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Yeast Population Dynamics in Spontaneous and Inoculated Alcoholic Fermentations of Zametovka Must

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Summary

Inoculated fermentations, which are more rapid and more reliable than spontaneous fermentations, and assure predictable wine quality, are nowadays prevalent in Slovenia's large-scale wine production. However, spontaneous fermentation strengthens local characteristics of wine and offers opportunities for technological innovation. In the 1999 vintage, spontaneous and inoculated fermentations of Zametovka (*Vitis vinifera*) grape must were studied. Zametovka is the main red variety in production of traditional Slovene red blend wine, Cvček. The diversity of yeast species and strains in both of the investigated fermentations was determined by molecular and traditional identification methods. The outset of alcoholic fermentation, yeast growth kinetics, and yeast population dynamics presents the main differences between the examined fermentations. Yeast population diversity was higher in the spontaneous process. Dominant yeast isolates from spontaneous fermentation were identified as *Candida stellata*, *Hanseniaspora uvarum* and *Saccharomyces cerevisiae*; whereas *Saccharomyces bayanus*, *Pichia kluyveri*, *Pichia membranifaciens* and *Torulaspora delbrueckii* were found less frequently. Dominant species in the inoculated fermentation was *Saccharomyces cerevisiae*; other species found in smaller numbers were *Candida stellata*, *Hanseniaspora uvarum* and *Debaryomyces hansenii* var. *hansenii*. Using PFGE, we were able to distinguish among 15 different *Saccharomyces cerevisiae* strains and three different *Saccharomyces bayanus* strains isolated from spontaneous fermentation, whereas, in the case of inoculated fermentation, only two *Saccharomyces cerevisiae* strains were found. Their chromosomal patterns coincide with the chromosomal patterns of the starter culture strains.

Key words: wine yeasts, red grape must fermentation, population dynamics, PFGE, PCR – RFLP of rDNA

Introduction

The fermentation of grape must into wine is a complex biochemical process involving interactions between

yeasts, bacteria, filamentous fungi and their viruses. Of these organisms, it is the yeasts that play a key role.

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During fermentation, yeasts utilize sugars and other constituents of grape must as substrate for their growth, converting them into ethanol, carbon dioxide and other metabolic end products that contribute to the chemical composition and sensory qualities of the wine (1). Diversity of the yeast species and strains also depends on the types of alcoholic fermentation. In general, spontaneous and inoculated alcoholic fermentations have been used in modern winemaking process. Even today, winemakers in many wineries, especially in traditional winegrowing areas, accept the potentially staggering risks involved in spontaneous fermentations to achieve stylistic distinction and vintage variability. In large-scale wine production, where rapid and reliable fermentations are essential for consistent wine flavour and predictable quality, the use of selected yeast starter culture of known ability is preferred (2,3). Yet 80 % of worldwide wine production is still produced with spontaneous fermentations (4). As the type and amount of metabolic products are also a consequence of the diversity and composition of microorganisms and their dynamics and frequency of occurrence, it is very important to know more about the succession of the entire microflora during the alcoholic fermentation process (5,6).

Diversity of yeast species and strains is monitored by physiological tests and different molecular methods. Especially over the last decade, with the rapid development of molecular biology, new techniques for the identification and typing of microorganisms have emerged (7). The restriction fragment length polymorphism of PCR-amplified fragments from the rDNA gene cluster (PCR RFLP of rDNA), have proved to be a fast and powerful technique for differentiating yeasts on the species level (8–11). On the other hand, pulsed field gel electrophoresis (PFGE) is a very successful method for recognizing different *Saccharomyces cerevisiae* strains during fermentations (6,12–15). PFGE was also successfully used to distinguish between *S. cerevisiae* and *S. bayanus* species (16–18).

Guillamón *et al.* (19) studied the relationship between the genetic distance and geographical or ecological origin of *Saccharomyces* wine yeast strains. They concluded that yeast strains conducting red wine fermentation were significantly grouped according to their geographical origin, independent of the wine type and the grapevine variety. Vezinhet *et al.* (20) also stated that wide distribution of some strains in the studied areas, and their presence over the years, constitute evidence for the occurrence of representative native strains in a wine region.

Therefore, the aim of our work was to obtain precise information about the differences in the dynamics of yeast species and strain populations during spontaneous and inoculated alcoholic fermentations of the Zametovka grape must in Dolenjska winegrowing district in the south-eastern part of Slovenia.

