*Miličević, B. [1], Lukić I.[2], Babić, J.[3], Šubarić, D.[3], Miličević, R.[4], Đurđica Ačkar[3]*

*scientific study*

# The influence of fermentation process with immobilized yeast cells on quality of tangerine distillates

**Summary**

*The aim of this work was to create an objective evaluation of the influence of fermentation process with immobilized yeast cells on final quality of tangerine distillates.*

*Gas-chromatography and sensory analysis were applied to detect aroma and quality of tangerine distillates. The obtained results have shown that fermentation with immobilized yeast cells is a promising approach in distillates (obtained from tangarines) production process.*

*Lower concentrations of volatile compounds, such as esters and higher alcohol were observed in distillates made with immobilized yeast cells. In addition, the type of fermentation process had a significant influence on final quality on tangerines distillates.*

***Keywords:*** *tangerine, immobilized yeast, fermentation, volatile compound, distillate, quality*

## Introduction

The total world production of citrus fruit grew tremendously during the last four decades. Oranges constitute the largest single portion of citrus produced and currently contribute over 60% of the total world production (Miyazaki et al. 2011). Tangerines are non-climacteric fruits which can be kept for long periods of time without apparently suffering biochemical changes. In addition, due to their anatomy, they can be peeled and segmented easily, practically without affecting their vesicular structure (Pretel et al., 1998; Restuccia et al., 2006).

Early-season fruit are harvested when their external peel color is still green and are commercially degreened after harvest by exposing them to ethylene for a few days (Porat et al., 1999). The optimum temperature for the degreening process was reported to be 25ºC (Eaks, 1977; Cohen, 1978), but in the last few years it has become common in Spain and other Mediterranean countries to degreen Satsuma and other early mandarins at somewhat lower temperatures of 18–22 ºC.

1 **Borislav Miličević**, Zvečevo d.d., Prehrambena industrija, Kralja Zvonimira 1, Požega
2 **Igor Lukić**, Veleučilište u Rijeci, Trpimirova 2, Rijeka
3 **Jurislav Babić**, Drago Šubarić, Đurđica Ačkar, Prehrambeno-tehnološki fakultet Osijek, F. Kuhača 18, Osijek
4 **Radoslav Miličević**, Fakultet sigurnosti na radu Zagreb, I. Lučića 5, Zagreb





Alcoholic beverages have been part of human society since the beginning of recorded history and have developed in various regions of the world to reflect natural climates and cultural traditions (Room et al., 2005). Quality of alcoholic beverages is influenced by many factors during the production process (the main are aroma substances). Many studies (Vila et al., 1998; Lilly et al., 2000; Yajima and Yokotsuka, 2001) have attempted to answer the question how the secondary aroma substances which are generated during the fermentation process, influenced on aroma and total quality of alcoholic beverages.

Immobilized yeast technology has attracted continual attention in the industry over the several last years. Reasons are numerous: faster fermentation rates and increased volumetric productivity, possibility of continuous operation, etc. Latest developments in alcoholic beverages production with immobilized yeast cells clearly indicate that different reactor and systems designs have great influence on quality of final product (Virkajarvi and Kronolof, 1998; Poncelet et al., 2001).

The aim of this work was to create an objective evaluation of the influence of fermentation process with immobilized yeast cells on final quality of mandarin distillates.

## Materials and Methods

### Pulp

Each set of samples of tangerines pulp is taken from the production process at "Zvečevo d.d. Food Industry", from the harvest of the year 2008. All samples were protected by $SO_2$ (30 mg/L).

### Fermented pulp

Sample No.1 is produced using classical technological procedure: fermentation with free yeast */selected yeast Feromol-Bouqet 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 ºC. The average duration of fermentation under these conditions was 18 days.

Samples No. 2 and 3 are produced using technological procedure as shown in the scheme in Fig. 1: fermentation with immobilized yeast cells /selected *yeast Feromol-Bouqet 125*, immobilized in Ca-alginate gel (Poncelet et al., 2001) / in internal loop gas-lift fermentor with alginate beads as yeast carriers and controlled thermal regime using outer refrigeration of fermen-

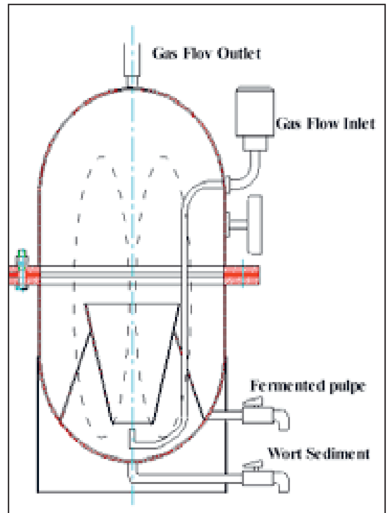


Figure 1: **Reactor for fermentation with immobilized yeast cells**

tors 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 32 hours for each set. The reusability of immobilized cultures was tested by replacing fermented pulp with fresh one every 2 days.

At the end of fermentation before sedimentation, all samples were taken. Therefore the samples were insufficiently clear and slightly dull, which is appropriate for the selected procedure for the production of distillate.

