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# Sparkling Wine Production by Immobilised Yeast Fermentation

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**Abstract**

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The prospects of sparkling wine production by the 'Champenoise' method using alginate-immobilised yeast cells were examined. Grape varieties dominant in quantity were selected within the group of recommended and permitted varieties of Kutjevo vineyards, located in the eastern part of continental Croatia. Research revealed that there are no influential variations in the principal physicochemical and sensory characteristics between sparkling wines obtained through immobilised yeast and traditional sparkling method. The analysis of aroma compounds showed minor differences between samples. Observed oenological parameters assessed in the final products did not show any relevant oenological differences, with the exception of alcohol content, which was slightly higher in sparkling wines made with yeast cells immobilised with calcium alginate beads. According to this research, the sensory properties of the produced sparkling wines, compared to sparkling wine produced with free yeast, did not show any significant differences. On the full-scale obtained results indicate that some of the selected varieties can be sorted as suitable for the production of sparkling wine using immobilised yeast cells.

**Keywords**: immobilised yeast cells; aroma; grape varieties; wine; quality

The sparkling wine (SW) production consists of two phases. In the first one, the base wine was obtained after applying white vinification. The second phase is conducted using two distinct methods: in the bottle (Champenoise method) or in isobaric tanks (Charmat method) (Stefenon *et al.* 2014).

The particularity of the Champenoise method consists in fermentation in a bottle of wine where sugar is up to 24 g/l and yeast have been added (Godia *et al.* 1991). When the fermentation has been completed, yeast cells are removed from the main wine body and deposited on the bottleneck by storing and turning the bottles in an inclined position. Next is the freezing of the bottlenecks and the removal of yeast sediment. The yeast sedimentation can take several weeks involving a considerable amount of labour to be devoted to. Thus, various alternatives are studied, i.e. chemical additives, the use of flocculent yeast strains, and use of immobilised yeast are some of them (Godia *et al.* 1991).

The immobilised yeast technology (IYT), compared with the traditional Champenoise method, presents several advantages related to the cost, the ability to control the fermentation, and minimised duration as a result of elimination of the riddling and disgorging steps and less storage room in the winery (Ntagas *et al*. 2003; Torresi *et al*. 2011). IYT compared to free cells seems to produce more glycerol, more es-

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ters, and lower amounts of both higher alcohols and acetaldehyde (Divies 1989; Tataridis *et al.* 2005).

The composition of aroma compounds is the sole intrinsic factor determining the quality of SW (Rapp & Mandery 1986; Etievant 1991; Pretorius *et al.* 2003). The majority of SW aroma compounds are formed during the first fermentation process (Rankine 1967; Nykänen 1986; Antonelli *et al.* 1999; Herjavec *et al.* 2003). During the second fermentation the interactions between the components present in the wine and in the yeast cells will cause a number of chemical and enzymatic reactions forming different chemical and aroma profile of SW (Gallardo-Chacón *et al.* 2010; Buxaderas & López-Tamames 2012; Stefenon *et al.* 2014). The technology used, the biological characteristics of the yeasts employed, and the chemical composition of SW resulting in the unique chemical and aroma profile of the final product modulate those reactions (Stefenon *et al.* 2014).

The objective of this study was to compare the quality of SW produced at an industrial scale by Champenoise method and by immobilised yeast technology (IYT). SW samples were produced from seven grape varieties (Welschriesling, Pinot blanc, Pinot gris, Pinot noir, Chardonnay, Rheinriesling, Traminer) from the oenological region of the Kutjevo vineyards, in the eastern part of Croatia.

## MATERIAL AND METHODS

***Base wine production***. The base wines were produced from the grapes appertaining to the following varieties: Welschriesling, Pinot blanc, Pinot gris, Pinot noir, Chardonnay, Rheinriesling, Traminer. Sugar content in the grape varieties varied between 93.0 and 98.5°Oe and total acids were in the range of 5.10–7.50 mg/l.

Samples of base wines were produced using a classical technological procedure; fermentation with selected yeast (Fermol® Bouquet – AEB Spa Brescia Ital; a yeast particularly valued for its ability to highlight primary and floral aromas, since it highlights the terpenic overtones of grapes grown in moderately warm climates), and controlled thermal regime, performed through the outer cooling of fermenters with running water, with the aim of keeping the average temperature in intervals of 16–22°C. The average duration of the fermentation of all grape varieties under these conditions was 42 days.

