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CHARACTERIZATION OF SOME INDIGENOUS *SACCHAROMYCES CEREVISIAE* ISOLATES OBTAINED DURING VINIFICATION OF 'KALECIK KARASI' AND 'EMIR' GRAPES GROWN IN CENTRAL ANATOLIA

CARACTERIZAÇÃO DE ISOLADOS INDÍGENAS DE *SACCHAROMYCES CEREVISIAE* OBTIDOS DURANTE A VINIFICAÇÃO DE UVAS DAS CASTAS 'KALECIK KARASI' E 'EMIR' CULTIVADAS NA REGIÃO CENTRAL DA ANATÓLIA

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SUMMARY

In this research, 20 *Saccharomyces cerevisiae* isolates obtained during spontaneous fermentation of 'Kalecik Karasi' and 'Emir' grapes were evaluated for their technological characteristics. All of the *S. cerevisiae* isolates could grow at the tested temperatures, pH values and sugar concentration. Most of the tested isolates (80%) produced the same level of H_2S than the control culture. All indigenous *S. cerevisiae* isolates showed similar carbon assimilation and fermentation profiles, with a few exceptions. Only one isolate (E/S6) was found to have proteolytic activity. The killer phenotype was detected in KK/FS11 isolate. KK/FS3, E/FS12, E/S7 and E/S8 isolates were neutral as to killer phenotype. While only three isolates could tolerate high concentration of ethanol (14%; v/v), 11 isolates could grow in the media containing 200 mg SO_2/L. In addition, some of the isolates were distinguished among the others because of their ability to secrete enzyme(s) which may provide advantages for producing wines with specific aromatic characteristics. When some of the tested technological characteristics and enzymatic profiles were evaluated together, four isolates (KK/S17, E/S6, E/S9 and E/FS12) distinguished from others with respect to their superior characteristics, which have potential for further investigation in order to produce wines with specific aroma for Capadoccia region.

RESUMO

Este trabalho incidiu na avaliação das características tecnológicas de 20 isolados de *Saccharomyces cerevisiae* obtidos durante a fermentação espontânea de uvas das castas 'Kalecik Karasi' e 'Emir'. Foi observado crescimento de todos os isolados de *S. cerevisiae* às temperaturas testadas, pH e concentração de açúcar. A maioria dos isolados testados (80%) produziu o mesmo nível de H_2S que a cultura controlo. A assimilação de carbono e os perfis de fermentação foram idênticos para a maioria dos isolados de *S. cerevisiae*. Apenas um isolado (E/S6) apresentou atividade proteolítica. O fenótipo *killer* foi detetado no isolado KK/FS11, enquanto os isolados KK/FS3, E/FS12, E/S7 e E/S8 se revelaram neutros. Apesar de só três isolados terem evidenciado tolerância a uma elevada concentração de etanol (14%; v/v), 11 isolados puderam crescer nos meios contendo 200 mg de SO_2/L. Para além disso, alguns dos isolados distinguiram-se dos restantes devido à sua capacidade de segregar enzima(s) que podem proporcionar vantagens na produção de vinhos com características aromáticas específicas. A avaliação conjunta das características tecnológicas e dos perfis enzimáticos permitiu distinguir quatro isolados (KK/S17, E/S6, E/S9 e E/FS12) pela sua superioridade. Em virtude deste potencial, considera-se que poderão ser alvo de uma investigação mais aprofundada a fim de produzir vinhos com aroma específico para a região da Capadoccia.

Key words: *Saccharomyces cerevisiae*, indigenous yeast, technological characteristics, spontaneous fermentation, wine.
Palavras-chave: *Saccharomyces cerevisiae*, levedura indígena, características tecnológicas, fermentação espontânea, vinho.

INTRODUCTION

The microbial process of must fermentation has been the subject of numerous studies, in which variability and diversity of wine yeast populations have been well-established (Esteve-Zarzoso *et al.*, 2000; Lee and Yu, 2007). These studies demonstrated that the main responsible agents of the alcoholic fermentation

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are strains of the species *Saccharomyces cerevisiae*. These strains are well adapted to must conditions and can grow under these conditions to complete the alcoholic fermentation (Esteve-Zarzoso *et al.*, 2000). *S. cerevisiae* strains are of great importance in industrial processes like alcoholic fermentations, but while the physiological characterization of the yeasts involved in oenological applications is common, there is less information about the genetic properties and the regulatory mechanisms of the enzymatic activities involved in flavour production. These metabolic activities have great technological importance because they can determine the organoleptic properties of wine (Schuller and Casal, 2005).

One of the most significant advances in winemaking has been the control of the microbiological process by grape must inoculation using selected yeasts. Today, the use of indigenous wine yeasts selected from each wine production region is widespread (Lopes *et al.*, 2007). These indigenous yeasts are presumed to be more competitive than commercial yeasts because they are better adapted to the ecological and technological conditions of their own region. Therefore, they have potential of dominating the fermentation and might become the most important biological agent responsible for the winemaking. Additionally, selection of the appropriate local yeasts would assure the production of quality premium wines maintaining the differential properties of their own region and preserving its natural biodiversity (Lopes *et al.*, 2007). Criteria for the selection and development of yeasts for wine fermentation have evolved over many years and were discussed by Regodon *et al.* (1997), Rainieri and Pretorius (2000), Schuller and Casal (2005) and Suarez-Lepe and Morata (2012). Basically, these criteria can be covered under three categories: (i) properties that affect the performance of the fermentation process; (ii) properties that determine wine quality and character; (iii) properties associated with the commercial production of wine yeasts. Within each category, there are properties of varying degrees of significance and importance, some being essential and some being desirable (Fleet, 2008).

The cultivation of grapes and the practice of winemaking in Anatolia date back to 4,000 B.C. Wines produced from 'Emir' and 'Kalecik Karasi' grape varieties grown in Central Anatolia are known by their high quality, distinct aroma and flavor profile. While 'Emir' is known as one of the best white grape variety for wine production, 'Kalecik Karasi' is grown for the production of good quality red wines in Turkey. The aim of the present study was to assess the technological potential of indigenous *S. cerevisiae* isolates in this region and identify those that could be used as starter cultures in vinification process.

MATERIAL AND METHODS

Yeast isolates

In this study, 20 indigenous *S. cerevisiae* isolates obtained during spontaneous wine fermentations of 'Kalecik Karasi' (KK) and 'Emir' (E) grape varieties grown in Central Anatolia; Cappadocia, were used. The identification of these isolates were previously performed, by using API ID 32C (bioMérieux, France) and some complementary identification tests (Senses-Ergul and Ozbas, 2014).

