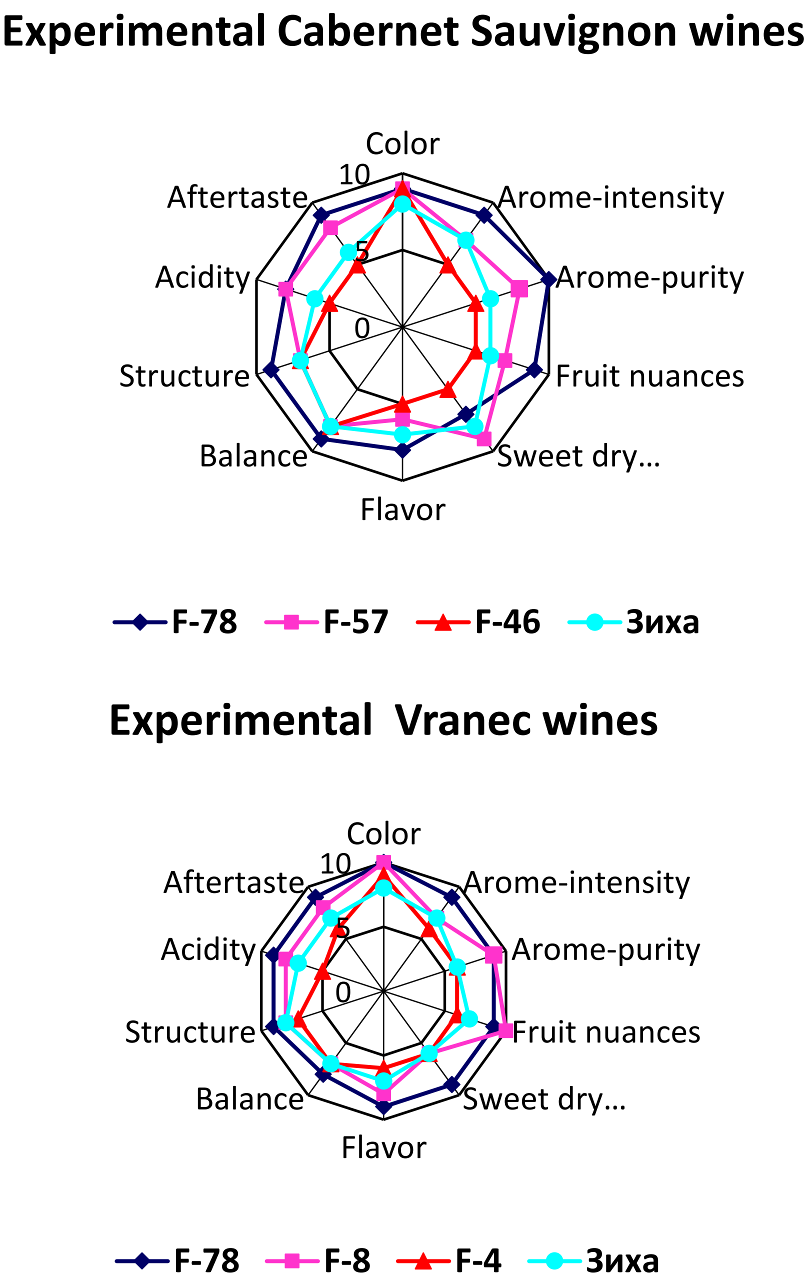


Fig. 3: Spider diagrams for organoleptic profile of wines produced from Vranec and Cabernet Sauvignon grape variety and fermented by ten second-stage selected yeast strains

This fact was supported by the oxidation protection and the slightly higher acidity of the trial. A major importance had the behavior of the third stage selected yeast strain F-78 which helped the extraction of the color matter.[32,35] We could assume that this strain has low ß-glycosidase activity and it is suitable for red wines.

The oeno-chemical parameters and sensory profile of the semi-industrial wine from Vranec variety confirmed that the selected local yeast strain F-78 carries through an intensive, pure and complete fermentation. Vranec wine produced in semi-industrial conditions had intense color, intense "fruit-spicy" flavour, firm body and aging potential. This strain is recommended for production of quality regional red wines from Tikveš wine-growing region in Macedonia.

Conclusion

Generally speaking, organoleptic profiles of experimental wines fermented with autochthonous yeast strains revealed superiority in comparison with control wines produced with commercial strain SiHa which was more obvious in the local grape variety Vranec. Finally, this study can be used as general recommendation for specific regional wines production.

