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*original scientific paper*

# Biodiversity of Yeasts During Plum Wegierka Zwykla Spontaneous Fermentation**

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**Summary**

The study comprises an analysis of the yeast microbiota that participated in the spontaneous fermentation of crushed Wegierka Zwykla plum fruit, which is the raw material for slivovitz production in the mountain region in the south of Poland. *Saccharomyces cerevisiae* yeast strains were differentiated by means of the killer sensitivity analysis related to a killer reference panel of 9 well-known killer yeast strains. The first phase of the fermentation was dominated by the representatives of *Kloeckera apiculata* and *Candida pulcherrima* species, which reached their maximum concentration ($1.4 \cdot 10^6$ CFU/mL) after 48 h of the process. Almost all yeasts isolated during the following days were classified as *S. cerevisiae* and the killer sensitivity analysis revealed a high population diversity of this species and the presence of 14 different strains that changed quantitatively and qualitatively throughout the fermentation period.

*Key words:* plums microbiota, indigenous *S. cerevisiae*, killer sensitivity, strain diversity, slivovitz

## Introduction

Natural (spontaneous) alcoholic fermentation of grape juice in the traditional wine production technology is carried out by a mixture of different yeast species. The initial concentration of yeast population in freshly extracted grape must ranges between $10^3$–$10^6$ CFU/mL. The count of *Saccharomyces cerevisiae* cells is usually very low. The early stages of the alcoholic fermentation are dominated by non-*Saccharomyces* species, especially *Kloeckera apiculata*/*Hanseniaspora uvarum*, which constitute from 50 to 75 % of the total yeast count, and *Candida* (especially *C. stellata* and *C. pulcherrima*). The growth of *Debaryomyces*, *Zygosaccharomyces*, *Pichia*, *Hansenula* and *Kluyveromyces* species occurs to a lesser extent (*1–3*).

Qualitative and quantitative composition of the yeast microbiota in fermenting musts depends mainly on the following factors: region of the fruit origin, production procedure, type of the produced beverage, initial cell concentration, temperature, pH, $SO_2$ and ethanol concentration (*1,2*).

Non-*Saccharomyces* spp. can proliferate to the final population of $10^6$–$10^9$ CFU/mL in fermenting musts and mashes. It is accepted that such a high content of cells can significantly affect the analytical composition and quality of the beverage. Therefore, the physiological and metabolic properties of the non-*Saccharomyces* yeasts could be of practical importance (*3*).

It is accepted that the content of wild non-*Saccharomyces* yeasts begins to decrease rapidly after two days of their intensive activity in contrast to *Saccharomyces* species, which takes over the fermentation process (4). Microorganisms of this species do not form a uniform group either. Research by means of PCR-RFLP (*1,5*), PFGE

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methods (*6*) and the killer sensitivity analysis (*7*) showed a high diversity within *S. cerevisiae* species. Individual strains differ in the genotypic and/or biochemical profile and their concentration changes throughout the fermentation process.

The presence of the killer strains can affect the population differentiation of the microorganisms during fermentation and as a consequence influence the quality of the alcoholic beverages. The killer yeasts are able to kill sensitive yeasts by secreting proteinaceous or glycoproteinaceous toxins that they themselves are immune to (*7,8*).

The genes responsible for this »killer« phenomenon may be carried on a chromosome (*S. cerevisiae* KHS, KHR, *Williopsis mrakii*), on a double-stranded RNA (dsRNA) (*S. cerevisiae* K1, K2, K28, *Ustilago maydis*, *Hanseniaspora uvarum*) or on a linear double-stranded DNA (dsDNA) (*Kluyveromyces lactis*, *Pichia inositovora*, *Pichia acaciae*) (*9–11*).

The research conducted so far has shown that the killer sensitivity analysis is an exceptionally useful method for the discrimination of yeasts at the strain level (*7,8*). For this purpose a reference panel of different well-known yeast killer strains has been applied. This method is considered significantly cheaper but less powerful than molecular methods used for the classification of this group of microorganisms.

The aim of the present study was to characterize qualitatively and quantitatively the yeast microbiota that participate in the individual stages of the spontaneous fermentation of crushed Wegierka Zwykla plums. The plums were picked from an orchard located in the submontane region in the south of Poland, where the plum brandy Śliwowica Łącka has been produced according to traditional recipes since the 18th century (*12*). The killer sensitivity analysis was used for the differentation of *S. cerevisiae* strains.