***Sparkling wine production***. Secondary fermentation ('Champenoise method') was carried out in standard 750 ml sparkling wine bottles filled with base wine plus sugar at 22 g/l. Five batches of sparkling wine were manufactured with immobilised commercial selected yeast Fermol®Reims Champagne. Ca-alginate gel beads as yeast carriers were produced using a technological procedure as shown in previous publications (Gaserud 1998; Poncelet *et al.* 2001), and five batches were produced by the so-called traditional method. For Ca-alginate immobilisation, a 3% solution of Ca-alginate in growth medium was prepared. 250 g of wet yeast cells was suspended in 300 ml of the prepared solution and a yeast suspension was dropwise added to a cross-linking solution containing $CaCl_2$ (3%) with syringe to obtain spherical beads with diameter less than 2 mm. Bottles were closed with bidules and crown caps and positioned horizontally on the floor of a cave with a constant temperature of 13–16°C. For each batch, 5 different bottles were produced.

Oenological, sensory, and aroma analyses of the resulting products were performed after 12 months of aging (nine months is the minimum aging period established by a regulation, European Commission 1990).

Three bottles of each batch were disgorged for performing all the tests. Bottles were filled with the sparkling wine itself in order to produce Brut sparkling wines (Liqueur d'expédition was not added). For the oenological analysis, samples were previously degassed by magnetic stirring.

***Oenological analysis of wine***. Density, alcohol content, total extract, total sugar, total titratable acidity, volatile acidity, total and free $SO_2$, total nitrogen, and ash content were determined according to European Commission (1990), OIV (2008), and Croatian law (Anonymous 1996 & 2000).

***Analysis of aroma compounds***. The analysis was done on a Hewlett Packard 5890 gas chromatograph with a split/splitless injector and a FID detector. For the headspace analysis of wine, a Hewlett Packard HP 7694 sampler was used. Compounds of interest were resolved on a Stabilwax (Restec, USA) capillary column (30 m × 0.25 mm i.d. × 0.25 mm) with the following parameters: initial oven temperature of 30°C was kept for 4 min, then raised by 10°C/min to 100°C followed by 25°C/min to 250°C, and kept at 250°C for 7 minutes. Injection port temperature was kept at 180°C, pressure was 137.89 kPa, and carrier gas (nitrogen) flow was 3 ml/minutes. Detector temperature was 250°C.

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A headspace sampler was equipped with a standard 1 ml loop. Carrier gas pressure was 17 psi, vial pressure was 7 psi, and injection time was 0.20 minutes. Samples were heated at 100°C for 10 minutes. For the analysis of wine distillates, a Stabilwax (Restec, USA) capillary column (30 m × 0.25 mm i.d. × 0.25 mm) was used, the initial oven temperature was kept at 35°C for 7 min, then raised by 10°C/min to 80°C followed by 25°C/min to 200°C, and kept at 200°C for 4 minutes. Quantitative data was obtained using 1-pentanol as an internal standard. Qualitative analysis was carried out by combined GC-mass spectrometry (GC/MS) with Stabilwax (Restec, USA) capillary column as well as the comparison of mass spectra and retention index (RI) with reference substances or library data.

***Sensory evaluation***. Sensory evaluation of wine samples was executed using the Buxbaum model of positive rating (Satora & Tuszynski 2010). This model is developed on 4 sensory characteristics (colour, clearness, odour, and taste) with a maximum of 20 points. A panel of ten trained professional judges made all the evaluations.

***Statistical analysis***. Statistical analyses of experimental data were performed using MS Excel (Microsoft Office 2007 Professional) and Statistica 12 (StatSoft, USA). All measured data were expressed as mean ± standard deviation. The experimental data were subjected to analysis of variance (ANOVA) at a 5% significance level and main effects to ANOVA at a 95% confidence level.

## RESULTS AND DISCUSSION

***Oenological analysis of wine***. The results obtained in the chemical analysis of wine, reported in Table 1, show that the quantity of alcohol in all analysed samples corresponded to the requirements of wine regulations (Anonymous 1996). All sparkling wine samples manufactured by immobilised yeast cells