References

1. Niimi J., Boss P.K. and Bastian S.E.P., Sensory profiling and quality assessment of research Cabernet Sauvignon and Chardonnay wines; quality discrimination depends on greater differences in multiple modalities, *Food Res. Int*., **106**, 304–316 **(2018)**

2. Baidya D., Chakraborty I. and Saha J., Table wine from tropical fruits utilizing natural yeast isolates, *J. Food Sci. Technol*., **53(3)**, 1663–1669 **(2016)**

3. Comitini F., Capece A., Ciani M. and Romano P., New insights on the use of wine yeasts, *Curr. Opin. Food Sci*., **13**, 44–49 **(2017)**

4. Arici M., Ozulku G., Yildirim M.R., Sagdic O. and Durak M.Z., Biodiversity and technological properties of yeasts from Turkish sourdough, *Food Sci. Biotechnol*., **27(2)**, 499-508 **(2018)**

5. Capozzi V., Garofalo C., Chiriatti M.A., Grieco F. and Spano G., Microbial terroir and food innovation: The case of yeast biodiversity in wine, *Microbiol. Res*., **181**, 75–83 **(2015)**

6. González-Neves G., Favre G., Gil G., Ferrer M. and Charamelo D., Effect of cold pre-fermentative maceration on the color and composition of young red wines cv. Tannat, *J. Food Sci. Technol*., **52(6)**, 3449–3457 **(2015)**

7. Blanco P., Mirás-Avalos J.M., Suárez V. and Orriols I., Inoculation of Treixadura musts with autochthonous *Saccharomyces cerevisiae* strains: Fermentative performance and influence on the wine characteristics, *Food Sci. Technol. Int.*, **19(2)**, 177-186 **(2012)**

8. Sidari R., Caridi A. and Howell K.S., Wild *Saccharomyces cerevisiae* strains display biofilm-like morphology in contact with polyphenols from grapes and wine, *Int. J. Food Microbiol*., **189**, 146–152 **(2014)**

9. Liu N., Song Y.Y., Qin Y., Gong X. and Liu Y.L., Chromatic characteristics and anthocyanin compositions of cabernet sauvignon wines: Influence of indigenous *Saccharomyces cerevisiae* strains in Ningxia, China, *Food Sci. Biotechnol.*, **24(6)**, 1973-1978 **(2015)**

10. Avizcuri J.M., Sáenz-Navajas M.P., Echávarri J.F., Ferreira V. and Fernández-Zurbano P., Evaluation of the impact of initial red wine composition on changes in color and anthocyanin content during bottle storage, *Food Chem*., **213**, 123–134 **(2016)**

11. Varela C., Barker A., Tran T., Borneman A. and Curtin C., Sensory profile and volatile aroma composition of reduced alcohol Merlot wines fermented with *Metschnikowia pulcherrima* and *Saccharomyces uvarum*, *Int. J. Food Microbiol*., **252**, 1–9 **(2017)**

12. Escott C., Del Fresno J.M., Loira I., Morata A., Tesfaye W., Del Carmen González M. and Suárez-Lepe J.A., Formation of polymeric pigments in red wines through sequential fermentation of flavanol-enriched musts with non-*Saccharomyces yeasts*, *Food Chem*., **239**, 975–983 **(2018)**

13. Sun S.Y., Gong H.S., Jiang X.M. and Zhao Y.P., Selected non-*Saccharomyces* wine yeasts in controlled multi starter fermentations with *Saccharomyces cerevisiae* on alcoholic fermentation behaviour and wine aroma of cherry wines, *Food Microbiol.*, **44**, 15-23 **(2014)**

14. Lick E., König B., Kpossa M.R. and Buller V., Sensory expectations generated by colours of red wine labels, *J. Retailing Consumer Service*, **37**, 146–158 **(2017)**

15. Cameleyre M., Lytra G., Tempere S. and Barbe J.C., 2-Methylbutyl acetate in wines: Enantiomeric distribution and sensory impact on red wine fruity aroma, *Food Chem*., **237**, 364–371 **(2017)**

16. Nicolli K.P., Biasoto A.C.T., Souza-Silva É.A., Guerra C.C., Dos Santos H.P., Welke J.E. and Zini C.A., Sensory, olfactometry and comprehensive two-dimensional gas chromatography analyses as appropriate tools to characterize the effects of wine management on wine aroma, *Food Chem*., **243**, 103–117 **(2018)**

17. Puertas B., Guerrero R.F., Jurado M.S., Jiménez M.J. and Cantos-Villar E., Evaluation of alternative winemaking processes for red wine color enhancement, *Food Sci. Technol. Int.*, **14**, 21-27 **(2008)**

18. Ilieva F., Kostadinović Veličkovska S., Dimovska V., Mirhosseini H. and Spasov H., Selection of 80 newly isolated autochthonous yeast strains from the Tikveš region of Macedonia and their impact on the quality of red wines produced from Vranec and Cabernet Sauvignon grape varieties, *Food Chem*., **216**, 309-315 **(2017)**

