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Miličević, Borislav; Mesić, Josip; Lukić, Igor; Svitlica, Brankica; Jozinović, Antun; Šubarić, Domagoj

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# INFLUENCE OF IMMOBILIZED YEASTS ON AROMA PROFILE OF ROSE MUSCAT OF POREČ AND MUSCAT BLANC DISTILLATES

## UTJECAJ IMOBILIZIRANIH KVASACA NA AROMATSKI PROFIL DISTILATA SORTI MUŠKAT RUŽA POREČKI I MUŠKAT BIJELI

MILICEVIC, Borislav; MESIC, Josip; LUKIC, Igor; SVITLICA, Brankica;  
JOZINOVIC, Antun & SUBARIC, Domagoj

**Abstract:** *In this research main goal was to investigate influence of immobilized cell fermentation on aroma of distillates produced from two grape varieties commonly grown in Croatian region Istria (Rose Muscat of Poreč and Muscat Blanc). Distillate samples were produced both by classical and immobilized yeast technology. Aroma profile was determined using GC/FID analysis. Results showed that immobilized cell technique gives distillates with higher ethanol and lower ester contents. The immobilized cell technique seems to be promising technique for production of quality marcs distillates.*

**Key words:** *Immobilized yeast cells, marcs distillate, aroma*

**Sažetak:** *Cilj istraživanja je bio utvrditi utjecaj fermentacije imobiliziranim kvascima na aromu destilata dobivenih od dvije sorte grožđa koje se obično uzgajaju u hrvatskoj regiji Istri (Muškat ruža porečki i Muškat bijeli). Destilati su proizvedeni klasičnim i imobiliziranom kvascima. Profil arome određen je korištenjem GC/FID analize. Fermentacija imobiliziranim kvascima daje destilate s višim udjelom etanola i nižim sadržajem estera. Proizlazi da je fermentacija imobiliziranim kvascima pogodna za proizvodnju kvalitetnih destilata.*

**Ključne riječi:** *Imobilizirani kvasci, destilat masulja, aroma*



**Author's data:** Borislav Miličević PhD, Faculty of Food Technology Osijek, F. Kuhača 20, 31000 Osijek; Josip Mesić Msc., jmesic@vup.hr, Brankica Svitlica PhD, Polytechnic in Požega, Vukovarska 17, 34000 Požega; Igor Lukić PhD, Institute of agriculture and tourism Poreč, K.Huguesa, 8, 52440 Poreč; Antun Jozinović PhD, Faculty of Food Technology Osijek; Domagoj Šubarić PhD, Faculty of agrobiotechnical sciences Osijek, V. Preloga 1, 31000 Osijek, Croatia

## 1. Introduction

The production of grape marc distillates by traditional alembic distillation is a part of the national identity for many Mediterranean countries. Pedological properties of soil, as well as favourable climatic conditions, in Croatian region Istra, in addition to the traditional production procedures and choice of the appropriate grappe varieties result in unique quality of grappe marc distillates. The quality of grappe marc distillates is influenced by many factors [9, 19, 20, 22, 24], the main are volatile aroma substances. Aroma compounds, their levels, odour attributes and thresholds are most important for quality and authenticity of distilled spirits and liqueurs [12]. The composition of volatile aroma compounds in distilled spirits has been widely investigated using gas chromatography and mass spectrometry [1, 2, 7, 15, 16]. The typical flavour of grappe marc distillates results from the raw material compounds, as well as compounds developed during processing process. Many authors [5, 8, 25] have attempted to answer the question how the secondary aroma substances generated during the fermentation process influenced aroma and total quality of alcoholic beverages, as well as to investigate the fermentation process with immobilized yeasts. Immobilized yeast technology has attracted continual attention in the industry over the last decade. Latest developments in technology with immobilized yeast cells clearly corroborate that use of different reactor and systems practis [11, 18] excellent results in a quality can be accomplished. All these achievements are promising but still have numerous technological issues to be studied. The aim of this research was to study an impact of immobilized cell fermentation on aroma of marc distillates produced from two grape varieties commonly grown in Istra - Croatia (Rose Muscat of Poreč and Muscat blanc)