Table 1. Oenological analysis of sparkling wine samples produced by the traditional method and using immobilised yeast cells (mean values ± standard deviation); all results are average of three measures

| | Welschriesling | Pinot noir | Pinot gris | Chardonnay | Rheinriesling | Traminer | Pinot blanc |
|---|---|---|---|---|---|---|---|
| **Traditional method** | | | | | | | |
| Density (20/20°C) (g/ml) | 0.9935 ± 0.00 | 0.9915 ± 0.00 | 0.9914 ± 0.00 | 0.9928 ± 0.00 | 0.9932 ± 0.00 | 0.9950 ± 0.00 | 0.9923 ± 0.00 |
| Alcohol (% vol.) | 13.50 ± 0.01 | 13.01 ± 0.02 | 13.39 ± 0.01 | 13.07 ± 0.02 | 13.64 ± 0.04 | 13.08 ± 0.02 | 12.83 ± 0.05 |
| Total extract (g/l) | 22.85 ± 0.01 | 21.60 ± 0.02 | 21.30 ± 0.00 | 20.60 ± 0.00 | 22.56 ± 0.10 | 21.80 ± 0.00 | 21.28 ± 0.02 |
| Total sugar (g/l) | 4.30 ± 0.00 | 4.65 ± 0.00 | 4.34 ± 0.01 | 3.78 ± 0.00 | 3.80 ± 0.00 | 4.90 ± 0.00 | 3.60 ± 0.00 |
| Total acidity (g/l) | 5.98 ± 0.01 | 5.30 ± 0.00 | 5.31 ± 0.01 | 5.49 ± 0.03 | 6.09 ± 0.02 | 5.19 ± 0.02 | 5.38 ± 0.03 |
| Volatile acidity (g/l) | 0.23 ± 0.02 | 0.18 ± 0.01 | 0.19 ± 0.00 | 0.20 ± 0.00 | 0.23 ± 0.03 | 0.21 ± 0.02 | 0.19 ± 0.02 |
| Ash (g/l) | 1.97 ± 0.02 | 1.64 ± 0.04 | 1.74 ± 0.00 | 1.42 ± 0.00 | 1.82 ± 0.00 | 1.90 ± 0.10 | 1.42 ± 0.03 |
| Free $SO_2$ (mg/l) | 23.20 ± 0.00 | 11.24 ± 0.04 | 17.04 ± 0.07 | 17.09 ± 0.09 | 16.99 ± 0.01 | 21.76 ± 0.00 | 16.20 ± 0.00 |
| Total $SO_2$ (mg/l) | 155.35 ± 0.04 | 119.33 ± 0.58 | 118.40 ± 0.00 | 125.25 ± 0.05 | 149.00 ± 0.00 | 149.75 ± 0.20 | 124.33 ± 0.03 |
| Total nitrogen (mg/l) | 240.30 ± 0.26 | 260.50 ± 0.50 | 260.00 ± 0.00 | 260.00 ± 0.31 | 245.30 ± 0.00 | 249.00 ± 0.00 | 245.20 ± 1.04 |
| **Immobilised yeast cells** | | | | | | | |
| Density (20/20°C) (g/ml) | 0.9925 ± 0.00 | 0.9909 ± 0.00 | 0.9907 ± 0.00 | 0.9921 ± 0.00 | 0.9922 ± 0.00 | 0.9930 ± 0.00 | 0.9911 ± 0.00 |
| Alcohol (% vol.) | 13.64 ± 0.06 | 13.62 ± 0.03 | 13.47 ± 0.03 | 13.37 ± 0.03 | 13.84 ± 0.01 | 13.38 ± 0.03 | 13.02 ± 0.03 |
| Total extract (g/l) | 22.57 ± 0.03 | 21.10 ± 0.00 | 20.15 ± 0.00 | 20.06 ± 0.00 | 22.01 ± 0.01 | 21.12 ± 0.02 | 21.03 ± 0.05 |
| Total sugar (g/l) | 3.22 ± 0.03 | 3.45 ± 0.00 | 3.25 ± 0.00 | 3.64 ± 0.06 | 3.69 ± 0.01 | 3.70 ± 0.00 | 3.03 ± 0.05 |
| Total acidity (g/l) | 5.73 ± 0.03 | 5.00 ± 0.00 | 5.10 ± 0.00 | 5.18 ± 0.02 | 5.61 ± 0.01 | 5.15 ± 0.00 | 5.10 ± 0.00 |
| Volatile acidity (g/l) | 0.21 ± 0.01 | 0.16 ± 0.06 | 0.17 ± 0.03 | 0.18 ± 0.00 | 0.22 ± 0.03 | 0.20 ± 0.00 | 0.19 ± 0.01 |
| Ash (g/l) | 1.96 ± 0.01 | 1.62 ± 0.03 | 1.70 ± 0.00 | 1.32 ± 0.03 | 1.70 ± 0.00 | 1.80 ± 0.00 | 1.40 ± 0.17 |
| Free $SO_2$ (mg/l) | 23.02 ± 0.03 | 11.32 ± 0.03 | 17.14 ± 0.01 | 17.00 ± 0.00 | 17.30 ± 0.10 | 21.26 ± 0.01 | 16.40 ± 0.17 |
| Total $SO_2$ (mg/l) | 153.33 ± 0.03 | 118.52 ± 0.02 | 118.20 ± 0.00 | 124.20 ± 0.00 | 119.30 ± 0.26 | 149.96 ± 0.01 | 124.83 ± 0.03 |
| Total nitrogen (mg/l) | 230.00 ± 0.00 | 260.00 ± 0.00 | 249.00 ± 0.00 | 257.23 ± 2.13 | 244.00 ± 0.00 | 247.00 ± 0.00 | 243.80 ± 0.34 |