## Materials and Methods

### *Plums and plum orchard*

Wegierka Zwykla plums were picked in September 2002 in a plum orchard located on the borderland between the town of Łącko and the village of Czarny Potok (Poland). The orchard with young trees (7–8 years old, about 3 meters high) is situated on a gentle slope facing north-eastwards and occupying an area of about 3500 $m^2$. The plantation is surrounded mainly by apple orchards and there are farm buildings on its north-east side. The weather conditions in the month before picking the fruit were characterized by a temperature of 11 to 25 °C, small to moderate overcast, baffling, mainly south and east wind (3–23 m/s) and a total rainfall of 110 mm.

### *Spontaneous fermentation*

The plums were sorted (mechanically injured, decayed and rotten fruits were removed), cut in quarters and weiged out to 2.5 kg per 5-L sterile glass flask. The fruits were pressed so that the juice covered their surface, then the flasks were stopped with fermentation tubes with glycerine. The tests were repeated three times. Samples of fermenting juice were taken every 2 days during the beginning of fermentation (stage 1, density (g/L): 1.075–1.100, 0 to 6th day) and every 3–4 days during the central stage of fermentation (stage 2, density (g/L): 1.020–1.040, from 7th to 19th day) and the last stage of fermentation (stage 3, density (g/L): 993–1.000, from 20th to 30th day) (*1*). After appropriate dilution ($10^{-2}$–$10^{-6}$) samples were spread (1 mL each) onto WL nutrient agar (Fluka Chemie, Buchs, Switzerland) specific for growth of species of the genus *Saccharomyces*, and lysine agar selective medium (Oxoid, Basingstoke, UK) on which only the non-*Saccharomyces* yeasts can grow (*1,13*). After incubation at 25 °C for 4–5 days, colonies were enumerated and 10 colonies from each medium were isolated (with the exception of the first two samplings when, because of high amount of growing colonies, 20 of them were isolated). The fermentation was carried out for 30 days until stabilization of the sample mass was reached. Species identification was carried out using API 20C AUX system (bioMérieux) and some additional biochemical tests (*14*).

### *Killer sensitivity analysis*

All the isolates identified as indigenous strains of *S. cerevisiae* (52 strains) were analysed and their killer sensitivity patterns were determined related to a killer reference panel of 9 well-known yeast killer strains: *S. cerevisiae* CBS 7302 (K1), *S. cerevisiae* CBS 6505 (K2), *S. cerevisiae* CBS 7903 (K3), *Pichia anomala* CBS 5759 (K4), *Debaryomyces vanrijiae* var. *vanrijiae* CBS 4072 (K5), *Pichia membranifaciens* var. *membranifaciens* CBS 7373 (K7), *Pichia anomala* CBS 1982 (K8), *Williopsis saturnus* var. *mrakii* CBS 1707 (K9), *Kluyveromyces lactis* var. *drosophilarum* CBS 2896 (K10).

### *Assay for phenotypic sensitivity*

Killer sensitivity was assayed using seeded-agar-plate technique (*7,8*). YEPD-MB agar plates containing 3 mg of methylene blue per 100 mL were buffered to pH= 4.6 with 0.5 M phosphate-citrate buffer. Each wild yeast culture was suspended in sterile water (~$10^5$ cells/mL) and plated as a lawn onto YEPD-MB agar. These seed plates were streaked with thick smears of a 48-hour killer reference culture and then incubated at 18 °C for 48 h. If the killer reference strain was surrounded by a clear zone of inhibition fringed with blue colour, the lawn wild yeast was declared sensitive. The lawn strain was considered killer resistant if there was no clear zone of inhibition fringed with blue colour (*7*).

## Results

### *Yeast microbiota analysis during plum spontaneous fermentation*

A total of 167 yeast colonies were isolated, 51 from lysine medium and 116 from WL nutrient agar. Yeasts isolated from lysine medium were identified by API 20C AUX system. All 116 yeasts isolated from WL nutrient agar were tested for utilisation of maltose, sucrose and lactose as carbon sources, and the inability to use lysine and nitrate as nitrogen sources. From morphology of

vegetative cells and asci, as well as from metabolic data, 52 strains of genus *Saccharomyces* were recognized (*15,16*).