Material and Methods

Grapevine variety and harvest

Zametovka (*Vitis vinifera*) is the main red variety in the production of traditional Slovene red blend wine,

Cvicek, and belongs to the ecological-geographical group of the *Pontica* varieties. Cvicek's geographical origin is under the special protection of the Slovene Wine Law (21).

Only healthy and undamaged grapes were gathered from the selected vineyard located in Dolenjska winegrowing district so SO₂ was not added. Immediately after harvesting, they were transported to the Agricultural Institute of Slovenia in Ljubljana, one hour away from the wine region.

Fermentation and post fermentation handling

Not to destroy the indigenous yeast population, the crushed grapes were not treated with SO₂; pH of the must was 3.03. It was divided into two tanks of 50 L; in the first tank, must was inoculated with a commercial starter culture of *S. cerevisiae* – Fermicru VR5 (Italy) (0.20 g lyophilised yeast *per* L). In the second, must was not inoculated. Fermentation temperature was controlled by a cooling coil and was kept between 18 and 20 °C. Must of the spontaneous fermentation was macerated for three days and must of the inoculated fermentation for two days; after maceration, must was pressed and the fermentations continued. Both fermentations ended after ten days when sugar concentration fell below 5.0 g/L. Final ethanol volume fractions were 8.90 and 9.71 % for the wine produced by inoculated and spontaneous fermentation, respectively. Fermentation processes were characterized also by changes in sugar concentration (g/L). Hewlett-Packard 1110 liquid chromatography was applied to determine sugars. Separation was achieved on a Bio-Rad HPX-87H column (mobile phase 0.007 M H₂SO₄). Sugars were detected based on refractive index and UV absorption measurements.

Three weeks after the fermentations were completed, Zametovka wines were racked and blended with red and white grapevine varieties of Modra frankinja and Kraljevina, in accordance with traditional technology. Spontaneous and inoculated fermentations of both were carried out concomitantly with those of Zametovka. The Cvicek obtained after blending wines of spontaneous or inoculated fermentations contained the following proportions of varieties: 60 % Zametovka, 20 % Modra frankinja and 20 % Kraljevina wine in accordance with Regulations (22).

Sampling

During each fermentation, eight samples were taken: at the time 0, meaning immediately after the tanks had been filled with must and maceration/fermentation had begun, and again after 24, 48, 72, 96, 144, 192 and 240 hours. Samples were plated on yeast malt (YM) agar (0.3 % yeast extract, 0.3 % sugar extract, 0.5 % pepton, 1.0 % glucose and 2.0 % agar) at different serial decimal dilution in triplicate (10⁻¹ to 10⁻⁷). Plates were incubated at 28 °C for two days. From each sample 36–42 colonies were randomly picked from the countable plate. The number of isolated yeast strains in each fermentation was 324, comprising a total of 648 isolates. These were maintained in 10 % of glycerol at –80 °C.

Pulsed field gel electrophoresis (PFGE)

The chromosomal DNA of yeast strains was prepared according to the procedure published by Carle and Olson (23) as modified by Raspor *et al.* (24). The yeast chromosomes were separated by CHEF apparatus (LKB Pulsaphor™ System, Uppsala, Sweden) at 170 V for 15 h with 60 s pulse time, 8 h with 90 s pulse time, and 1 h with 100 s pulse time in 1 % agarose gel in 0.5 x TBE buffer chilled at 12 °C. The chromosome size marker employed was *S. cerevisiae*, YPH 755 (Roche Diagnostics, Mannheim, Germany). The agarose gels were stained with ethidium bromide (0.5 µg/mL) and subsequently documented by Gel Doc 2000 (BIO-RAD system, Hercules, USA).

PCR RFLP of 18S + ITS ribosomal DNA

DNA was isolated according to Raspor *et al.* (24). The DNA amplifications were performed in Perkin Elmer PCR System 2400. They were carried out in a 20 µL reaction volume containing 1 x PCR buffer (Promega, Madison, USA), 2 mM of each dNTP, 2 mM MgCl₂, 0.5 pmol of each primer and 1 U of Taq DNA polymerase (Promega, Madison, USA). The primer pair used for amplification of the 18S-ITS1-5.8S-ITS2 rDNA region was: NS1 (5' GTAGTCATATGCTTGCTC 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') (25). The PCR conditions were as follows: the initial denaturing cycle of 5 min at 95 °C, followed by 35 cycles consisting of 30 s at 95 °C, 30 s at 60 °C and 3 min at 72 °C, and the final extension step of 7 min at 72 °C. PCR products were restricted individually with endonucleases *CfoI*, *MspI*, *HaeIII*, and *RsaI* (Roche Diagnostics, Mannheim, Germany), following the manufacturer's instructions and separated by electrophoresis in 1.5 % agarose gel and 1 x TAE buffer, stained with ethidium bromide (0.5 µg/mL), and photographed by Gel Doc 2000.