A commercial yeast strain, *S. cerevisiae* Fermirouge No. 7303 (INRA, Narbonne, France) and a wine isolate, *S. cerevisiae* IFO 2359, were used as control cultures in the tests performed for determination of technological characteristics. In the killer activity experiments, *S. cerevisiae* NCYC 232 (killer strain, K_1), *S. cerevisiae* NCYC 738 (killer strain, K_2) and *S. cerevisiae* NCYC 1006 (sensitive strain, S) were also used as control cultures.

Determination of growth at different temperatures, low pH and high sugar concentration

The ability of the indigenous *S. cerevisiae* isolates to grow at different temperatures (12, 16, 20, 37 and 42 °C) was tested according to Nikolaou *et al.* (2006). The capability of the isolates to grow at low pH values (3.0 and 4.0) was determined according to Caridi *et al.* (2002). Growing of the isolates at high sugar concentration (30 °Brix) was examined according to Iranzo *et al.* (1998). For this purpose, the ºBrix of the broth medium was adjusted by adding glucose. All of these experiments were performed by inoculating 24 h-old cultures into Yeast extract peptone dextrose (YEPD) broth (yeast extract 10.0 g, peptone 20.0 g and glucose 20.0 g per L) prepared according to the aim and incubating the cultures for 3-7 days. Ability to grow at low pH values and high sugar concentration tests were performed at 28 ºC.

Determination of some sugar assimilation and fermentation characteristics

Assimilation of different sugars was tested by API ID 32C test system (bioMérieux, France) using manufacturer's instructions. Fermentation of glucose, fructose, saccharose, maltose, galactose, lactose, mellibiose and mannitol were investigated by using media with Durham tubes including 2% (w/v) of each tested sugar. After inoculation of activated yeast cultures to the media and incubation of them at 28 ºC

for up to 28 days, positive results were evaluated according to the growth and gas formation into the Durham tubes (Yarrow, 2000).

Determination of enzymatic activities

Enzyme profiles of the tested yeast isolates were detected by using API-ZYM system (bioMérieux, France). API-ZYM is a miniaturized, semiquantitative test system, used for screening 19 different enzyme activities. For the application of the test, manufacturer's instruction was followed. Enzyme activity was graded from 0 to 5 by comparing the developed colour with the API-ZYM colour reaction chart.

Determination of proteolytic activity

Proteolytic activities of the isolates were tested according to Harrigan (1998) by using 10% reconstituted skim milk added (w/v) Milk agar media. In this method, activated *S. cerevisiae* cultures were inoculated on the media by streaking across the surface of the agar medium. Petri dishes were incubated at 28 ºC for 2-14 days. After incubation, colonies surrounded by a clear zone were recorded as positive results.

Determination of hydrogen sulphide, foam and acetic acid productions

Hydrogen sulphide (H_2S) productions of the tested *S. cerevisiae* isolates were determined by colony colour formation on inoculated BIGGY agar (Oxoid, Hampshire, UK). Petri dishes inoculated by streaking across the surface of the agar medium were incubated at 28 ºC for 5 days. The degree of browning associated with yeast growth on the BIGGY agar plate was scored using the following scale: 1=white, 2= cream, 3=light brown, 4=brown, 5=dark brown, 6=black (Spiczki *et al.*, 2001; Orlić *et al.*, 2005). In these experiments, a *Candida krusei* strain from our culture collection was used as positive control.

Foam production of the *S. cerevisiae* isolates was determined according to Regodon *et al.* (1997) and Nikolaou *et al.* (2006). For this purpose, sterilized must obtained from fresh grapes was used as inoculation medium. The inoculation level of each *S. cerevisiae* isolate was 10^6 cfu/mL. Inoculated grape musts were incubated at 25 ºC for 3 days. The test results were grouped according to the foam height produced by the yeast isolates. These were: F0 (foam height lower than 2 mm), F1 (foam height between 2-4 mm) and F2 (foam height higher than 4 mm).

Acetic acid production of the tested *S. cerevisiae* isolates were examined according to Caridi *et al.* (2002). YEPD agar medium containing 0.5% (w/v) $CaCO_3$ was used in the tests. Activated *S. cerevisiae* cultures were inoculated on media by streaking across the surface of the agar medium. Petri dishes were incubated at 25 ºC for 10 days. After incubation, colonies surrounded by a clear zone were recorded as positive regarding acetic acid production.

Determination of ethanol and sulphur dioxide tolerances

The capabilities of the indigenous yeast isolates to grow and to begin fermentation in the presence of different concentrations of ethanol (0, 8, 10, 12, and 14 %, v/v) or SO_2 (0, 25, 50, 75, 100, 150, and 200 mg/L) were determined according to Parish and Caroll (1987) by using Yeast Nitrogen Base (YNB, BD Difco, USA) broth prepared according to the aim. Results were recorded as positive or negative after 72 h of incubation at 30 ºC.

Determination of killer activity and interaction with starter culture

Killer activity was determined by using the agar diffusion technique on Yeast extract peptone dextrose-methylene blue (YEPD-MB) agar as described by Ozcelik and Donmez (1993) and Delfini and Formica (2001). Interaction with starter culture was also tested on YEPD-MB agar medium according to the method described by Izgu *et al.* (1997).

RESULTS AND DISCUSSION

Growth at different temperatures, low pH and high sugar concentration

Growth abilities at different temperatures, low pH and high sugar concentration of the tested *S. cerevisiae* isolates are presented in Table I.

All isolates obtained during spontaneous fermentation of 'Kalecik Karasi' grapes were able to grow at all temperatures in the studied range. However, six of the nine *S. cerevisiae* isolates (E/FS8, E/FS10, E/FS12, E/S5, E/S6 and E/S9) obtained during spontaneous fermentation of 'Emir' grapes can weakly grow at 42 ºC (Table I). E/S6 isolate weakly grew at 12 ºC. All of the 'Emir' isolates grew well at 20 and 37 ºC. It is thought that *S. cerevisiae* isolates obtained during vinification of 'Emir' grapes were adapted to cooler temperatures as a result of the fermentation temperature applied in white wine production process.