The initial concentration of fungal microorganisms averaged ca. $0.8 \cdot 10^6$ CFU/mL and the representatives of *K. apiculata* and *C. pulcherrima* species constituted over 80 % of total yeasts. Moreover, relatively high amounts of *Rhodotorula mucilaginosa* and *R. graminis* (9 %) and *Aureobasidium* sp. (5 %) were present, which dominated on the fresh but not fully ripe fruits (Fig. 1).

After two days of fermentation a rapid growth of yeast population up to the level of $1.4 \cdot 10^6$ CFU/mL was observed. Simultaneously, the anaerobic conditions created caused the complete extinction of aerobic microorganisms. The rising concentration of ethanol resulted also in the elimination of some fermentative strains or facultative anaerobes, *e.g. Aureobasidium* spp. The fermenting must was dominated by two species: *K. apiculata* and *C. pulcherrima*, which constituted over 98 % of the total population of microorganisms.

The increase of the yeast microbiota continued during the next 48 hours until the maximum of $10.3 \cdot 10^6$ CFU/mL was achieved. The qualitative composition of the yeasts present in the fermented must underwent a complete change too. Because of the increasing concentration of ethanol and other fermentation products the non-*Saccharomyces* cell count was strongly reduced, the environment was however inhabited by the *S. cerevisiae* strains that could tolerate higher concentrations of ethyl alcohol. After 4 days of fermentation their share rose up to 99.8 %, in spite of the fact that they were undetectable during the first two days of the process.

It has to be noted that the number of microorganisms in the must was decreasing from the 5th day of the fermentation. On the 10th day it was reduced by ~50 % ($4.3 \cdot 10^6$ CFU/mL). In samples that were isolated on this stage of the fermentation no significantly higher presence of microorganisms other than *S. cerevisiae* was observed (Fig. 1).

Until the end of the process the content of yeasts was decreasing in logarithmic rate and on the 30th day achieved the level of $1.9 \cdot 10^4$ CFU/mL. The samples that were stored further were characterized also by a low concentration of living yeast cells.

Throughout the fermentation process a low amount of moulds (*Aspergillus*, *Penicillium*) was isolated. Their growth was probably inhibited. However, conidial stages occurred, so that the concentration of these microorganisms in all samples remained on a constant level.

### *Killer sensitivity patterns of isolated indigenous Saccharomyces cerevisiae strains*

Killer sensitivity analysis during the fermentation revealed the presence of 14 different killer toxin sensitivity patterns, which were marked with consecutive letters of alphabet according to the increasing killer resistance (Table 1). The most numerous group was formed by cultures sensitive to at least seven different toxins (54 % of total *S. cerevisiae* strains).

During the turbulent fermentation stage (first 6 days), when there was a significant quantity of non-*Saccharomyces* cells present in the must, the patterns from A to H dominated and the strain E occurred most often (over half of all isolated *S. cerevisiae*). The concentration of sensitive cells was decreasing gradually during the next days, however, more and more killer resistant yeasts appeared. From the 20th day of the fermentation mainly the strains from K to N (immune to the lethal activity of the majority of killer toxins) were identified. An exception was the presence of the strains A and C, the concetration of which was maintained in the fermenting must at a similar level throughout the fermentation process (Fig. 2).