### Distillate

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

### Sensory analyses

A sensory analysis of samples was performed according to the method of positive scoring factor according to the German DLG model (Koch, 1986).

This model was based on 4 sensorial experiences, which are marked with grades 0 to 5, including 0, while the average grade is multiplied by the significance factor.

Sensory assessment was conducted in two repetition cycles. Each group had ten testers, selected by selection procedure (Jellinek, 1985).

### Chemical analysis of distillates

Fundamental analytical techniques were applied for the evaluation of the distillates quality. In industrial control laboratories these techniques represent the basis for the determination of the quality parameters.

Chemical analysis of the distillates included ethanol, total extract, total acidity, total $SO_2$, total aldehydes, total esters, higher alcohols, furfural and methanol analysis (AOAC 1995).

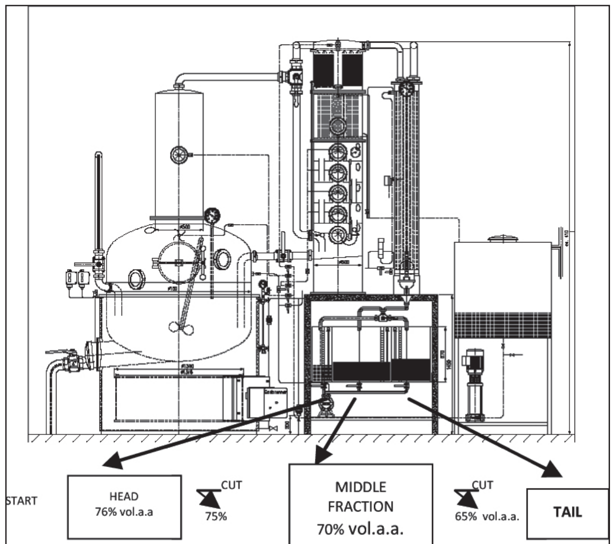

Figure 2: **Distillation protocol: Column proces with rectification index 1**

### Analyses of aroma substances

Gas chromatography (GC) analyses were performed on a Chrompack 437A gas chromatograph with a split /splitless injector and an FID detector. For analysis of distillates a Chrompack Poraplot capillary column (25 m x 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 standards.





## Results and Discussion

### Sensory analyses

Table 1 shows the results of conducted sensory analyses of distillates. Sensory assessment of samples was conducted according to the method of positive scoring with weight factors 3 to 9, according to German *DLG* model (Koch, 1986). The scoring was conducted by a group of ten professional testers with extensive experience in sensory assessment of distillates. Total sensory assessment ranged in the interval from 96.10 to 98.50; which indicates significant differences among samples. Furthermore, they can be addressed to different characteristics of fermentation protocol, since other factors in the production of distillates were the same for all samples. According to the estimated sensory characteristics, statistically significant differences (P=0.05) among distillates were found (Dawies, 1964). The samples No. 2 and No. 3, produced by fermentation protocol with immobilized yeast cells, were the ones with slightly weaker sensory quality in comparison to the sample No. 1 which is produced using classical technological procedure of fermentation with free yeast. The samples No. 2 and No. 3 also had pleasant aroma with slightly sharp alcoholic odour tones, and without characteristic off-flavour tones described by many authors (Perez-Lopez and Carbonell-Barrachina 2006.; Nykanen and Nykanen, 1991.; Yajima and Yokotsuka 2001.)

Table 1 **Sensory analyses of distillates - *German DLG model***

| *Sample* | *Assessment characteristics* | | | | TOTAL *(max 100 points)* |
|---|---|---|---|---|---|
| | Colour *(max 15 points)* | Clearness *(max 15 points)* | Odour *(max 25 points)* | Taste *(max 45 points)* | |
| **1** | 15.00 | 15.00 | 24.50 | 44.00 | 98.50 |
| **2** | 14.70 | 14.70 | 23.50 | 43.20 | 96.10 |
| **3** | 14.70 | 14.70 | 24.50 | 42.40 | 96.30 |

### Chemical analyses

In Table 2 chemical and physico-chemical characteristics have been shown. From the results obtained it is evident that defined characteristics of produced distillates are within referential values (Nykanen and Suomalainen 1983, Nykanen and Nykanen 1991).

The results obtained show that sample No. 1 had a lower share of alcohol ranging from

68,51 % a.a. volume while samples No. 2 and No. 3 had a higher amount of ethanol (70,60 and 71.50 % ) in total volume in relation to the optimal proportion of 70,00 % v (Nykanen and Suomalainen 1983). It's important to stress that lower share of alcohol may cause the reduction some of aroma substances in distillates (Conner et al. 1998).

The presence of free $SO_2$ in distillates ranging from 3.75 to 4.33 mg/L is the result of added $SO_2$ during production which is important for the protection of pulp from non-controlled fermentation process and oxidation. Free $SO_2$ may bind acetaldehyde which develops during distillation which may result to intensive stuffy odour (Guan and Pieper, 1998.).