Physiological testing

Instruction for the preparation of materials, selection of tests and testing conditions were followed as described by Barnett *et al.* (26).

Sensory evaluation

After blending wines of Zameťovka, Kraljevina and Modra frankinja, which were all concomitantly fermented as spontaneous and inoculated processes, eight panelists evaluated the sensory properties of two final Cvček wines. Two samples represented our spontaneous and inoculated fermentations and four samples were randomly selected. The wines were evaluated in one replication by modified 20 point Buxbaum method (27) and a duo test (28) performed on two wines from our experiment. With the Buxbaum method, the following score rank was used: maximum four points for appearance, maximum four points for aroma and bouquet, maximum ten points for taste and maximum two points for overall impression. The results from both analyses were not statistically processed, and as far as this work is concerned, they will be considered only to support a part of the discussion.

Results

Kinetics of yeasts growth and sugars utilization

In the initial 48 hours of spontaneous and inoculated fermentations the concentration of total sugars decreased from 176 to 116 g/L and from 176 to 174 g/L, respectively (Fig. 1). During the first 48 hours of spontaneous fermentation, the number of total yeasts in the must ($1.1 \cdot 10^6$ cfu/mL) was 50-fold lower than the number of total yeasts in the inoculated one ($5.2 \cdot 10^7$ cfu/mL) (Fig. 1). After 72 hours, total sugars decreased from 176 to 100 g/L in the inoculated fermentation, and from 176 to 154 g/L in the spontaneous one. The dynamics of inoculated fermentation was more intense in the early stage of the process (until the 96th hour of fermentation), whereas the dynamics of the spontaneous fermentation was more intense during the later stages (after the 72nd hour of fermentation) (Fig. 1).

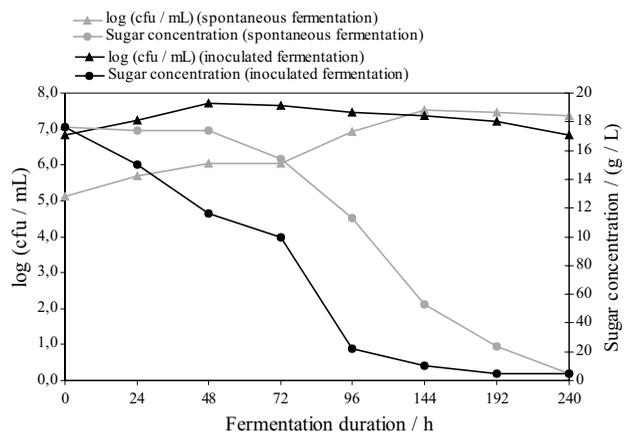


Fig. 1. The kinetics of yeasts growth and sugars utilization during spontaneous and inoculated fermentations of Zameťovka grape must

Yeast species diversity during both wine fermentations

Identification of pure cultures was carried out combining molecular methods and physiological tests. The yeast isolates isolated at the time 0 and after 48, 96, 144, 192 and 240 hours of spontaneous process and at the time 0 and after 48 and 240 hours of those inoculated were identified. Using PFGE, the yeast isolates were divided into six non-*Saccharomyces* groups and two *Saccharomyces* groups. The yeast isolates of two *Saccharomyces* groups were identified as *Saccharomyces cerevisiae* and *Saccharomyces bayanus* by PFGE and only confirmative physiological tests (26) were done for the representatives of these two groups. For the species identification of six non-*Saccharomyces* groups the representatives of these groups were chosen and PCR – RFLP of 18S + ITS rDNA was employed. The species-specific restriction patterns were obtained and compared to those of the type strains of 54 species that are most frequently isolated from the grape/must/wine system (26) and represent a database in our laboratory. Fig. 2 shows the restriction patterns of six isolates obtained with *RsaI* and *MspI* enzymes. Four yeast species out of six were identi-

fied as *Hanseniaspora uvarum*, *Pichia kluyveri*, *Pichia membranifaciens* and *Debaryomyces hansenii* var. *hansenii*. The species identity was further confirmed by 7–12 selected physiological tests, suggested for confirmation of individual species by Barnett *et al.* (26). For the representatives of two non-*Saccharomyces* groups that were not identified by molecular methods it was necessary to perform physiological tests required for identification of individual species (26) and they were identified as *Candida stellata* and *Torulaspora delbrueckii*.