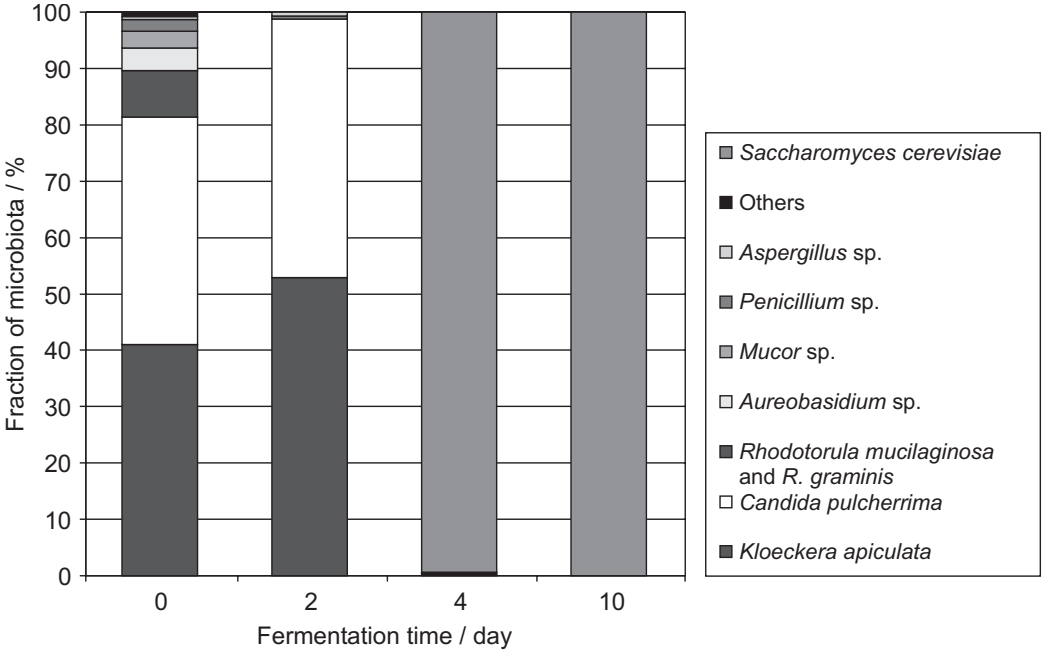


**Fig. 1.** Qualitative profile of the fungal microbiota isolated during plum must spontaneous fermentation

Table 1. Killer sensitivity patterns and their frequencies of appearance in indigenous *S. cerevisiae* populations associated with plum must spontaneous fermentation

| Pattern symbol | Specific killer sensitivity response | Number of strains | Frequency of appearance / % |
|---|---|---|---|
| A | K1,K2,K3,K4,K7,K8,K9,K10 | 6 | 12.0 |
| B | K1,K2,K4,K5,K7,K8,K9,K10 | 2 | 4.0 |
| C | K1,K3,K4,K5,K7,K8,K9,K10 | 8 | 16.0 |
| D | K1,K3,K4,K5,K7,K9,K10 | 1 | 2.0 |
| E | K1,K4,K5,K7,K8,K9,K10 | 10 | 20.0 |
| F | K1,K2,K4,K7,K8,K10 | 2 | 4.0 |
| G | K1,K4,K5,K7,K9,K10 | 1 | 2.0 |
| H | K1,K4,K5,K8,K9,K10 | 3 | 6.0 |
| I | K1,K4,K7,K8,K9,K10 | 2 | 4.0 |
| J | K1,K4,K7,K8,K10 | 2 | 4.0 |
| K | K3,K5,K7,K9 | 5 | 10.0 |
| L | K2,K3,K4 | 1 | 2.0 |
| M | K3,K5,K7 | 6 | 12.0 |
| N | K7 | 1 | 2.0 |