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Table 2. Effect of wine type and yeast immobilisation on oenological properties of sparkling wines (multivariate analysis of covariance)

| | Variety | | Method | |
|---|---|---|---|---|
| | *F*-values | *P* | *F*-values | *P* |
| Density (20/20°C) | 19.207 | 0.00000 | 32.341 | 0.00000 |
| Alcohol | **47.264** | 0.00000 | **79.650** | 0.00000 |
| Total extract | **168.48** | 0.00000 | **135.28** | 0.00000 |
| Total sugar | 8.7562 | 0.00000 | **97.158** | 0.00000 |
| Total acidity | **122.51** | 0.00000 | **154.86** | 0.00000 |
| Volatile acidity | 4.3136 | 0.00245 | 3.3404 | *0.07638* |
| Ash | **88.639** | 0.00000 | 11.041 | 0.00214 |
| Free $SO_2$ | **3861.6** | 0.00000 | 0.06527 | *0.79990* |
| Total $SO_2$ | **39.883** | 0.00000 | 7.2538 | 0.01090 |
| Total nitrogen | **84.706** | 0.00000 | 32.372 | 0.00000 |

*P* — level of significance; bold values represent significant effect at $P < 0.05$

had a raised amount of alcohol ranging from 13.02 to 13.84%, in relation to the amount of 12.83–13.64% in wines produced using the classical technological procedure. The impact of yeast immobilisation was statistically significant (Table 2). It is important to stress that the raised amount of alcohol in wines may cause the reduction of aroma compounds (Escalona *et al.* 1999).

Immobilised cells gave wines with lower contents of total extract (20.06–22.57 g/l). The amount of total extract in all wines produced using the classical technological procedure ranged from 21.30 to 23.56 g/l. It is important to stress that total extract in new sparkling wine is also composed of substances among which predominantly are yeast cells the extract of which accounts for 60–70% of total lees. The differences in the amount of total extract between sparkling wine samples are statistically significant, but all in conformity with the characteristics of quality wines obtained from the examined grape varieties (Yokotsuka *et al.* 1997; Ribereau-Gayon *et al.* 1998).

In sparkling wine samples produced with immobilised cells, slightly lower values of total and volatile acidity were noted (5.00–5.73 g/l and 0.16–0.22 g/l) than in wines which were produced using the classical technological procedure (5.30–6.09 and 0.18–0.23 g/l), where volatile acidity was not significantly changed (Table 2). Differences in total and volatile acidity after the end of the second fermentation were similar to those observed by other researchers (Yajima & Yokotsuka 2001; Mallouchos *et al.* 2003).

The amount of total sugars (3.60–4.90 g/l) is significantly higher in all sparkling wines produced using the classical technological procedure in relation to the value in sparkling wine samples produced using immobilised yeast cells (3.03–3.70 g/l). These results are related to the content of ethanol in sparkling wine (Diviès *et al.* 1994; Delfini *et al.* 2001). All wine samples, however, are in the category of dry (seco) wines, since sugar content in all samples is below 5 g/l (Moreno & Peinado 2012). The presence of free $SO_2$ in all sparkling wine samples, ranging from 11.24 to 23.20 mg/l, corresponds to results of Antonelli *et al.* (1999) and was not influenced by the immobilisation. From the results obtained, it is evident that the defined physicochemical properties of produced sparkling wines are within reference values (Vila *et al.* 1998). Immobilisation resulted in statistically significant differences in alcohol, total sugar, total extract, and total acidity values (Table 2). On the basis of the results obtained in this research, the samples of Rheinriesling and Welschriesling of Kutjevo vineyards, located in the eastern part of continental Croatia, correspond to the properties of sparkling wines suitable for production using the technological procedure with immobilised yeast cells.