## 2. Material and methods

Grape Marc Samples. Grape (*Vitis vinifera* L.) varieties included in the investigation were Istrian, Muscat Blanc and Rose Muscat of Poreč, , obtained in harvest 2018. Samples Muscat Blanc and Rose Muscat of Poreč were produced using classical technological procedure: fermentation with free yeast /selected yeast Fermol-Bouquet 125/ and controlled thermal regime using outer refrigeration of fermenters with running water, with the aim of keeping the average temperature in intervals of 18-20 °C. The average duration of fermentation under these conditions was 21 days. Samples Muscat Blanc and Rose Muscat of Poreč were produced using technological procedure as shown in Figure 1: fermentation with yeast cells /selected yeast Fermol-Bouquet 125 immobilized in Ca-alginate gel [6,18], in internal loop gas-lift fermenter with alginate beads as yeast carriers and controlled thermal regime using outer refrigeration of fermenters with running water, with the aim of keeping the average temperature in intervals of 18-20 °C. The average duration of fermentation under these conditions was 72 hours for each set. The reusability of immobilized cultures [21] was not tested. All samples were taken at the end of fermentation before sedimentation, therefore the samples were insufficiently clear and slightly dull, which is appropriate for the selected procedure for the distillate production. (Figure 1.)

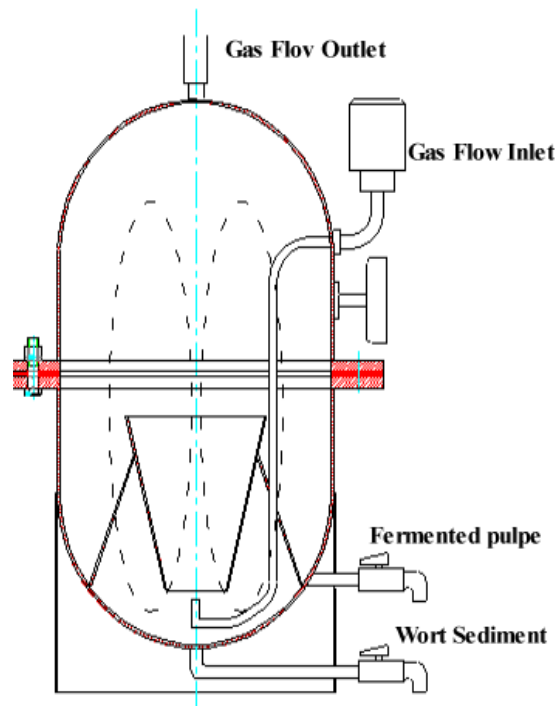


Figure 1. Reactor for fermentation with immobilized yeast cells

Selected samples were distilled in copper clip distillation device, according to the procedure, as shown in Figure 2.