Table I

Growth of the indigenous *S. cerevisiae* isolates at different temperatures, low pH and high sugar concentration

Crescimento dos isolados indígenas de S. cerevisiae a diferentes temperaturas, baixo pH e elevada concentração de açúcar

İsolate nº	Temperature (˚C)					pH			Sugar concentration
	12	16	20	37	42	6.2*	4.0	3.0	30 ˚Brix
KK/FS3	+	+	+	+	+	+	+	+	+
KK/FS4	+	+	+	+	+	+	+	+	+
KK/FS7	+	+	+	+	+	+	+	+	+
KK/FS8	+	+	+	+	+	+	+	+	+
KK/FS11	+	+	+	+	+	+	+	+	+
KK/FS12	+	+	+	+	+	+	+	+	+
KK/S8	+	+	+	+	+	+	+	+	+
KK/S12	+	+	+	+	+	+	+	+	+
KK/S13	+	+	+	+	+	+	+	+	+
KK/S16	+	+	+	+	+	+	+	+	+
KK/S17	+	+	+	+	+	+	+	+	+
E/FS8	+	+	+	+	w	+	+	+	+
E/FS10	+	+	+	+	w	+	+	+	+
E/FS12	+	+	+	+	w	+	+	+	+
E/S5	+	+	+	+	w	+	+	+	+
E/S6	w	+	+	+	w	+	+	+	+
E/S7	+	+	+	+	+	+	+	+	+
E/S8	+	+	+	+	+	+	+	+	+
E/S9	+	+	+	+	w	+	+	+	+
E/S10	+	+	+	+	+	+	+	+	+
Commercial strain	+	+	+	+	+	+	+	+	+
S. cerevisiae IFO	+	+	+	+	+	+	+	+	+

***:** control for pH test; +: positive growth, w: weak growth.

Temperature is defined as one of the factors that vary from one winemaking process to another and influences the production of volatile acidity and metabolites during fermentation, but also yeast viability (OIV, 2012). In some wine producing regions, temperatures during harvesting and fermentation can be above 35 ºC. It has been suggested that using starter strains able to grow at high temperatures is important to prevent stuck and sluggish fermentations performed in wineries without cooling systems (Regodon *et al.*, 1997; Nikolaou *et al.*, 2006). It is thought that especially indigenous *S. cerevisiae* strains, which are able to grow at low temperatures (12 °C and 16 °C), can have a potential to be used as a white wine starter culture. Growth characteristic of the yeast strains at different temperatures is known as an important aspect towards the climatic conditions where fermentation occurs.

Growth ability at low pH is usually considered as an important technological characteristic for yeast strains, because of its effect on decreasing the possibility of malolactic fermentation and positive effect on aromatic profile (Ough, 1992). In this research, all of the *S. cerevisiae* isolates originating from spontaneous wine fermentations of 'Kalecik Karasi' and 'Emir' grapes were able to grow at pH 3.0 and 4.0 (Table I). It has been stated that the effect of pH on fermentation is indirect. The inhibition effect of SO_2 on yeasts is related with the pH of the must. It is known that inhibition effect of SO_2 is significantly lower at pH values above 3.5. Furthermore, low pH values influence fermentation by inhibiting the growth of competitive microorganisms other than starter cultures. In addition, low pH values are important for the uptake of some aminoacids into yeast cells and activating transport/transfer of these aminoacids by supplying protons (Ough, 1992; Jackson, 2000). In a study performed by Serra *et al.* (2005) to assess the influence of environmental conditions, that is temperature and pH, on the growth of two hybrid strains, it was stated that temperature was the factor which had the main influence on yeast growth and the choice of pH value for yeast growth was more determining for the strain *S. cerevisiae* than for the strain *S. bayanus var. uvarum*.

Glucose and fructose are known as the main carbon and energy sources required for wine fermentation. It has been accepted that total sugar concentration of mature grapes ranges between 20-25% at harvest. However, because of the osmotic effect of high sugar concentration on yeast growth, the beginning of

fermentation can be delayed. It has also been stated that possibility of incomplete fermentation is highly increased at sugar concentrations above 20-25% (Jackson, 2000). In the present study, all tested indigenous isolates and control cultures were able to grow at 30 °Brix (Table I). In a similar research performed in Spain, oenological characteristics and enzymatic activities of 74 *Saccharomyces* strains were investigated and it has been reported that all of the tested *Saccharomyces* strains were able to grow in media at 30 °Brix (Iranzo *et al.*, 1998). In addition, musts obtained from grapes grown in regions with hot climates are known to have higher sugar concentration. It has been reported that it is essential to use starter cultures tolerating high concentrations of sugar and completing fermentation for the vinification of musts obtained from these regions (Caridi *et al.*, 2002).

Assimilation and fermentation of some sugars

In carbon assimilation tests, only three *S. cerevisiae* isolates (KK/FS3, KK/FS11 and KK/S16) showed different assimilation profiles compared to those of the control cultures. It was found that control cultures and 85% of the tested *S. cerevisiae* isolates were able to assimilate galactose, saccharose, raffinose, maltose and glucose. In addition; *S. cerevisiae* KK/FS3 isolate assimilated palatinose and trehalose. Besides, *S. cerevisiae* KK/FS11 could also assimilate palatinose but could not assimilate maltose. Differently, one *S. cerevisiae* isolate (KK/S16) assimilated also glycerol but could not assimilate raffinose (data not shown). Carbon assimilation is an important criterion in the taxonomy and identification of yeasts, which depends on the utilisation of organic carbon compounds as an energy source and for growth (Jimoh *et al.*, 2012).

When sugar fermentation profiles of the tested *S. cerevisiae* isolates were compared with those of the control cultures, all indigenous isolates except for *S. cerevisiae* KK/FS11 had the same profiles than the control cultures. Despite all isolates fermented galactose, saccharose, mellibiose, glucose and fructose, they were not able to ferment maltose, mannitol and lactose. Differently, *S. cerevisiae* KK/FS11 was not able to ferment mellibiose (data not shown). It is known that sugars in grape must are mainly glucose and fructose. However, it is also reported that galactose, mannose, cellobiose, mellibiose, raffinose, arabinose, rhamnose, xylose, ribose and fucose can be found in musts in low amounts. Besides, the amount of saccharose in mature grapes and musts is also considerably low (Ough and Amerine, 1988; Ough, 1992; Jackson, 2000). Determination of capability of yeast strains to ferment and assimilate different sugars is important for physiological differentiation of these strains. Additionally, the ability of the yeasts to metabolize sugars other than glucose and fructose indicates that yeasts can use these sugars in must during fermentation as substrates only if they are in low amounts (Delfini and Formica, 2001; Caridi *et al.*, 2002; Capello *et al.*, 2004). In a similar study performed in Italy, indigenous strains were screened to determine their fermentation characteristics. It was reported that *S. cerevisiae* strains isolated during spontaneous fermentation of wine showed differences in fermentation of saccharose, galactose, maltose, and raffinose. These differences in fermentation profiles of the *S. cerevisiae* strains were considered during the selection of wine yeasts with suitable fermentation characteristics (Caridi *et al.*, 2002).