***Sensory analysis***. Flavour perception is the result of multiple interactions within a wide range of chemical and sensory receptors located in the olfactory epithelium. The volatiles that reach the pituitary via the nose comprise the odour of a product, but when the chemicals reach the pituitary via retronasal stimulation, i.e. through the mouth, we talk about the flavour of the product. Sensory analysis can be used to evoke, measure, analyse, and interpret the reactions to stimuli perceived through the senses. Sensory analysis still remains an efficient tool for assessing the properties of sparkling wine (Ferreira 2010). The results of the sensory analysis of sparkling wines are shown in Figure 1. Sensory evaluation was done by the rating system according to the Buxbaum model (Satora & Tuszynski 2010). The sparkling wines were submitted to a jury of 10 trained wine experts (oenology senior sensory staff). The aim of

Table 3. Effect of wine type and yeast immobilisation on sensory properties of sparkling wines

| | Variety | | Method | |
|---|---|---|---|---|
| | *F*-values | *P*-values | *F*-values | *P*-values |
| Overall sensory score | 2.1348 | 0.07461 | 0.14676 | 0.70403 |

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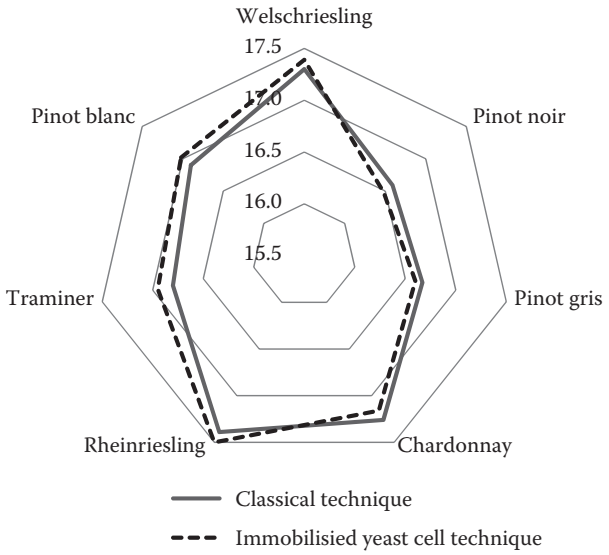


Figure 1. Sensory characteristics of sparkling wine samples

the sensory analysis was the selection of sparkling wines with prospects of suitable quality for production using immobilised yeast cells in secondary fermentation.

Observing the results from Figure 1 and Table 3, it may be noted that sensory characteristics of wines were very good and were not influenced by the production method. This is in agreement with findings of other researchers (Fumi *et al*. 1987; Gòdia *et al*. 1991; Yokotsuka *et al*. 1997).

However, the sample of Pinot noir sparkling wine was given the lowest rating while the best rating was given to Rheinriesling, singling this grape variety out as the best one for the production of wine using immobilised yeast cells, which is in accordance with the results of physicochemical analysis.

***Analysis of volatile ingredients***. In all wine samples (produced by the classical procedure of fermentation Champenoise method and produced by the procedure with immobilised yeast cells), aroma compounds were identified and quantitatively determined as shown in Table 4. Volatile compound compositions of the wines produced with immobilised and free cells was almost identical. This coincides with data from Yokotsuka *et al*. (1997).

The amounts of methanol in samples produced by the procedure with immobilised yeast cells were very small (0.08–0.68 mg/l) compared to the results obtained in similar researches (Gnekow & Ough 1976).

The amount of acetaldehyde in wines ranged from 40.96 mg/l to 130.77 mg/l (Table 5) and from 40.66 mg/l to 130.57 mg/l (Table 6). Acetaldehyde is a product of alcoholic fermentation and its amount in wine, according to data from literature, is usually within limits ranging from 10 to 300 mg/l (Fleet & Heard 1993). The final concentration of this compound depends on fermentation conditions (Moreno & Peinado 2012); however, the yeast immobilisation did not have any significant influence in this research. It is responsible for the intensive aroma of biologically aged wines, and the raised amount of acetaldehyde originates from pyruvate after decarboxylation and decreased activity of NADH alcohol dehydrogenase.