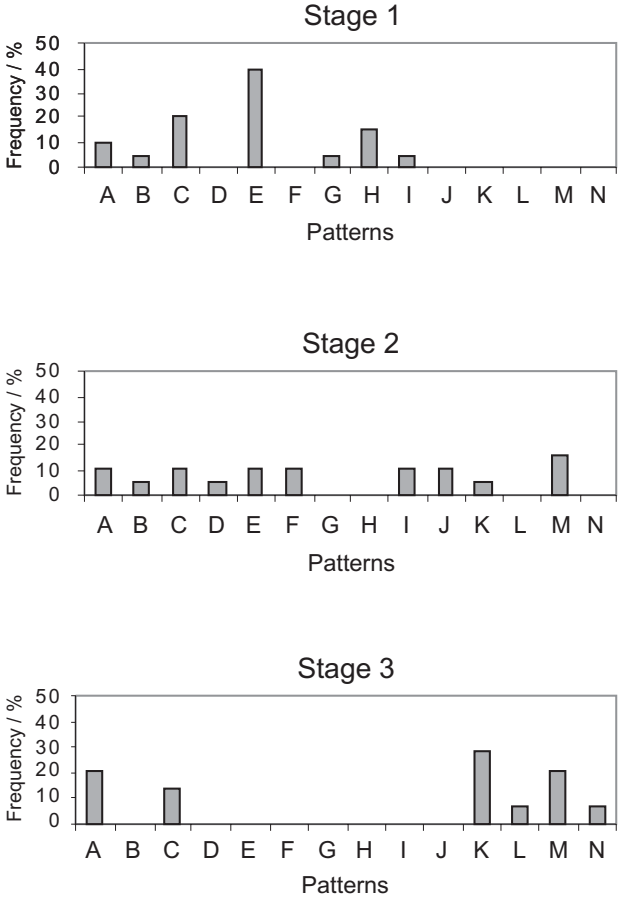


**Fig. 2.** Population dynamics of *S. cerevisiae* strains during plum must spontaneous fermentation
Stage 1 – the beginning of fermentation (0–6th day)
Stage 2 – central stage of fermentation (7th–19th day)
Stage 3 – the last stage of fermentation (20th–30th day)

Only the N strain, practically immune to almost all used toxins except K7, occurred in the must on the last day before the destillation of samples (30th day). Such a low sensitivity to killer toxins as well as to the high concentrations of ethanol and fermentation by-products is probably connected with the properties of cell membranes, which can effectively protect these microorganisms against disadvantageous conditions of the environment.

## Discussion

So far literature reports have given little information on the plum fruit microbiota and the available items include mainly plum pathogen profile (*17*). There is also no research connected with microorganisms that participate in the spontaneous fermentation of plum musts. On the basis of the obtained results, we can claim that qualitative composition of yeasts and fungi that occurred on the surface of plums is similar to that present on the other fruits, for example grapes (*18,19*), and the plum spontaneous fermentation – to the process of wine production by epiphytic microbiota (*1,2,5,13,20,21*).

The non-fermented must contained yeast microbiota with a composition similar to that found on the surface of the fruit during harvest (*3,15,18*). During next days successive changes of yeast population composition occurred, which was mainly connected with the increase of ethanol concentration and decrease of nutrient components (*1,20,21*). The microorganisms that dominated during the initial stages, classified as *K. apiculata* and *C. pulcherrima*, are able to grow without disturbance if the ethyl alcohol concentration is lower than 5.5 % (*22*). The ethanol tolerance can increase when compounds like sorbitol are present in the fermentation must. The polyhydroxy alcohol mentioned above is one of the major con-

stituents in the plum flesh (*23,24*) and can have a bioprotective effect on the wild yeast cell membranes (*25*). At the same time sorbitol is used as the carbon source by *C. pulcherrima* (*26*), which can stimulate strong multiplication of this species during the initial stages of spontaneous fermentation.

After 48 hours of fermentation, when the amount of ethanol increased, the non-*Saccharomyces* population started to decay rapidly, which created an environmental niche that was inhabited by *S. cerevisiae* strains. The concentration of indigenous yeast strains on the surface of fruit was extremely low (*1,18,27,28*) and they constituted almost 99.8 % of the total isolated yeasts only after the third or fourth day of the spontaneous fermentation.

The killer sensitivity analysis turned out to be an exceptionally useful tool for the differentation of individual indigenous strains of *S. cerevisiae*. During this study heterogenicity of *S. cerevisiae* culture was observed, which resulted in the isolation of 14 different killer patterns with different participation in the individual stages of fermentation.

Apart from increasing ethanol concentration, the presence of killer toxins in fermenting fruit must is the other important factor that determined the growth of individual yeast strains (*7,29*). It is worth noticing that the count of the killer resistant strains increased as the process continued. This tendency can indicate a gradual elimination of sensitive cells from the must, which favours the growth of killer immune cultures (*7*). Among the identified killer sensitive strains, killer toxin producers also occurred (*30*).

Molecular polymorphism of *S. cerevisiae* can affect highly cell physiological characteristics and fermentative profile, hence each isolated microorganism can influence the organoleptic character of the produced beverage in a different way (*6,31*). Therefore, further research is needed that would combine killer sensitivity profile of isolated yeasts with their enological characteristics.

## Conclusion

Śliwowica Łącka is a beverage made during spontaneous fermentation of Wegierka Zwykla plum fruit by the epiphytic microbiota. This complex process is begun by *K. apiculata* and *C. pulcherrima* species, the non-*Saccharomyces* yeasts died as ethanol concentration increased and were replaced by indigenous *S. cerevisiae* strains. Within *S. cerevisiae* species strong polymorphism occurred and on the basis of the killer sensitivity analysis 14 different patterns were identified.