Enzymatic characterization and proteolytic activity

Results of the enzymatic activities of the yeast isolates are given in Table II. According to the results, it was found that leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α- glucosidase enzymes were displayed by all of the indigenous *S. cerevisiae* isolates. It is known that yeasts can produce many extracellular enzymes that are involved in the transformation of the substrates in grape must. It has been reported that enzymes produced during fermentation can have a positive effect on the formation of typical aroma of wines, while enzymes produced by some other yeast strains can affect wine production in a negative way (Delfini and Formica, 2001; Fiore, 2003). Protease, xylanase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-glucosidase are defined as enzymes playing an important role in the aroma formation of wines (Delfini and Formica, 2001; Fiore, 2003; Nikolaou *et al.*, 2006, 2007). Esterases are involved in the formation of aroma compounds, contributing to the secondary aroma after fermentation process (Nikolaou *et al.*, 2006). In this study, it was found that 85% of the tested indigenous *S. cerevisiae* isolates produced esterase (level 1-2) and 80% of them produced esterase lipase (level 1-2). Lipases are known as one of the subclasses of esterases. Fatty acids and sterols can be released from must by the activity of extracellular lipase of wine yeasts. These compounds are known to act as survival factors against negative conditions such as high ethanol concentration and as precursors of sensory active ethyl ester derivatives (Delfini and Formica, 2001). In the study, it was found that 40% of the tested *S. cerevisiae* isolates (KK/FS3, KK/FS4, KK/FS7, KK/FS12, KK/S13, KK/S17, E/FS8,

E/FS12) were able to produce lipase (level 1). Besides, it was verified that both of the tested control cultures (wine strains) were unable to produce lipase.

Table II

Enzymatic profiles of indigenous *S. cerevisiae* isolates

Perfis enzimáticos dos isolados indígenas de S. cerevisiae

Isolate nº	Enzyme no.																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
KK/FS3	0	2	2	1	4	1	1	0	0	4	1	0	1	0	3	0	0	0	0
KK/FS4	0	2	0	1	5	0	0	0	0	4	1	0	0	0	3	0	0	0	0
KK/FS7	1	2	1	1	4	1	1	1	0	4	1	1	1	0	3	1	0	1	1
KK/FS8	0	2	0	0	5	0	1	0	0	5	3	0	0	0	3	0	0	0	0
KK/FS11	0	0	2	0	4	0	1	0	0	5	2	1	1	0	1	0	0	0	1
KK/FS12	1	0	0	1	4	0	0	0	0	3	1	1	1	0	2	0	0	0	1
KK/S8	0	2	1	0	4	0	1	0	0	4	1	0	0	0	3	0	0	0	0
KK/S12	0	1	1	0	3	0	0	0	0	5	1	0	0	0	2	0	0	0	0
KK/S13	0	0	0	1	3	1	2	0	0	4	1	2	0	0	3	0	0	2	2
KK/S16	0	2	1	0	5	2	1	0	0	2	1	0	0	0	2	0	0	0	0
KK/S17	0	2	2	1	5	2	1	0	1	5	2	1	1	0	2	0	0	0	0
E/FS8	0	2	2	1	5	1	1	0	0	3	1	0	1	0	3	0	0	0	0
E/FS10	0	2	1	0	5	1	2	0	0	4	1	0	0	0	3	0	0	0	0
E/FS12	0	2	1	1	5	1	2	0	0	3	1	1	0	0	2	0	0	0	0
E/S5	0	1	2	0	5	1	2	0	0	3	1	0	0	0	2	0	0	0	0
E/S6	0	1	2	0	5	2	1	1	0	3	1	0	0	0	4	0	0	0	0
E/S7	0	2	1	0	5	2	1	0	0	4	1	0	0	0	1	0	0	0	0
E/S8	0	1	1	0	5	1	2	0	0	5	3	0	0	0	3	0	0	0	0
E/S9	0	1	1	0	5	1	2	0	0	3	1	0	0	0	4	0	0	0	0
E/S10	1	2	2	0	5	0	1	0	0	5	1	0	0	0	5	0	0	0	0
Commercial strain	0	2	2	0	5	1	2	0	0	5	1	0	0	0	5	0	0	0	0
S. cerevisiae IFO 2359	0	2	2	0	5	1	2	0	0	5	1	0	0	0	5	0	0	0	0

1: Alkaline phosphatase, 2: Esterase (C4), 3: Esterase lipase (C8), 4: Lipase (C14), 5: Leucine arylamidase, 6: Valine arylamidase, 7: Cystine arylamidase, 8: Trypsin, 9: α-chymotrypsin, 10: Acid phosphatase, 11: Naphtol-AS-BI-phosphohydrolise, 12: α-galactosidase, 13: β-galactosidase, 14: β-glucuronidase, 15: α-glucosidase, 16: β-glucosidase, 17: N-acetyl- β-glucoaminidase, 18: α-mannosidase, 19: α-fucosidase.

The nitrogen composition of grape must, concerning proteins and peptides, is estimated as 1-13% and 20.8% of total nitrogen, respectively (Delfini and Formica, 2001; Nikolaou *et al.*, 2006, 2007). These compounds may be degraded through activities of leucine-, valine- and cystine-arylamidase. In our study, it was verified that all of the *S. cerevisiae* isolates produced leucine arylamidase (level 3-5). Besides, 65% of the tested isolates produced valine arylamidase (level 1-2), and 85% of them produced cystine arylamidase (level 1-2). It has been reported that low molecular weight peptides, and especially amino acids, can be produced and may have direct involvement in forming fermentation bouquet, through their catabolism by the activity of these enzymes during fermentation (Delfini and Formica, 2001; Nikolaou *et al.*, 2006, 2007).