During the initial 48 hours of spontaneous fermentation, no *S. cerevisiae* strain was isolated (Fig. 3). The species *C. stellata* was prevalent at the beginning of fermentation. As the process continued, the proportions of species *C. stellata* and *P. kluyveri* decreased, while the proportion of *H. uvarum* increased. After 96 hours of fermentation, the amount of total sugars decreased by 63 g/L and the share of *S. cerevisiae* was 45.0 %. After 144 hours, total sugars in the must decreased by 123 g/L and the share of *S. cerevisiae* was 87.5 %. After 192 hours of fermentation, the share of non-*Saccharomyces* yeasts was only 7.5 %. The species *S. cerevisiae* completed the fermentation. The species *S. bayanus* was isolated after 96 and 192 hours of the process with shares of 5.0 and 2.5 %, respectively (Fig. 3).

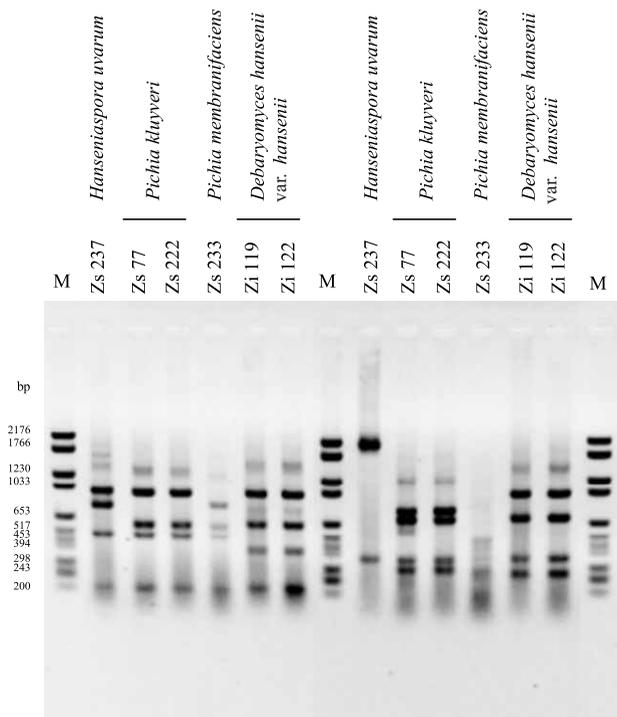


Fig. 2. The restriction enzyme patterns of amplified 18S + ITS rDNA of six yeast isolates obtained with *RsaI* (left side of the middle molecular size marker pattern) and *MspI* enzymes (right side of the middle molecular size marker pattern) (Lane M: molecular size marker of DNA, number VI (Roche Diagnostics, Mannheim, Germany)).

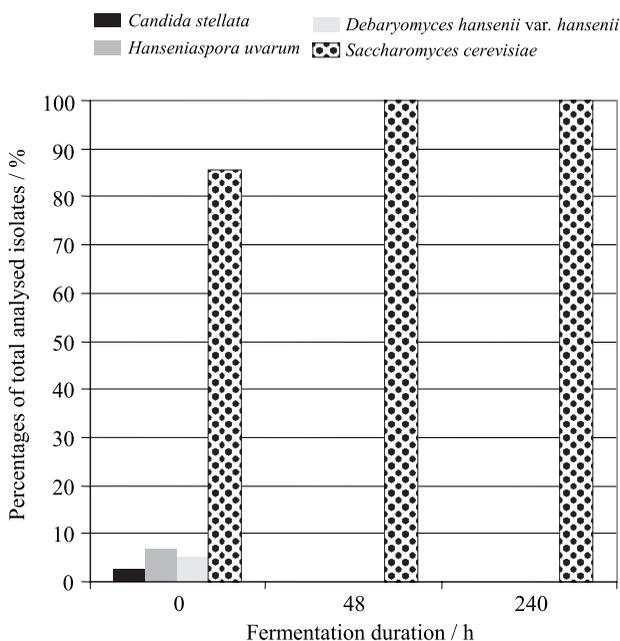


Fig. 4. The frequencies of occurrence of different yeast species during inoculated fermentation of Zametovka grape must.