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## References

1. M.J. Torija, N. Rozes, M. Poblet, J.M. Guillamon, A. Mas, Yeast population dynamics in spontaneous fermentations: Comparison between two different wine-producing areas over a period of three years, *Antonie van Leeuwenhoek*, *79* (2001) 345–352.
2. L. Granchi, D. Ganucci, A. Messini, M. Vincenzini, Oenological properties of *Hanseniaspora osmophila* and *Kloeckera corticis* from wines produced by spontaneous fermentations of normal and dried grapes, *FEMS Yeast Res. 2* (2002) 403–407.
3. D.E. Zohre, H. Erten, The influence of *Kloeckera apiculata* and *Candida pulcherrima* yeasts on wine fermentation, *Process Biochem. 38* (2002) 319–324.
4. G.H. Fleet, G.M. Heard: Yeast – Growth During Fermentation. In: *Wine Microbiology and Biotechnology*, Hartwood Academic Publishers, Chur (1994) pp. 27–54.
5. G. Beltran, M.J. Torija, M. Novo, N. Ferrer, M. Poblet, J.M. Guillamon, N. Rozes, A. Mas, Analysis of yeast populations during alcoholic fermentation: A six year follow-up study, *Syst. Appl. Microbiol. 25* (2002) 287–293.
6. A. Versavaud, P. Courcoux, C. Roulland, L. Dulau, J.-N. Hallet, Genetic diversity and geographical distribution of wild *Saccharomyces cerevisiae* strains from the wine-producing area of Charentes, France, *Appl. Environ. Microbiol. 61* (1995) 3521–3529.
7. M. Sangorrin, I. Zajonskovsky, M. van Broock, A. Caballero, The use of killer biotyping in an ecological survey of yeast in an old patagonian winery, *World J. Microbiol. Biotechnol. 18* (2002) 115–120.
8. G.A. da Silva, The occurence of killer, sensitive, and neutral yeasts in Brazilian Riesling Italico grape must and the effect of neutral strains on killing behaviour, *Appl. Microbiol. Biotechnol. 46* (1996) 112–121.
9. G.M. Walker, A.H. McLeod, V.J. Hodgson, Interactions between killer yeasts and pathogenic fungi, *FEMS Microbiol. Lett. 127* (1995) 213–222.
10. W.-B. Chen, Y.-F. Han, S.-H. Jong, S.-H. Chang, Isolation, purification and characterization of a killer protein from *Schwanniomyces occidentalis*, *Appl. Environ. Microbiol. 67* (2000) 3058–3063.
11. M. Ciani, F. Fatichenti, Killer toxin of *Kluyveromyces phaffi* DBVPG 6076 as a biopreservative agent to control apiculate wine yeasts, *Appl. Environ. Microbiol. 67* (2001) 3058–3063.
12. T. Tuszynski, P. Satora, Microbiological characteristics of the Wegierka Zwykla plum orchard in submontane region, *Pol. J. Food Nutr. Sci.* 12/53 (2003) 43–48.
13. C.L. Pallmann, J.A. Brown, T.L. Olineka, L. Cocolin, D.A. Mills, L.F. Bisson, Use of WL Medium to profile native flora fermentations, *Am. J. Enol. Vitic. 52* (2001) 198–203.
14. J.A. Barnett, R.W. Payne, M. Yarrow, *Yeasts: Characteristics and Identification*, Cambridge University Press, Cambridge (1983).
15. G.M. Heard, G.H. Fleet, Growth of natural yeast flora during the fermentation of inoculated wines, *Appl. Environ. Microbiol. 50* (1985) 727–728.
16. B. Esteve-Zarzoso, A. Gostincar, R. Bobet, F. Uruburu, A. Querol, Selection and molecular characterization of wine yeasts isolated from the »El Penedes« area (Spain), *Food Microbiol. 17* (2000) 553–562.
17. N. Ale-Agha, Prunus-Arten und ihre Pilzfloren (an Blättern, Früchten) im Duisburger Norden, *Med. Fac. Landbouww. Univ. Gent 62/3b* (1997) 943–949.
18. R.R. Davenport, Microecology of yeasts and yeast-like organisms associated with an English vineyard, *Vitis, 13* (1974) 123–130.
19. K.J. Moore, M.G. Johnson, J.R. Morris, Indigenous yeast microflora on Arkansas white Riesling (*Vitis vinifera*) grapes and in model must system, *J. Food Sci. 53* (1998) 1725– 1728.
20. L. Cocolin, L.F. Bisson, D.A. Mills, Direct profiling of the yeast dynamics in wine fermentations, *FEMS Microbiol. Lett. 189* (2000) 81–87.