It has been showed that alkaline phosphatase enzyme can combine with tannins in grapes and musts and precipitate (Adams and Harbertson, 1999). When the effect of tannins on wine aroma is considered, it is thought that yeasts capable of producing alkaline phosphatase can be effective on the astringency of wines. In this study, only three tested *S. cerevisiae*

isolates (KK/FS7, KK/FS12, E/S10) were found to produce alkaline phosphatase. It has also been reported that acid phosphatase has a role in the uptake of thiamine into the cell and this is known as an effective factor in ethanol metabolism (Thomson *et al.*, 2005). In our study, it was found that all of the tested *S. cerevisiae* isolates (level 2-5) and control cultures (level 5) produced acid phosphatase.

Glucosidases are responsible for hydrolyzing the glycosidically bound monoterpenes and converting them into free odorous forms, thus enhancing the aromatic character of wines (Nikolaou *et al.*, 2007). In our study, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities were detected in all of the tested *S. cerevisiae* isolates. Only one indigenous isolate (KK/FS7) was found to produce β-glucosidase (level 1).

Additionally; some of the enzymes screened were produced by only a few tested *S. cerevisiae* isolates. For example, *S. cerevisiae* KK/FS7 and KK/S13 were the only isolates producing α-mannosidase and α-fucosidase, together. Besides, α-fucosidase was also produced by *S. cerevisiae* KK/FS11 and KK/FS12. Only six isolates (30%) (KK/FS7, KK/FS11, KK/FS12, KK/S13, KK/S17 and E/FS12) produced α-galactosidase. β-galactosidase activity was detected for six (30%) of the indigenous *S. cerevisiae* isolates. Only two *S. cerevisiae* isolates (KK/FS7 and E/S6) had trypsin activity. Also, α-chymotrypsin was only produced by *S. cerevisiae* KK/S17 isolate. In this research it was determined that some of tested indigenous *S. cerevisiae* isolates (KK/FS3, KK/S16, KK/S17, E/FS8, E/FS10, E/FS12, E/S7, E/S9 and E/S10) had similar enzyme profiles as the control cultures with few exceptions (Table II).

In the present study, only one *S. cerevisiae* isolate (E/S6) was found to have the proteolytic activity. It was also found that none of the control cultures had proteolytic activity. Proteolytic activity is known as one of the rarely encountered characteristic for *Saccharomyces* strains (Iranzo *et al.*, 1998; Strauss *et al.*, 2001; González *et al.*, 2004). It has been reported that yeast proteases and peptidases are important during the wine production because of their contribution to the nutritional value and formation of aroma. In addition, proteases and peptidases are known to have an important role in wine production stages such as clarification of must and wine (Dizzy and Bisson, 2000; Delfini and Formica, 2001). It is thought that this indigenous *S. cerevisiae* isolate should be further investigated in order to be used as a starter culture in wine production because of its potential in producing a characteristic aromatic profile.

Hydrogen sulphide, foam and acetic acid productions

Hydrogen sulphide (H_2S) is one of the undesirable compounds which has a great effect on wine flavour. It was found that 80% of the *S. cerevisiae* isolates produced the same amount (level 3) of H_2S than the commercial wine strain used as control. Besides, 20% of the tested isolates produced higher amounts (level 4) of H_2S compared to that of the control strain. According to the applied test, none of the tested *S. cerevisiae* isolates produced the highest amount (level 6) of H_2S (Table III).

Table III

H_2S production by indigenous *S. cerevisiae* isolates

Produção de H_2S pelos isolados indígenas de S. cerevisiae

Isolate nº	Level of H_2S production
KK/FS3	Light brown (3)
KK/FS12	Light brown (3)
KK/S8	Light brown (3)
KK/S12	Light brown (3)
KK/S13	Light brown (3)
KK/S16	Light brown (3)
KK/S17	Light brown (3)
E/FS8	Light brown (3)
E/FS10	Light brown (3)
E/FS12	Light brown (3)
E/S5	Light brown (3)
E/S6	Light brown (3)
E/S7	Light brown (3)
E/S8	Light brown (3)
E/S9	Light brown (3)
E/S10	Light brown (3)
Commercial strain	Light brown (3)
KK/FS4	Brown (4)
KK/FS7	Brown (4)
KK/FS8	Brown (4)
KK/FS11	Brown (4)
S. cerevisiae	Dark brown (5)
IFO 2359	
C. krusei	Dark brown (5)

In a study performed for the selection of indigenous *S. cerevisiae* isolates suitable for wine production, H_2S production of 32 *S. cerevisiae* isolates were tested and it was reported that all of the tested isolates produced H_2S (Lopes *et al.*, 2007). Also, Spiczki *et al.* (2001) reported that all of the tested *S. cerevisiae, S. paradoxus* and *S. bayanus* strains were found to

produce H_2S at the level of 5 and it is recommended the strains to be genetically modified before using as starter culture for wine fermentation.

Foam production test was performed in sterilized must obtained from fresh grape, containing a total reducing sugar of 148.36 g/L and at a pH of 3.83. All of the tested *S. cerevisiae* isolates produced the same amount of foam (>4 mm, level F2) than the control cultures used (data not shown). Foam production levels of IFO and commercial strains were found as 9 and 12 mm, respectively. The lowest foam production level was obtained with KK/FS3 isolate as 4 mm, while the highest one was 15 mm for KK/S16 isolate. Depending on the composition of grape must for this study, KK/FS3, KK/FS11, E/FS10, E/S6 and E/S9 isolates were determined to be producing low level of foam (between 4-6 mm) compared to other *S. cerevisiae* isolates tested in the study. Low amount of foam production is one of the preferred technological characteristic for starter cultures used in wine fermentation. In a similar study, Nikolaou *et al.* (2006) considered foam production as one of the main criteria for the selection of wine yeasts and reported that tested *S. cerevisiae* strains produced foam height between 1.5-3.0 mm.

Acetic acid is recognized as one of the most important by-products of wine production that negatively affects aromatic profile of wine when its concentration reaches more than 500-600 mg/L (Paraggio and Fiore, 2004). Results of the acetic acid production test were found to be negative for all of the indigenous *S. cerevisiae* isolates and also for control cultures (data not shown). Conversely, Caridi *et al.* (2002) reported that 9% of the tested 46 *S. cerevisiae* strains produced acetic acid. In another study, 80 *S. cerevisiae* strains were characterized for acetic acid production. It was showed that a significant variability in production levels was determined among the strains (Paraggio and Fiore, 2004).