19. Ilieva F., Kostadinović Veličkovska S., Dimovska V. and Spasov H., The impact of some wine-making practices on the quality of Vranec red wines from Macedonia produced by the newly-selected local strain "F-78", *Food Chem*., **194**, 1123-1131 **(2016)**

20. Boulton R., The relationships between total acidity, titratable acidity and pH in wines, *Am. J. Enol. Viticul*., **31(1)**, 76-80 **(1980)**

21. Singleton V.L. and Rossi A. Jr., Colorimetry and total phenolics with phosphomolybdic phosphotungstic acid reagents, *Am. J. Enol. Viticul*., **16(3)**, 144-158 **(1965)**

22. Kostadinović Veličkovska S., Mirhosseini H. and Bogeva E., Isolation of anthocyanins by high-speed countercurrent chromatography and application of colour activity concept to different varieties of red grape pomace from Macedonia, *J. Nutr. Food Sci*., **3**, 1–7 **(2013)**

23. Kostadinović S., Wilkens A., Stefova M., Ivanova V., Vojnoski B., Mirhosseini H. and Winterhalter P., Stilbene levels and antioxidant activity of Vranec and Merlot wines from Macedonia: Effect of variety and enological practices, *Food Chem*., **135**, 3003–3009 **(2012)**

24. Perrone B., Giacosa S., Rolle L., Cocolin L. and Rantsiou K., Investigation of the dominance behavior of *Saccharomyces cerevisiae* strains during wine fermentation, *Int. J Food Microbiol*., **165**, 156–162 **(2013)**

25. Morales M.L., Fierro-Risco J., Callejón R.M. and Paneque P., Monitoring volatile compounds production throughout fermentation by *Saccharomyces* and non-*Saccharomyces* strains using headspace sorptive extraction, *J. Food Sci. Technol*., **54(2)**, 538–557 **(2017)**

26. Borrull A., Lopez-Martínez G., Miro-Abella E., Salvado Z., Poblet M. and Cordero-Otero R., New insights in the physiological state of *Saccharomyces cerevisiae* during ethanol accumulation for producing sparkling wines, *Food Microbiol*., **54**, 20-29 **(2016)**

27. Medina K., Boido E., Dellacassa E. and Carrau F., Growth of non-*Saccharomyces* yeasts affects nutrient availability for *Saccharomyces cerevisiae* during wine fermentation, *Int. J. Food Microbiol.*, **157**, 245-250 **(2012)**

28. Maturano Y.P., Mestre M.V., Esteve-Zarzoso B., Nally M.C., Lerena M.C., Toro M.E., Vazquez F. and Combina M., Yeast population dynamics during prefermentative cold soak of Cabernet Sauvignon and Malbec wines, *Int. J. Food Microbiol*., **199**, 23–32 **(2015)**

29. Puértolas E., Álvarez I. and Raso I., Changes in Phenolic Compounds of Aragón Red Wines During Alcoholic Fermentation, *Food Sci. Technol. Int*., **17(2)**, 77-86 **(2011)**

30. Lesschaeve I., The use of sensory descriptive analysis to gain the better understanding of consumer wine language, In 3rd international wine business and marketing research conference, Montpellier, France **(2006)**

31. Puertas B., Guerrero R.F., Jurado M.S., Jiménez M.J. and Cantos-Villar E., Evaluation of alternative winemaking processes for red wine color enhancement, *Food Sci. Technol. Int*., **14**, 21-27 **(2008)**

32. Moreno J., Moreno-García J., López-Muñoz B., Mauricio J.C. and García-Martínez T., Use of a flor velum yeast for modulating colour, ethanol and major aroma compound contents in red wine, *Food Chem*., **213**, 90–97 **(2016)**

33. Callejon R.M., Clavijo A., Ortigueira P., Troncoso A.M., Paneque P. and Morales M.L., Volatile and sensory profile of organic red wines produced by different selected autochthonous and commercial *Saccharomyces cerevisiae* strains, *Anal. Chimica Acta*, **660**, 68–75 **(2010)**

34. Şener H. and Yildirim H.K. Influence of different maceration time and temperatures on total phenols, colour and sensory properties of Cabernet Sauvignon wines, *Food Sci. Technol. Int*., **19(6)**, 523-533 **(2012)**

35. Morata A., Loira I., Heras J.M., Callejo M.J., Tesfaye W., González C. and Suárez-Lepe J.A., Yeast influence on the formation of stable pigments in red winemaking, *Food Chem*., **197**, 686-69 **(2016)**.

(Received 04th September 2018, accepted 05th November 2018)