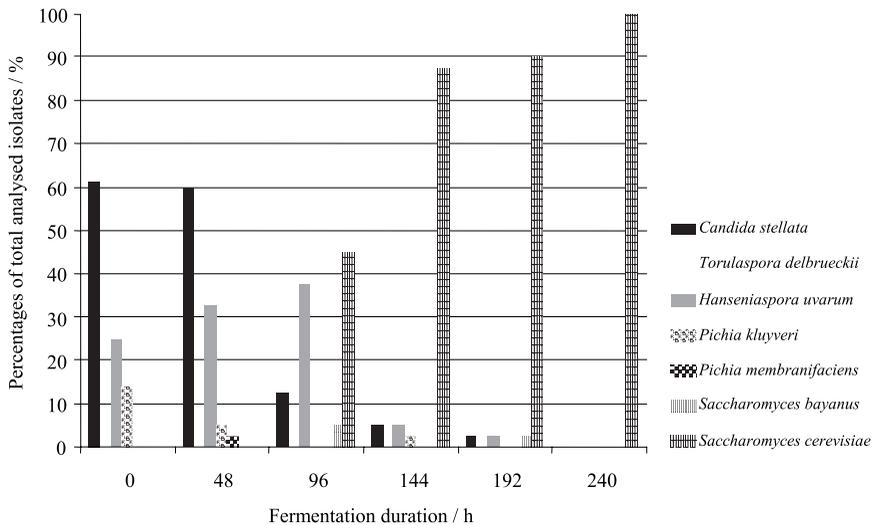


Fig. 3. The frequencies of occurrence of different yeast species during spontaneous fermentation of Zametovka grape must

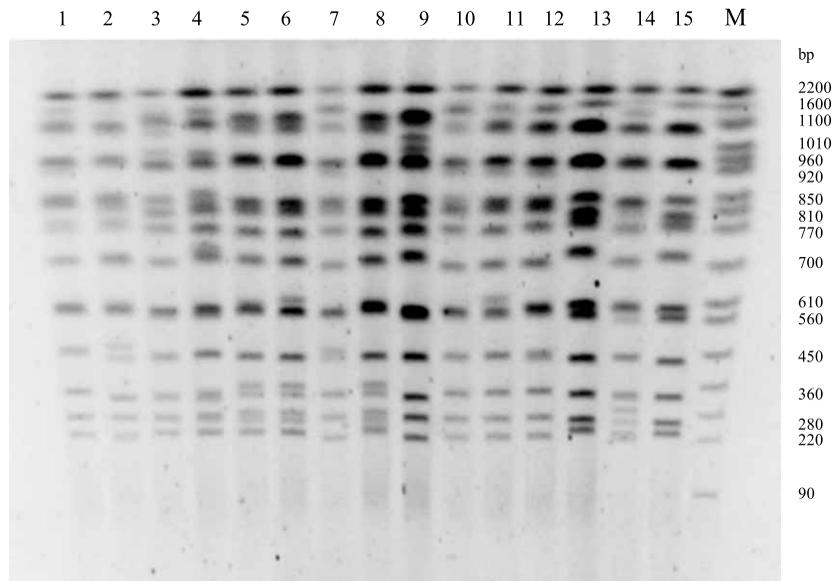


Fig. 5. The karyotypes of 15 randomly selected *S. cerevisiae* strains isolated after ten days of spontaneous fermentation (Lanes 1–12) and after two days of inoculated fermentation (Lanes 13–15) (Lane M: marker *S. cerevisiae*, Roche Diagnostics, Mannheim, Germany (YPH 755); karyotype Ssc1: Lanes 1, 7, 10, 12; karyotype Ssc3: Lanes 3, 9; karyotype Ssc6: Lane 11; karyotype Ssc7: Lane 2; karyotype Ssc8: Lane 4; karyotype Ssc9: Lanes 5, 6, 8; karyotype Sic1: Lanes 13, 15; karyotype Sic2: Lane 14).

At the time 0 of inoculated fermentation, 14.3 % of non-*Saccharomyces* isolates were identified as *C. stellata*, *H. wourum* and *D. hansenii* var. *hansenii* (Fig. 4). After 48 hours of fermentation the species of *S. cerevisiae* prevailed.