Ethanol and SO_2 tolerance

The results related to ethanol tolerance are given in Table IV. Maximum ethanol concentration to grow and begin fermentation was 12% (v/v) for the control cultures. Among the tested 20 indigenous *S. cerevisiae* isolates, three of them (KK/FS3, KK/FS7 and KKS17) were found to grow and begin fermentation in the presence of maximum ethanol concentration (14%, v/v), tested. All other *S. cerevisiae* isolates were able to grow or to begin fermentation at a maximum ethanol concentration of 12% (v/v). It is a well-known aspect that in the commercial production of alcohol by fermentation there is a certain alcohol concentration which cannot be exceeded, regardless of the amount of sugar supplied to the yeast cell. It is assumed that for every yeast strain, there is a maximum alcohol concentration above which the yeast ceases to function (Gray, 1941). In the literature, it can be seen that the capability to grow and start fermentation at ethanol concentrations between 10-12% (v/v) were accepted as one of the most important selection criteria for wine yeasts (Parish and Caroll, 1987; Iranzo *et al.*, 1998; Perez-Coello *et al.*, 1999; Paraggio and Fiore, 2004; Nikolaou *et al.*, 2006). In another study, ethanol tolerance of wine yeasts was investigated and 106 of 632 yeast strains were found to grow at 10% (v/v) ethanol concentration (Benítez *et al.*, 1983). Lopes *et al.* (2007) reported that 32 indigenous *S. cerevisiae* strains were able to begin fermentation at 5 and 10% (v/v) ethanol concentration. Besides, it was reported that only three of the tested *S. cerevisiae* strains were able to ferment at 15% (v/v) ethanol concentration. In the present study, only three of indigenous *S. cerevisiae* isolates were able to grow and begin fermentation at 14% (v/v) ethanol concentration. It is thought that these isolates may have a potential to be used as a starter culture for producing wines with high ethanol concentration.

The results of the sulphur dioxide tolerance of the *S. cerevisiae* isolates are presented in Table IV. It was found that 55% of *S. cerevisiae* isolates were able to grow and begin fermentation at all of the SO_2 concentrations tested. All of the *S. cerevisiae* isolates were able to grow and begin fermentation at a maximum SO_2 concentration of 75 mg/L. When concentration of SO_2 was increased to 100 mg/L, 70% of the tested *S. cerevisiae* isolates had the ability to grow and begin fermentation. For the control commercial and IFO strains, the maximum SO_2 concentrations to grow and begin fermentation were 100 and 75 mg SO_2/L, respectively. It is known that it is possible to control activity of non-*Saccharomyces* strains during fermentation by the addition of SO_2, which has a toxic effect on most of the yeast species. It has also been reported that resistance to SO_2 must be considered as one of the main criteria for the selection of a wine starter culture. For the selection of a wine starter culture, yeast strains growing and beginning fermentation at 100-300 mg SO_2/L were mostly preferred (Parish and Caroll, 1987; Regodon *et al.*, 1997; Iranzo *et al.*, 1998; Perez-Coello *et al.*, 1999; Spiczki *et al.*, 2001; Nikolaou *et al.*, 2006). However, it is also recognized that adding SO_2 to wine raises a number of issues, especially as it relates to prevent excessive doses, mainly for health reasons but also because of their impact on aroma. It has also been reported that EU legislation has gradually reduced the maximum level allowed to 160 mg/L for

most red wines and 210 mg/L fort the majority of white wines (Reg. EC nº 1622/2000; Ribéreau-Gayon *et al.*, 2006).

Table IV

SO_2 tolerance of indigenous *S. cerevisiae* isolates

Tolerância ao SO_2 pelos isolados indígenas de S. cerevisiae

	Ability to grow / to begin fermentation			
	Ethanol concentration (v/v, %)	**SO_2 concentration (mg/L)**		
Isolate nº	**14**	**100**	**150**	**200**
KK/FS3	+/+	-/-	-/-	-/-
KK/FS4	-/-	-/-	-/-	-/-
KK/FS7	+/+	-/-	-/-	-/-
KK/FS8	-/-	-/-	-/-	-/-
KK/FS11	-/-	+/+	+/+	+/+
KK/FS12	-/-	+/+	-/-	-/-
KK/S8	-/-	-/-	-/-	-/-
KK/S12	-/-	+/+	-/-	-/-
KK/S13	-/-	+/+	+/+	+/+
KK/S16	-/-	-/-	-/-	-/-
KK/S17	+/+	+/+	-/-	-/-
E/FS8	-/-	+/+	+/+	+/+
E/FS10	-/-	+/+	+/+	+/+
E/FS12	-/-	+/+	+/+	+/+
E/S5	-/-	+/+	+/+	+/+
E/S6	-/-	+/+	+/+	+/+
E/S7	-/-	+/+	+/+	+/+
E/S8	-/-	+/+	+/+	+/+
E/S9	-/-	+/+	+/+	+/+
E/S10	-/-	+/+	+/+	+/+
Commercial strain	-/-	+/+	-/-	-/-
S. cerevisiae IFO 2359	-/-	-/-	-/-	-/-

+/+: The isolate has the ability to grow and begin fermentation. -/-: The isolate has not the ability to grow nor to begin fermentation

Killer activity and interaction with starter culture

Yeast strains can be classified into four phenotypes with respect to their killer activity. These phenotypes are: killer (K); killer-sensitive (K-S); sensitive (S); neutral (N) (Delfini and Formica, 2001). Several killer toxin types has been described for *S. cerevisiae*, however K2-type toxin has proved to be the most important one in winemaking because it is active at pH values during wine production (Marquina *et al.*, 2002). In the present study, it was found that ten *S. cerevisiae* isolates were sensitive, four isolates were neutral, five isolates were killer-sensitive and only one isolate was killer (Table V). Control cultures used in the study were also found to be sensitive to both of the killer strains tested.

The use of selected *S. cerevisiae* strains with the killer factor in wine fermentation is suggested to be effective in suppressing undesirable wild yeast strains or in avoiding stuck fermentations caused by indigenous killer yeasts (Kapsopoulou *et al.*, 2008).

In a study performed by da Silva (1996), killer characteristics of yeast strains were investigated and a total of 85 *S. cerevisiae* strains isolated from 'Riesling Italico' grape must produced in Brazil were tested. It was reported that 24.7% of the yeast strains were killer and 7.1% of them were killer-sensitive. In a study performed by Lopes *et al.* (2007) in North Patagonia, yeast strains isolated during fermentation of Malbec wine were tested regarding their killer profile. Of the 32 indigenous *S. cerevisiae* strains, 30 of them were reported to be sensitive, one of them was neutral and one of them was killer.