Among the higher alcohols, 1-propanol, 1-butanol, 2-methyl-1-propanol, 1-hexanol, 3-methyl-1-butanol and 2-phenyl ethanol were identified, where 1-propanol, 1-butanol, and 2-methyl-1-propanol have a high threshold of sensitivity and thus do not affect the wine aroma considerably (Lambrechts & Pretorius 2000). The amount of 1-hexanol in wines produced by the procedure with immobilised yeast cells was also under the threshold of sensory sensitivity, except in the sample of Welschriesling, where it was 5.22–7.42 mg/l. Since 1-hexanol content is negatively correlated with the aroma of Riesling wines (Moreno & Peinado 2012), it can be stated that the yeast immobilisation had a favourable effect in this regard due to a reduction of hexanol content from 7.42 and 7.01 mg/l to 5.22 and 5.01 mg/ml, respectively (Table 4). The immobilisation had a significant effect on hexanol content(Table 5). 3-methyl-1-butanol is the most important aliphatic alcohol synthesised by yeasts during alcoholic fermentation. It accounts for almost 70% of all fractions of higher alcohols. The amount of 3-methyl-1-butanol in wines ranged from 129 to 219 mg/l. This corresponds to Rapp and Mandery (1986), where the amount of 3-methyl-1-butanol in wine was 45–490 mg/l. Higher alcohols have a beneficial effect on the complexity of wine if they do not exceed the amount of 300 mg/l (Rapp & Mandery 1986).

The amount and composition of esters affect sensory properties of wine giving it fresh fruity aroma (Etievant 1991; Frank *et al*. 2011). The most abundant ester in wine is ethyl acetate (Moreno & Peinado 2012). The amount of ethyl acetate in wine produced with immobilised yeast cells ranged from 40.78 mg/l to 45.33 mg/l. This corresponds to the data from literature (Lambrechts & Pretorius 2000), according to which the amount of ethyl acetate ranges from 30 mg/l to 200 mg/l. The threshold of sensory sensitivity is 160 mg/l, but even below this

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Table 4. Aroma compounds in sparkling wine samples produced by the traditional method and using immobilised yeast cells (mean values ± standard deviation)