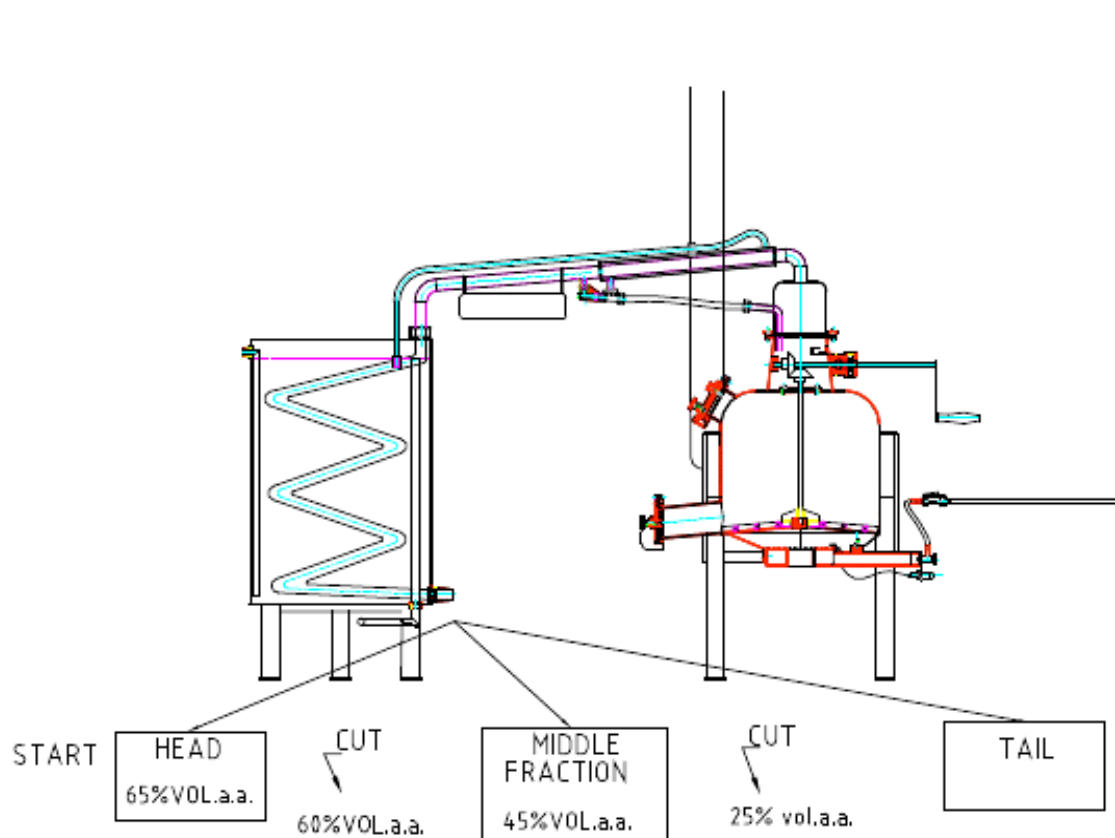


Figure 2. Procedure of distillation

The samples containing approximately 45 % vol. alcohol were taken from the middle fraction, or with recommended alcohol concentration in distillates, while the first (head) and the last (tail) fraction were not used. All selected samples were distilled according to the same traditional distillation protocol.

The major volatile components were analyzed on the basis of the European Community Reference Methods for the analysis of spirits using gas chromatography [3, 4]. Gas chromatography (GC) analyses were performed on a Chrompack 437A gas chromatograph with a split /splitless injector and a FID detector. For analysis of distillates a Chrompack Poraplot capillary column (25 m × 0,25 µm i.d. 0,25 µm) was used. Initial oven temperature was kept at 35 °C for 7 min, then raised at 10 °C/min to 80 °C followed by 25 °C/min to 180 °C, and kept for 4 min at 180 °C. Qualitative analysis was done by comparing the standard retention times (analytical grade from Merck, Germany) with the corresponding peaks of samples. The quantification was carried out by comparing the peak areas to those of the Merck standardhods for the analysis of spirits using gas chromatography [3, 4].

### 3. Results and discussion

The behaviour of the majority of the volatile compounds determined in middle fractions of Muscat Blanc and Rose Muskat of Poreč marc distillates are presented in Tables 1

Assessment characteristics	<i>Rose Muscat of Poreč</i>	<i>Muscat blanc</i>	<i>Rose Muscat of Poreč *</i>	<i>Muscat blanc*</i>
Total extract (g/ L)	0.064±0.01	0.034±0.02	0.018±0.02	0.011±0.03
Free SO <sub>2</sub> (mg/L)	3.54±0.02	3.85±0.01	4.04±0.03	4.20±0.02
Total acidity (mg/L)	457.60±0.03	485.00±0.02	247.00±0.02	351.20±0.02
Furfural (mg/L a.a.)	0.06±0.02	0.04±0.02	tr.	tr.
Benzaldehyde (mg/L)	2.29±0.03	1.67±0.02	1.59±0.02	1.49±0.06
2-Furancarboxaldehyde, 5(hydroxymethyl)	4.13±0.01	4.19±0.01	5.96±0.03	4.57±0.01
Ethanol (% vol.)	45.01±0.02	48.99±0.03	45.84±0.02	49.90±0.01
Methanol (mg/L a.a.)	0.12±0.02	0.14±0.02	0.08±0.02	tr.
1-propanol (mg/L)	2.75±0.01	4.70±0.03	1.00±0.01	3.70±0.02
1-butanol (mg/L)	0.15±0.01	0.20±0.02	0.09±0.01	0.10±0.03
isobutyl alcohol (mg/L)	3.62±0.01	3.90±0.03	3.42±0.02	3.60±0.032
isoamyl alcohol (mg/L)	8.79±0.01	9.90±0.01	5.39±0.03	6.39±0.03
2-phenyl ethanol (mg/L)	2.97±0.01	4.52±0.01	2.75±0.01	3.45±0.01
Linalool (mg/L)	0.91±0.02	0.61±0.03	0.98±0.02	0.65±0.02
α-Terpineol (mg/L)	2.18±0.02	1.58±0.01	2.85±0.01	2.45±0.01
Nerolidol (mg/L)	0.06±0.02	0.05±0.02	0.08±0.02	0.07±0.02
Limonene (mg/L)	0.11±0.03	0.11±0.01	0.19±0.02	0.19±0.01
β-Caryophyllene	4.45±0.02	4.33±0.01	5.38±0.01	5.15±0.02