In the present study, *S. cerevisiae* isolates determined as killer (KK/FS11) or neutral (KK/FS3, E/FS12, E/S7, E/S8) may be important in terms of their technological characteristics because of their potential to be selected as a starter culture for wine production.

Table V

Killer activity profiles of indigenous *S. cerevisiae* isolates

Perfis de atividade killer dos isolados indígenas de S. cerevisiae

Isolate no.	Killer activity	Resistivity against tested kiler toxins		Phenotype	Inhibition effect on starter culture
		K_1 toxin	K_2 toxin		
KK/FS3	-	+	+	N	-
KK/FS4	-	-	-	S	-
KK/FS7	-	-	-	S	-
KK/FS8	-	-	-	S	-
KK/FS11	+	+	+	K	+
KK/FS12	-	-	-	S	-
KK/S8	-	-	-	S	-
KK/S12	-	-	-	S	-
KK/S13	-	-	-	S	-
KK/S16	-	-	-	S	-
KK/S17	-	-	-	S	-
E/FS8	+	-	+	K-S	-
E/FS10	+	-	+	K-S	+
E/FS12	-	+	+	N	-
E/S5	-	-	-	S	-
E/S6	+	-	+	K-S	+
E/S7	-	+	+	N	-
E/S8	-	+	+	N	-
E/S9	+	-	+	K-S	-
E/S10	+	-	+	K-S	+
Commercial strain	-	-	-	S	*
S. cerevisiae IFO 2359	-	-	-	S	-

K: killer, S: sensitive, K-S: killer-sensitive, N: neutral, *: not tested.

CONCLUSIONS

Despite the availability of several commercial *S. cerevisiae* strains intended for wine production, strains isolated from wine producing regions are usually known to adapt more easily to their own climatic conditions, grapes and also partially responsible for particular characteristics that frequently identify specific wines and regions. Thus, some indigenous *S. cerevisiae* isolates in the microflora of Cappadocia, an important winery region of Turkey, was studied in order to characterize indigenous yeast isolates that could be used in wine production. Some of these isolates stand out with their specific enzymatic profiles which can be of great importance in order to produce wines with sensorial characteristics specific of Cappadocia region of Turkey.

When some of the tested characteristics of indigenous *S. cerevisiae* isolates were evaluated together, four isolates, KK/S17, E/FS12, E/S6 and E/S9 gave promising results in terms of their peculiar characteristics and may be selected for further investigations as wine starters. KK/S17 isolate was distinguished among other 'Kalecik Karasi' (KK) isolates by its ability to grow at high concentrations of SO_2 (100 mg/L) and ethanol (14%, v/v) and to produce low level of H_2S. When tested enzymatic characteristics were taken into account, it was found that KK/S17 isolate was able to synthesize enzymes like esterase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase and α-glucosidase, which are reported to be enzymes responsible for producing specific wine aroma. Concerning the technological characteristics tested, all 'Emir' (E) isolates were able to tolerate relatively high concentrations of SO_2 (200 mg/L) and ethanol (12%, v/v) and to produce the same degree of H_2S with commercial isolate. All (E) isolates were found to synthesize most of the enzymes tested. However, E/FS12 isolate was determined as being a rare isolate producing lipase. Another important characteristic of E/FS12 isolate distinguishing it from others was its neutral killer phenotype. Among (E) isolates, E/S9 was found to be the only one which had killer profile but no inhibition effect on commercial strain. Therefore, E/S9 is thought to be a promising isolate which can also be used in a co-culture with the commercial strain. It is known that proteolytic activity is a rarely encountered characteristic among *S. cerevisiae* strains. In addition to its positive enzymatic profile and other important technological characteristics for wine production, it is thought that E/S6 must be further investigated as a wine starter.

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REFERENCES

Adams D.O., Harbertson J.F., 1999. Evolution of skin and seed tannins in Pinot Noir berries during ripening: Application of a simple assay to a difficult problem. *In: Rave99, 2nd Joint Burgondy-California-Oregon Winemaking Symposium*, pp. 26-29.

Benítez T., del Castillo L., Aguilera A., Conde J., Cerdá-Olmedo E., 1983. Selection of wine yeasts for growth and fermentation in the presence of ethanol and sucrose. *Appl. Environ. Microbiol.*, **45**, 1429-1436.

Capello M.S., Bleve G., Grieco F., Dellaglio F., Zacheo G., 2004. Characterization of *Saccharomyces cerevisiae* strains isolated from must of grape grown in experimental vineyard. *J. Appl. Microbiol*., **97**, 1274-1281.

Caridi A., Cufari A., Ramondino D., 2002. Isolation and clonal pre-selection of enological *Saccharomyces. J. Gen. Appl. Microbiol.,* **48**, 261-267.

da Silva G.A., 1996. The occurrence of killer, sensitive, and neutral yeasts in Brazilian Riesling Italico grape must and the effect of neutral strains on killing behaviour. *App. Microbiol. Biotechnol.*, **46**, 112-121.

Delfini C., Formica J.V., 2001. *Wine Microbiology: Science and Technology*. 496 p. Marcel Dekker Inc., New York.

Dizzy M., Bisson L.F., 2000. Proteolytic activity of yeast strains during grape juice fermentation. *Am. J. Enol. Vitic.*, **51**, 155-167.

Esteve-Zarzoso B., Gostíncar A., Bobet R., Uruburu F., Querol A., 2000. Selection and molecular characterization of wine yeasts isolated from "El Penedes" area (Spain). *Food Microbiol*., **17**, 553-562.

Fiore C., 2003 Enzymatic activities in various yeast species and strains of wine origin. *In: Book of Abstracts of the 23rd International Specialised Symposium on Yeasts*.

Fleet G.H., 2008, Wineyeasts for the future. *FEMS Yeast Res*, **8**, 979–995.

González J.A., Gallardo C.S., Pombar A., Rego P., Rodríguez L.A., 2004. Determination of enzymatic activities in ecotypic *Saccharomyces* and non-*Saccharomyces* yeasts. *Electron. J. Environ. Agric. Food Chem.*, **3**, 743-750.

Gray W., 1941. Studies on the alcohol tolerance of yeasts. *J Bacteriol*, **42**, 561-574.