| Aroma compounds (mg/l) | Welschriesling | Pinot noir | Pinot gris | Chardonnay | Rheinriesling | Traminer | Pinot blanc |
|---|---|---|---|---|---|---|---|
| **Traditional method** | | | | | | | |
| Methanol | 0.14 ± 0.01 | 0.67 ± 0.01 | 0.33 ± 0.01 | 0.29 ± 0.02 | 0.16 ± 0.03 | 0.18 ± 0.02 | 0.28 ± 0.01 |
| Linalool | 0.97 ± 0.01 | 0.79 ± 0.01 | 1.16 ± 0.02 | 0.99 ± 0.02 | 0.89 ± 0.03 | 1.11 ± 0.01 | 0.95 ± 0.02 |
| 1-Propanol | 20.56 ± 0.02 | 9.97 ± 0.01 | 28.10 ± 0.01 | 18.94 ± 0.01 | 17.11 ± 0.01 | 20.14 ± 0.02 | 9.84 ± 0.01 |
| 2-Methylpropan-1-ol | 43.17 ± 0.02 | 24.63 ± 0.03 | 31.35 ± 0.01 | 26.68 ± 0.02 | 42.71 ± 0.03 | 33.52 ± 0.01 | 29.9 ± 0.02 |
| 1-Butanol | 0.65 ± 0.01 | nd | 0.85 ± 0.01 | 0.55 ± 0.01 | nd | 0.73 ± 0.01 | 0.49 ± 0.01 |
| 1-Pentanol | 199.36 ± 0.01 | 168.99 ± 0.01 | 133.08 ± 0.01 | 155.55 ± 0.01 | 219.99 ± 0.01 | 163.88 ± 0.01 | 179.99 ± 0.01 |
| 2-Phenyl ethanol | 32.04 ± 0.01 | 17.15 ± 0.01 | 43.37 ± 0.02 | 55.42 ± 0.01 | 35.74 ± 0.01 | 15.71 ± 0.02 | 38.82 ± 0.01 |
| 1-Hexanol | 7.42 ± 0.01 | 3.98 ± 0.02 | 4.99 ± 0.01 | 5.71 ± 0.01 | 7.01 ± 0.02 | 5.81 ± 0.01 | 5.36 ± 0.01 |
| Acetaldehyde | 42.30 ± 0.03 | 40.96 ± 0.01 | 130.77 ± 0.01 | 109.51 ± 0.02 | 50.57 ± 0.01 | 44.31 ± 0.01 | 55.69 ± 0.02 |
| Ethyl acetate | 54.51 ± 0.01 | 62.82 ± 0.02 | 75.53 ± 0.02 | 59.81 ± 0.01 | 54.73 ± 0.01 | 74.79 ± 0.02 | 68.45 ± 0.01 |
| 3-Methylbutyl acetate | 2.74 ± 0.01 | 1.22 ± 0.01 | 2.45 ± 0.01 | 1.82 ± 0.01 | 1.29 ± 0.02 | 3.12 ± 0.01 | 1.68 ± 0.01 |
| Ethyl hexanoate | 0.953 ± 0.01 | nd | 0.76 ± 0.01 | nd | nd | 0.32 ± 0.01 | nd |
| Ethyl octanoate | nd | 1.50 ± 0.01 | 3.66 ± 0.01 | 0.53 ± 0.01 | 0.52 ± 0.01 | 1.93 ± 0.03 | 1.02 ± 0.03 |
| Ethyl decanoate | 1.33 ± 0.01 | 0.42 ± 0.01 | 0.88 ± 0.03 | 0.23 ± 0.03 | 0.37 ± 0.02 | 0.18 ± 0.01 | 0.11 ± 0.03 |
| Ethyl lactate | 3.86 ± 0.01 | 2.69 ± 0.03 | 5.98 ± 0.01 | 8.74 ± 0.01 | nd | nd | nd |
| **Immobilised yeast cells** | | | | | | | |
| Methanol | 0.08 ± 0.01 | 0.68 ± 0.01 | 0.29 ± 0.01 | 0.19 ± 0.01 | 0.11 ± 0.03 | 0.16 ± 0.01 | 0.23 ± 0.01 |
| 3,7-Dimethyl-1,6-octadien-3-ol | 0.98 ± 0.03 | 0.81 ± 0.01 | 1.06 ± 0.02 | 0.97 ± 0.02 | 0.83 ± 0.01 | 1.03 ± 0.03 | 0.94 ± 0.03 |
| 1-Propanol | 20.56 ± 0.01 | 9.97 ± 0.02 | 28.10 ± 0.02 | 18.94 ± 0.02 | 17.11 ± 0.01 | 20.14 ± 0.04 | 9.84 ± 0.01 |
| 2-Methylpropan-1-ol | 43.17 ± 0.03 | 24.63 ± 0.01 | 31.35 ± 0.03 | 26.68 ± 0.01 | 42.71 ± 0.03 | 33.52 ± 0.01 | 29.9 ± 0.01 |
| 1-Butanol | 0.65 ± 0.01 | nd | 0.85 ± 0.01 | 0.55 ± 0.01 | nd | 0.73 ± 0.01 | 0.49 ± 0.02 |
| 3-Methyl-1-butanol | 190.23 ± 0.01 | 163.29 ± 0.01 | 129.03 ± 0.01 | 158.55 ± 0.01 | 211.47 ± 0.03 | 161.37 ± 0.01 | 177.95 ± 0.01 |
| 2-Phenyl ethanol | 32.04 ± 0.01 | 17.15 ± 0.01 | 43.37 ± 0.02 | 55.42 ± 0.01 | 35.74 ± 0.01 | 15.71 ± 0.03 | 38.82 ± 0.01 |
| 1-Hexanol | 5.22 ± 0.01 | 2.93 ± 0.03 | 2.92 ± 0.01 | 1.71 ± 0.02 | 5.01 ± 0.01 | 3.81 ± 0.01 | 2.36 ± 0.03 |
| Acetaldehyde | 42.33 ± 0.01 | 40.66 ± 0.03 | 130.57 ± 0.01 | 108.51 ± 0.01 | 50.17 ± 0.03 | 44.31 ± 0.01 | 55.66 ± 0.02 |
| Ethyl acetate | 41.11 ± 0.01 | 42.02 ± 0.01 | 45.33 ± 0.02 | 41.85 ± 0.01 | 40.78 ± 0.01 | 42.73 ± 0.03 | 41.44 ± 0.02 |
| 3-Methylbutyl acetate | 2.74 ± 0.01 | 1.22 ± 0.02 | 2.45 ± 0.01 | 1.82 ± 0.02 | 1.29 ± 0.01 | 3.12 ± 0.01 | 1.68 ± 0.01 |
| Ethyl hexanoate | 0.953 ± 0.01 | nd | 0.76 ± 0.01 | nd | nd | 0.32 ± 0.01 | nd |
| Ethyl octanoate | nd | 1.50 ± 0.01 | 3.66 ± 0.01 | 0.53 ± 0.01 | 0.52 ± 0.01 | 1.93 ± 0.01 | 1.02 ± 0.01 |
| Ethyl decanoate | 1.33 ± 0.01 | 0.42 ± 0.02 | 0.88 ± 0.02 | 0.23 ± 0.01 | 0.37 ± 0.01 | 0.18 ± 0.03 | 0.11 ± 0.02 |
| Ethyl lactate | 3.86 ± 0.01 | 2.69 ± 0.01 | 5.98 ± 0.01 | 8.74 ± 0.02 | nd | nd | nd |