*21.* L. Cocolin, M. Manzano, S. Rebecca, G. Comi, Monitoring of yeast population changes during a continuous wine fermentation by molecular methods, *Am. J. Enol. Vitic. 53* (2002) 24–27.

*22.* M. Ciani, F. Maccarelli, Oenological properties of non-*Saccharomyces* yeasts associated with wine-making, *World J. Microbiol. Biotechnol. 14* (1998) 199–203.

*23.* C. Nergiz, H. Yildiz, Research on chemical composition of some varieties of european plums (*Prunus domestica*) adapted to the Aegean district of Turkey, *J. Agric. Food Chem. 45* (1997) 2820–2823.

*24.* H.T. Sabarez, W.E. Price, J. Korth, Volatile changes during dehydratation of d'Agen prunes, *J. Agric. Food Chem. 48* (2000) 1838–1842.

*25.* K.F. Cooper, M.J. Mallory, R. Strich, Oxidative stress-induced destruction of the yeast C-type cyclin Ume3p requires phosphatidylinositol-specific phospholipase C and the 26S proteasome, *Mol. Cell. Biol. 19* (1999) 3338–3348.

*26.* P. Satora: Aspekty mikrobiologiczne otrzymywania spirytusów śliwkowych w rejonie Łacka, *PhD Thesis*, Cracow (2004).

*27.* M. Constanti, M.M. Poblet, L. Arola, A. Mas, J.M. Guillamon, Analysis of yeast populations during alcoholic fermentation in a newly established winery, *Am. J. Enol. Vitic. 48* (1998) 339–344.

*28.* K. Pohve Jemec, N. Cadez, T. Zagorc, V. Bubic, A. Zupec, P. Raspor, Yeast population dynamics in five spontaneous fermentations of Malvasia must, *Food Microbiol. 18* (2001) 247–259.

*29.* F. Ramon-Portugal, M.L. Delia, P. Strehaiano, J.P. Riba, Mixed culture of killer and sensitive *Saccharomyces cerevisiae* strains in batch and continuous fermentations, *World J. Microbiol. Biotechnol. 14* (1998) 83–87.

*30.* M.P. Sangorrin, I.E. Zajonkovsky, C.A. Lopes, M.E. Rodriguez, M.R.G. de van Broock, A.C. Caballero, Killer behaviour in wild wine yeasts associated with Merlot and Malbec type musts spontaneously fermented from northwestern Patagonia (Argentina), *J. Basic Microbiol. 41* (2001) 105–113.

*31.* D. Nadal, B. Colomer, B. Pina, Molecular polymorphism distribution in phenotypically distinct populations of wine yeast strains, *Appl. Environ. Microbiol. 62* (1996) 1944–1950.

# Biološka raznolikost kvasaca tijekom spontane fermentacije šljiva Wegierka Zwykla

**Sažetak**

U radu je provedena analiza mikrobiota kvasaca, koji su sudjelovali u spontanoj fermentaciji zgnječenih šljiva Wegierka Zwykla, a koriste se kao sirovina za proizvodnju šljivovice u južnoj Poljskoj. Sojevi kvasca *Saccharomyces cerevisiae*, u usporedbi s devet dobro poznatih ubilačkih sojeva, mogli su se razlikovati prema stupnju osjetljivosti. U prvoj fazi fermentacije prevladavale su vrste *Kloeckera apiculata* i *Candida pulcherrima* koje su nakon 48 sati dostigle maksimalnu koncentraciju $1{,}4 \cdot 10^6$ živih stanica/mL. Skoro svi kvasci izolirani idućih dana klasificirani su kao vrsta *S. cerevisiae*, a analizom ubilačke osjetljivosti otkrivena je jaka populacijska raznolikost te vrste, kao i prisutnost 14 različitih sojeva koji su se kvantitativno i kvalitativno mijenjali tijekom fermentacije.