Yeast strain diversity during both wine fermentations

Yeast strain diversity within *S. bayanus* and *S. cerevisiae* was monitored using PFGE. The starter culture was analysed as well, and two *S. cerevisiae* strains were determined.

Fig. 5 shows karyotypes of *S. cerevisiae*, isolated after ten days of spontaneous fermentation and after two days of the inoculated one. Among the isolates from spontaneous fermentation, 15 different karyotypes of *S. cerevisiae* and 3 karyotypes of *S. bayanus* (Fig. 6) were

determined. From Fig. 6, it can be concluded that after 96 hours of fermentation, four strains (Ssc1, Ssc2, Ssc3 and Ssc9) occurred in the must in greater proportions. All four strains exhibited different patterns of increase and decrease in the total number of isolates per milliliter of must during fermentation. The share of the strain Ssc1 increased from 10.0 to 32.5 % between the 96th and 240th hour of fermentation and was the biggest at the end. The share of the strain Ssc2 increased highly from 7.5 to 30.0 % between the 96th and 144th hour of fermentation and this strain was dominant at the 144th hour, whereas its share decreased to 12.5 % by the 240th hour. The share of the strain Ssc3 increased from 5.0 to 15.0 % between the 96th and 144th hour of fermentation, between the 144th and 192nd hour it decreased to its initial level (7.5 %) and between the 192nd and 240th hour it increased to the same share as in the 144th hour of ferment-

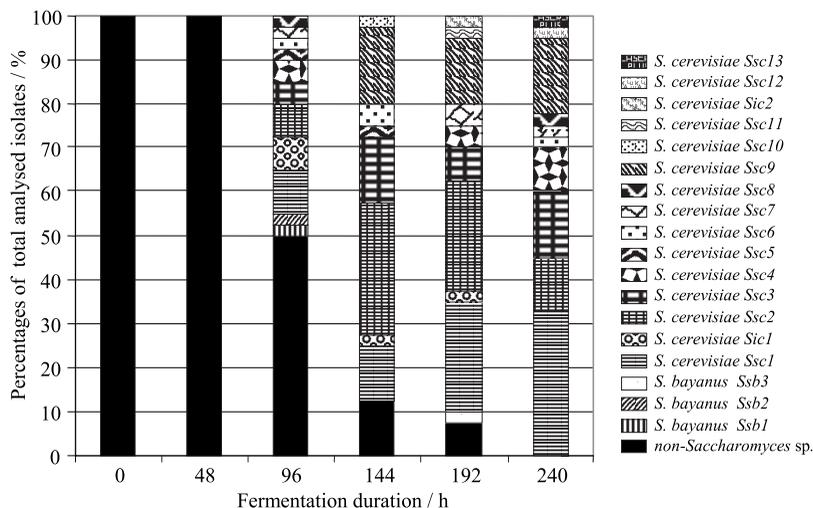


Fig. 6. The frequencies of occurrence of different *S. cerevisiae*, *S. bayanus* and non-*Saccharomyces* karyotypes during spontaneous fermentation of Zametovka grape must

tation (15.0 %). Ssc 9 reached maximum at the 144th hour (between 15.0 and 17.5 %). Two karyotypes (Sic1 and Sic2) of *S. cerevisiae* were identical with the karyotypes of the starter culture strains in spontaneous fermentation.

Fig. 7 displays the share of karyotypes in two *S. cerevisiae* strains from the starter culture that prevailed in the inoculated fermentation.

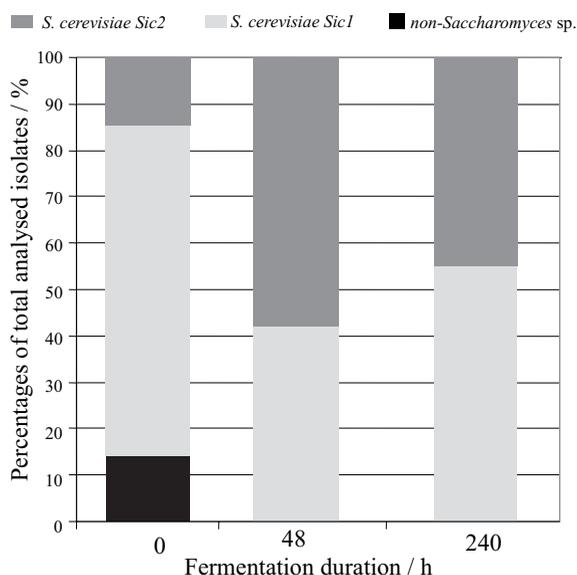


Fig. 7. The frequencies of occurrence of different *S. cerevisiae* and non-*Saccharomyces* karyotypes during inoculated fermentation of Zametovka grape must