nd – not detected

value it has a serious effect on wine aroma, giving a favourable effect (Moreno & Peinado 2012). The content of ethyl acetate was influenced by the sparkling procedure (Table 5). All other esters were within the limits of optimal values, except the samples of Welschriesling and Chardonnay, where ethyl lactate was slightly raised in comparison with the findings from literature (Nykänen 1986; Wondra & Berovič 2001). The ethyl lactate content is linked to malolactic fermentation and gives aromas of sour milk, yoghurt, and butter (Moreno & Peinado 2012).

3,7-Dimethyl-1,6-octadien-3-ol is a compound from the group of terpenes which does not change during alcoholic fermentation and is thus suitable for the characterisation of grape varieties (Rapp 1990). It is evident from this research that the grape variety

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Table 5. Effect of wine type and yeast immobilisation on aroma compounds in sparkling wine samples (multivariate analysis of covariance)

| Aroma compound | Variety | | Method | |
|---|---|---|---|---|
| | *F*-value | *P* | *F*-value | *P* |
| Methanol | **399.10** | 0.0000 | **38.287** | 0.0000 |
| 3,7-Dimethyl-1,6-octadien-3-ol | **47.889** | 0.0000 | 6.7105 | 0.01748 |
| 1-Propanol | **2376.7** | 0.0000 | 2.3581 | *0.14030* |
| 2-Methylpropan-1-ol | **78359** | 0.0000 | 6.7465 | 0.01722 |
| 1-Butanol | **1258.8** | 0.0000 | 1.0000 | *0.32926* |
| 3-Methylbutan-1-ol | **568.96** | 0.0000 | **29.516** | 0.00003 |
| 2-Phenyl ethanol | **15467** | 0.0000 | 1.4617 | *0.24076* |
| 1-Hexanol | **21.291** | 0.0000 | **142.54** | 0.0000 |
| Acetaldehyde | **33287** | 0.0000 | 9.1425 | 0.00671 |
| Ethyl acetate | 5.7753 | 0.00127 | **194.18** | 0.00000 |
| 3-Methylbutyl acetate | **3231.9** | | 3.2843 | 0.08499 |
| Ethyl hexanoate | – | – | – | – |
| Ethyl octanoate | **11932** | 0.0000 | 0.0000 | 1.00000 |
| Ethyl decanoate | **776.99** | 0.0000 | 1.8581 | *0.18800* |
| Ethyl lactate | **1250.2** | 0.0000 | 1.2578 | 0.27535 |

*P* — level of significance; bold values represent significant effect at $P < 0.05$

highly influences 3,7-dimethyl-1,6-octadien-3-ol content, unlike the sparkling procedure (Table 5). It has a very low perception threshold of 0.5–1 mg/l and contributes to olfactory aroma, giving floral notes. It is also recognised as a 'key component', since it has a special aroma which can vary according to the geographic origin, but is never masked (Moreno & Peinado 2012). The Riesling varieties are highly 'terroir-expressive', meaning that the character of Riesling wines is clearly influenced by the wine's place of origin.

The amount of 3,7-dimethyl-1,6-octadien-3-ol ranged from 0.79 to 1.16 mg/l, which fully corresponds to the character of grape varieties (López *et al*. 1999; Guarrera *et al*. 2005). It is evident from the results of the wine aroma analysis that the applied process of vinification ensures acceptable quality of sparkling wine. However, the samples of wines produced with immobilised cells are not of superior quality except the samples of Riesling varieties.

## CONCLUSIONS

The continental region of the Republic of Croatia is an area known by the production of high quality wines, and the research shown in this survey indicates that using specific grape varieties and specific procedures of fermentation with immobilised yeast cells, this region can produce quality sparkling wines.

The varieties Welschriesling and Rheinriesling may be sorted out among the selected varieties, because they have a suitable profile of aroma and moderate grape variety character, for the production of quality sparkling wine using immobilised yeast cells.

By the full-scale results, all the selected varieties can be distinguished as suitable for the production of sparkling wines. Since beads of Ca-alginate-immobilised yeast remained visually unchanged, further research may be directed to determine whether they can be reused.

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