ethyl lactate (mg/L)	0.17±0.02	0.41±0.02	n.d.	n.d.
ethyl octanoate (mg/L)	5.82±0.01	2.79±0.01	4.28±0.01	2.02±0.01
ethyl decanoate (mg/L)	1.84±0.02	1.91±0.02	n.d.	n.d.
ethyl acetate (mg/L)	6.57±0.01	6.60±0.01	4.49±0.02	4.82±0.01
Isoamyl acetate (mg/L)	9.61±0.01	9.41±0.02	9.02±0.02	8.45±0.01
acetaldehyde(mg/L)	5.10±0.02	4.75±0.03	4.55±0.01	3.15±0.02
Ethyl hexanoate (mg/L)	2.95±0.02	2.15±0.02	2.65±0.02	2.01±0.02
Methyl octanoate (mg/L)	0.56±0.03	0.46±0.02	0.46±0.03	0.35±0.03
2-Phenylethyl acetate (mg/L)	0.81±0.03	0.71±0.02	0.63±0.01	0.60±0.01
Methyl decanoate (mg/L)	0.34±0.01	0.30±0.02	0.25±0.02	0.23±0.01
Ethyl decanoate (mg/L)	28.45±0.01	28.54±0.01	28.40±0.01	28.40±0.01
Isoamyloctanoate (mg/L)	0.41±0.02	0.36±0.02	0.40±0.01	0.32±0.02
Ethylundecanoate (mg/L)	2.15±0.02	2.10±0.02	1.95±0.02	1.15±0.02
3-Methylbutyl dodecanoate (mg/L)	0.34±0.02	0.33±0.03	0.33±0.02	0.26±0.02
Ethyl dodecanoate (mg/L)	1.85±0.01	1.62±0.02	1.25±0.02	1.12.02
3-Methylphenyl butanoate (mg/L)	0.28±0.02	0.25±0.02	0.25±0.02	0.23±0.02
Undecanoic acid (mg/L)	2.96±0.02	2.83±0.02	2.93±0.02	2.88±0.02
Dodecanoic acid (mg/L)	0.29±0.02	0.28±0.02	0.23±0.02	0.21±0.01

Table 1. Selected chemical characteristics of mars distillates samples (mean ± standard error). \*fermented with immobilized yeast cells; n.d. – not detected; tr. – traces

The results obtained in this study revealed that it was possible to significantly affect the concentrations of many important volatiles in grape marc distillates by choosing the technology of immobilized cultures. From the obtained results (fig.1.), it is visible that fermentation with immobilized yeasts results in higher content of ethanol. For Rose Muscat of Poreč variety, this increase was from 45.01% vol. to 49.84% vol. in sample fermented with immobilized yeast cells, and for Muscat Blanc from 45.99% vol. to 49.90% vol. in sample fermented with immobilized yeast cells. The same trend was observed in our previous research [11]. Methanol content significantly is decreased with application of immobilized cell technology, which is preferably from the safety aspect of alcoholic beverages. [14, 17]. On the other hand, free SO<sub>2</sub> increased significantly (from 3.54 mg/L to 4.04 mg/L for Rose Muscat of Poreč and from 3.85 mg/L to 4.20 mg/L for Muscat Blanc), indicating that concentration of SO<sub>2</sub> added to pulp in order to control undesired fermentation and oxidation process could be reduced, since free SO<sub>2</sub> did not bind to acetaldehyde, giving stuffy odour [7]. Total extract and total acidity decreased due to immobilized cell technology application, which is consistent with our previous research [11]. According to [10, 12], most important classes of marc distillates are aroma compounds: alcohols, esters, aldehydes, ketones, etc. However, information regarding fermentation and distillation patterns of many minor constituents relevant for the aroma of spirits can be found in the available literature. [13]. More or less aroma profile of marc distillates given in Table 1. is consistent with the findings of our previous research [9]. The content of isoamyl acetate decreased by application of immobilised cells from 9.60 mg/L and

9.40 mg/L to 9.02 mg/L and 8.35 mg/L for Rose Muscat of Poreč and Muscat Blanc, respectively, indicating that immobilization could result in decreased quality characteristic of marc distillates. However, terpene content, which are also aroma compound of fresh grappe [13, 23], was significantly increased in samples produces using immobilized cell technique, probably due to fewer number secondary aroma reaction during fermentation.

Generally, ester contents decreased due to immobilized cell application, indicating that distillates produced by classical fermentation could have richer aroma.

#### 4. Conclusion

Istra, region of Republic of Croatia, is known by production of quality traditional marcs distillates, the results of this research indicate that fermentation with immobilized yeast cells results in increased ethanol content, and decreased content of methanol and esters. However the quality of marcs distillates is improved by immobilized technology application, compared to distillates produced in classical manner. Research showed that marcs distillates of the highest quality can be produced using immobilized technology, specific grappe varieties and specific traditional distillation procedures.

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