The results obtained in Table 2 show that samples No.2 and 3 had a higher value of aldehydes (305.00 to 307.00 mg/L a.a.) and lower proportion of higher alcohols (1830.71 to 1880.27 mg/L a.a.) and esters (690.00 to 728.23 mg/L a.a.). It is important to stress that during maturation of distillates the shares of aldehydes and higher alcohols may cause formation of larger quantity of acetals (Singelton 1995). The results of the physico-chemical analysis show that the distillates are of quite satisfactory quality. In particular, the samples No. 2 and 3 may be picked out as potentially good distillate for production of quality tangerines brandy.

Table 2 **Chemical analyses of distillates**

| *Assessment characteristics* | *sample 1* | *sample 2* | *sample 3* |
|---|---|---|---|
| Ethanol (% vol.) | 68.51 | 70.60 | 71.50 |
| Total extract (g/ L) | 0.044 | 0.034 | 0.014 |
| Total $SO_2$ (mg/L) | 4.33 | 3.95 | 3.75 |
| Total acidity (mg/L) | 247.60 | 185.00 | 184.20 |
| Aldehydes (mg/L a.a.) | 280.00 | 305.00 | 307.00 |
| Esters (mg/L a.a.) | 1378.23 | 728.23 | 690.00 |
| Higher alc. (mg/L a.a.) | 1962.14 | 1830.71 | 1880.27 |
| Furfural (mg/L a.a.) | 0.002 | 0.001 | Tr. |
| Methanol (mg/L a.a.) | 0.02 | Tr. | Tr. |

*Tr.-traces

## Analyses of aroma

According to the basic assumption that volatile substances have dominant impact on aroma of distillate, gas chromatographic (GC) analysis was performed.

As in most foods of commercial interest the components of citrus juice and essential oil are found in concentrations greater than 1%. Chosen aroma substances belong to the group of aroma substances responsible for the quality of tangerines and distillates produce from tangerines.

Table 3 shows the content of defined aroma substances in samples of distillates. According to the estimated chromatographic characteristics, greater differences among distillates were found. The monotherpene group is predominant in aroma profile of tangerines distillate of which limonene is the most abundant. This is in accordance with previous results (Chisholm & Jell, 2003; Sawamura et al. 2004).

However, there is a disagreement regarding to aroma activity of limonene and other aliphatic and monotherpenic aldehydes (especially citral), as well as those of esters and alcohols.

In comparison to the research that have been done so far (Elmaci and Altug, 2005; Perez-Lopez & Carbonell-Barrachina, 2006) lower concentrations of volatile compounds were observed in samples made by using protocol of fermentation with immobilized cultures.

Volatile substances which could destroy the distillate quality (Clark and Chamblee, 1992) were not identified in either of the samples produced by fermentation with immobilized cultures, 4-vinyl guaiacol a well-known indicator of thermal abuse and guaiacol, an indicator of microbial contamination haven't been noticed, not even in traces which proves the quality of the process.

Table 3 **Aroma compounds in distillate samples (mg/L)**

| *Aromacompounds (mg/L)* | *sample 1* | *sample 2* | *sample 3* |
|---|---|---|---|
| acetaldehyde | 13.76 | 13.96 | 14.11 |
| ethyl acetate | 56.57 | 54.20 | 44.31 |
| methanol | 0,025. | 0,038. | n.i. |
| 1-propanol | 24.75 | 24.70 | 18.20 |
| isobutyl alcohol | 23.62 | 19.39 | 15.37 |
| ethyl hexanoate | 2.47 | 1.57 | n.i. |
| isoamyl alcohol | 148.79 | 139.9 | 88.80 |
| ethyl lactate | 3.07 | 1.41 | n.i. |
| ethyl octanoate | 5.92 | 2.69 | 2.92 |
| ethyl decanoate | 1.74 | 0.91 | 1.18 |
| 2-phenyl ethanol | 2.97 | 3.52 | 3.45 |
| α-terpineol | 4.1 | 4.78 | 3.17 |
| linalool | 0.63 | 0.87 | 1.33 |
| terpinen-4-ol | 4.01 | 4.02 | 3.45 |
| thymol | 3,11 | 4.17 | 3.19 |
| limonene | 11.14 | 12.16 | 13.27 |
| α-pinene | 0.01 | 0.01 | 0.03 |

| | | | |
|---|---|---|---|
| *β*-pinene | n.i. | tr. | tr. |
| furaneol | n.i. | n.i. | n.i. |
| 4-vinyl guaiacol | n.i. | n.i. | n.i. |
| carvacrol | n.i. | n.i. | n.i. |
| guaiacol | n.i. | n.i. | n.i. |
| *p*-cymene, | n.i. | n.i. | n.i. |
| carvone, | n.i. | n.i. | n.i. |
| *p*-menthadiene-8 ol | tr. | tr. | tr. |
| *p*-menthen-1,8-diol | tr. | n.i. | n.i. |

*n.i. – not identified; *tr.-traces

## Conclusions

The obtained results have shown that fermentation with immobilized yeast cells has significant influence on aroma and quality of distillates.

Fermentation time was reduced and final distillates had satisfactory sensory and analytical profiles. System of immobilized yeast cells is not ideal, but is a promising approach in tangerine distillates production process.

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