Table 1. Scores of sensory evaluation by the 20 point Buxbaum method for six Cvček wines; Cvček 1: experimental wine of inoculated fermentation; Cvček 2: experimental wine of spontaneous fermentation; Cvček 3–6: randomly selected wines produced by inoculated fermentations

Panelists	Cvček wines					
	1	2	3	4	5	6
Panelist 1	15.9	16.2	16.8	16.3	16.1	16.8
Panelist 2	16.1	16.4	16.0	16.5	16.2	16.1
Panelist 3	16.1	16.3	15.5	15.1	15.6	16.2
Panelist 4	16.2	16.3	17.0	16.6	15.6	16.9
Panelist 5	16.3	16.1	16.4	15.9	15.7	15.6
Panelist 6	15.5	16.5	16.8	15.0	15.0	14.5
Panelist 7	17.1	17.0	17.0	17.2	17.2	17.5
Panelist 8	17.0	16.5	14.5	16.5	17.7	18.5
Average	16.3	16.4	16.2	16.1	16.1	16.5
Max	17.1	17.0	17.0	17.2	17.7	18.5
Min	15.5	16.1	14.5	15.0	15.0	14.5

Sensory evaluation

The results of the Buxbaum method for six Cvček wines (Table 1) indicate that the wines were relatively close to each other, particularly when comparing both wines from our experiment; 16.4 points *vs.* 16.3 points for wine of spontaneous fermentation. The results of the duo test (Table 2) were even more evident: for six panelists out of eight, the wine from spontaneous fermentation was better in sensory qualities than in the others produced by inoculated fermentation.

Discussion

The two most important differences between inoculated and spontaneous fermentations were observed as expected: a faster beginning and faster fermentation dynamics in inoculated fermentation. The lag phase of the inoculated process was shorter. The same was also observed in fermentations of Riesling, Chardonnay and Carinyena grape must by Egli *et al.* (5) and Constantí *et al.* (29). The concentration of yeasts determines the dynamics of the process, which is exhibited in the consumption rate of sugars from the must. A later beginning of wine fermentation may cause some problems in wine technology (4), therefore, in large-scale wine production, inoculation of the must with selected cultures of *S. cerevisiae* is used.

In spontaneous fermentation, the non-*Saccharomyces* species predominated during the initial 48 hours, as ethanol concentration was low and SO₂ was not employed. As was shown before, the non-*Saccharomyces* species originated from the surface of grapes (30,31) or winery equipment (30). Although *C. stellata* was not isolated from the grape berries of Zametovka variety (results not published), it prevailed in the must at the beginning of spontaneous fermentation. This yeast species probably entered the must through grapes/must handling, the selective effect of the alcoholic fermentation medium giving it priority (30). As the process proceeded, shares of the species *C. stellata* and *P. kluyveri* decreased, while the share of the species *H. uvarum* increased. If the reports from Constantí *et al.* (32) and Fleet (33) are considered, these results are fairly uncommon. Although the concentration of ethanol was not analysed during the fermentation, it could be assumed that the amount of ethanol formed before the 96th hour was not toxic to the species *H. uvarum*. According to some references, this species is more susceptible to ethanol than the species of *Candida* and *Pichia* (1,34). An increase in the share of *H. uvarum* can be ascribed to its faster growth when compared to *C. stellata* and *P. kluyveri* (1). The predominance

Table 2. Results of the duo test for experimental Cvček wines produced by inoculated and spontaneous fermentation

Wines	Panelists								Sum
	Panelist 1	Panelist 2	Panelist 3	Panelist 4	Panelist 5	Panelist 6	Panelist 7	Panelist 8	
Inoculated fermentation	x ^a		x						2
Spontaneous fermentation		x		x	x	x	x	x	6

Legend: ^a Panelists had to choose the better wine of two options. Their decisions are marked with x.

of *S. cerevisiae* and the vigorous phase of fermentation can be inhibited by *H. uvarum* strains (35). In our study, after 144 hours of spontaneous fermentation, the properties of the medium (sugar concentration, low pH, ethanol content) were advantageous for *S. cerevisiae*, which completed the fermentation.