Harrigan W.F., 1998. *Laboratory Methods in Food Microbiology*. 532 p. Academic Press, London.

Iranzo J.F.U., Pérez A.I.B., Cañas P.M.I., 1998. Study of oenological characteristics and enzymatic activities of wine yeasts. *Food Microbiol*., **15**, 399-406.

Izgu F., Altinbay D., Yucelis A., 1997. Identification and killer activity of a yeast contaminating starter cultures of *Saccharomyces cerevisiae* strains used in the Turkish baking industry. *Food Microbiol.*, **14**, 125-131.

Jackson R.S., 2000. *Wine Science: Principles, Practice, Perception*. 648 p. Academic Press, San Diego.

Jimoh S.O., Ado S.O., Ameh J.M., Whong C.M.Z., 2012. Characteristics and diversity of yeast in locally fermented beverages sold in Nigeria. *World J. Eng. Pure Appl. Sci.*, **2**, 40.

Kapsopoulou K., Barouti E., Makrioniti A., Kostaki K., 2008. Occurrence of *Saccharomyces cerevisiae* killer yeasts in wine-producing areas of Greece. *World J. Eng. Pure Appl. Sci.*, **24**, 1967-1971.

Lee C., Yu R., 2007. Selection and oenological comparison of Taiwan black queen grape wine yeasts. *J. Food Drug Anal.*, **15**, 299-309.

Lopes C.A., Rodríguez M.E., Sangorrin M., Querol A., Caballero A.C., 2007. Patagonian wines: the selection of an indigenous yeast starter, *J. Ind. Microbiol. Biotechnol.*, **34**, 539-546.

Marquina D., Santos A., Peinado J.M., 2002. Biology of killer yeasts. *Int. Microbiol.*, **5**, 65-71.

Nikolaou E., Soufleros E.H., Bouloumpasi E., Tzanetakis N., 2006. Selection of indigenous *Saccharomyces cerevisiae* strains according to their oenological characteristics and vinification results. *Food Microbiol.*, **23**, 205-211.

Nikolaou E., Andrighetto C., Lombardi A., Litopoulou-Tzanetaki E., Tzanetakis N., 2007. Heterogenity in genetic and phenotypic characteristics of *Saccharomyces cerevisiae* strains isolated from red and white wine fermentations. *Food Control*, **18**, 1458-1465.

OIV, 2012. Guidelines for the characterization of wine yeasts of the genus *Saccharomyces* isolated from vitivinicultural environments. Resolution OIV-Oeno 370-2012.

Orlić S., Očić N., Jeromel A., Huić K., Redžepović S., 2005. Selection of indigenous *Saccharomyces cerevisiae* strains from Kutjevo wine growing area at the laboratory scale. *Agric. Conspec. Sci.*, **70**, 93-97.

Ough C.S., 1992. *Winemaking Basics*. 336 p. Food Products Press, New York.

Ough C.S., Amerine M.A., 1988. *Methods for Analysis of Musts and Wines*. 400 p. Wiley-Interscience Publications, Canada.

Ozcelik F., Donmez S., 1993. Killer yeasts and the determination of killer characters of some yeasts. *Turk.* J. *Biol.*, **17**, 1-4.

Paraggio M., Fiore C., 2004. Screening of *Saccharomyces cerevisiae* wine strains for the production of acetic acid. *World J. Microbiol. Biotechnol.*, **20**, 743-747.

Parish M.E., Carroll D.E., 1987. Fermentation characteristics of *Saccharomyces cerevisiae* isolates from *Vitis rotundifolia* grapes and musts. *Am. J. Enol. Vitic.*, **38**, 45-48.

Perez-Coello M.S., Briones-Perez A.I., Ubeda-Iranzo J.F., Martin-Alvarez P.J., 1999. Characteristics of wines fermented with different *Saccharomyces cerevisiae* strains isolated from the La Mancha region. *Food Microbiol.*, **16**, 563-573.

Rainieri S., Pretorius I.S., 2000. Selection and improvement of wine yeasts. *Ann. Microbiol.*, **50**, 15-31.

Regodon J.A., Perez Valdes M.E., De Miguel C., Ramirez M., 1997. A simple and effective procedure for selection of wine yeast strains. *Food Microbiol.*, **14**, 247-254.

Regulation (EC) nº 1622/2000. Official Journal of the European Communities.

Ribéreau-Gayon P., Dubourdieu D., Doneche B., Lonvaud A., 2006. *Handbook of Enology*. *Vol. 1, The Microbiology of Wine and Vinifications.* 497 p. 2nd Edition. John Wiley&Sons Ltd, Chichester.

Schuller D., Casal M., 2005. The use of genetically modified *Saccharomyces cerevisiae* strains in the wine industry. *Appl. Microbiol. Biotechnol*., **68**, 292-304.

Senses-Ergul S., Ozbas Z.Y., 2014. Diversity of yeast species during spontaneous fermentation of Kalecik Karasi and Emir grapes grown in Cappadocia region of Turkey. *J. Pure Appl. Microbiol.*, **8**, 3537-3549.

Serra A., Strehaiano P., Taillandier P., 2005. Influence of temperature and pH on *Saccharomyces bayanus* var. *uvarum* growth; impact of a wine yeast interspecific hybridization on these parameters, *Int. J. Food Microbiol.*,**104**, 257-265.

Spiczki M., Romano P., Lipani G., Miklos I., Antunovics Z., 2001. Analysis of yeasts derived from natural fermentation in a Tokaj winery. *A. van Leeuw. J. Mıcrob.*, **79**, 97-105.

Strauss M.L.A., Jolly N.P., Lambrechts M.G., van Rensburg P., 2001. Screening for the production of extracellular hydrolytic enzymes by non-*Saccharomyces* wine yeasts. *J. Appl. Microbiol.*, **91**, 182-190.

Suarez-Lepe J.Á., Morata A., 2012. New trends in yeast selection for winemaking. *Trends in Food Sci Technol*, **23**, 39-50.

Thomson J.M., Gaucher E.A., Burgan M.F., de Kee D.W., Li T., Aris J.P., Benner S.A., 2005. Resurrecting ancestral alcohol dehydrogenases from yeast. *Nature Genetics*, **37**, 630-633.

Yarrow D., 2000. Methods for the isolation, maintenance and identification of yeasts. *In: The yeasts: a Taxonomic study*. Kurtzman C.P., Fell J.W. (eds.), Elsevier, Amsterdam.