Relatively high percentage (14.3 %) of non-*Saccharomyces* yeasts at the beginning of inoculated fermentation is probably due to immediate sampling after must was inoculated and the fact that neither grapes nor must were treated with SO₂. The same proportions of non-*Saccharomyces* and *Saccharomyces* yeasts in the early stages of inoculated fermentation of must without added SO₂ were demonstrated also by Constanti *et al.* (29) even after two days of starter culture inoculation.

The diversity of *S. cerevisiae* strains was also higher in a spontaneous process and from the variations of strain shares it was not possible to determine any rule that specified their occurrence. None of the isolated strains prevailed; therefore the population of spontaneous fermentation can be regarded as balanced and diversified. It has been shown by many authors that a succession of different strains in yeast populations existed during the course of the spontaneous process (12,36,37). Two karyotypes of *S. cerevisiae* were identical to the starter culture karyotypes. Since they occurred in the must after the pressing, they might have entered it from the winery equipment, even if all the equipment had been cleaned properly with a mixture of steam and hot water. The other isolated *S. cerevisiae* strains in spontaneous fermentation originated from the microflora present on the surface of the winery equipment, or on the grapes that originated in Dolenjska winegrowing district. Further investigations are needed to isolate the strains typical of the Dolenjska winegrowing district. However, the diversity of the strains *S. cerevisiae* in the inoculated process was rather modest; only two strains prevailed over the other yeast species and strains.

The yeast species' diversity as well as the strains' divergence in the species *S. cerevisiae* in spontaneous fermentations emphasizes originality, autochthonism, and geographical origin of the wine through the diversity of yeast primary and secondary metabolites (38,39). Therefore it can be assumed that the difference in the yeast population dynamics in both fermentations influences the sensory quality of the wine. This was also demonstrated in the sensory evaluation of both our wines, particularly during the duo test.

Determining the differences in the microbiology of spontaneous and inoculated alcoholic fermentations of Zametovka must is important for further enological practice in Cvček production that is restricted to Dolenjska winegrowing district. Winemakers, especially those who produce wines like Cvček under the special protection of the geographical origin paragraph, have to consider the question of whether or not to use starter cultures regardless of their geographical origin. The aim of the presented work was also to take the first step towards the isolation of geographically significant *S. cerevisiae* strains that will be used as starter cultures for Dolenjska winegrowing district.

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Dinamika populacije kvasca pri spontanim i induciranim alkoholnim fermentacijama vinskog mošta Zametovka

Sažetak

U današnjoj industrijskoj proizvodnji vina u Sloveniji prevladava inducirana fermentacija koja je brža i pouzdanija od spontane, a osigurava predvidivu kvalitetu vina. Spontana fermentacija pospješuje lokalne specifičnosti vina i omogućuje tehnološke inovacije. U berbi 1999. provedena je spontana i inducirana fermentacija mošta Zametovka (*Vitis vinifera*). Zametovka je glavna sorta crnog vina u proizvodnji Cvičeka, tradicionalnog slovenskog crnog vina. Raznolikost vrsta i sojeva kvasca u obje ispitivane fermentacije utvrđena je primjenom fizioloških i molekularnih testova. Glavne razlike između ispitivanih fermentacija sastojale su se u različitom početku alkoholne fermentacije, u kinetici rasta i dinamici populacije kvasca. Raznolikost u populaciji kvasca bila je veća pri spontanom procesu. Kao dominantni izolati kvasca pri spontanoj fermentaciji utvrđeni su *Candida stellata*, *Hanseniaspora uvarum* i *Saccharomyces cerevisiae* a rjeđe su se pojavljivali *Saccharomyces bayanus*, *Pichia kluyveri*, *Pichia membranifaciens* i *Torulaspota delbrueckii*. Pri induciranoj fermentaciji dominantna je vrsta *Saccharomyces cerevisiae*, a nađen je manji broj vrsta *Candida stellata*, *Hanseniaspora uvarum* i *Debaryomyces hansenii* var. *hansenii*. Koristeći gel-elektroforezu u pulsirajućem polju (PFGE), iz mošta pri spontanoj fermentaciji izolirano je 15 različitih sojeva *Saccharomyces cerevisiae* i tri različita soja *Saccharomyces bayanus*, a u induciranoj fermentaciji opažena su samo dva soja *Saccharomyces cerevisiae*. Njihova kromosomska struktura podudara se sa strukturom kromosoma soja starter-kulture.