Raspberry wine fermentation by immobilized yeast cells

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INTRODUCTION AND OBJECTIVE

Raspberry (*Rubus idaeus* L.), as a fruit species widely grown in Serbia, is a great source of components of important biological value. This fruit presents great source of polyphenolic compounds, particularly flavonoids such as anthocyanin pigments, which give raspberries their characteristic colour (Liu, 2007).

In recent years, the production of wine from berry fruits has increased. Fruit wines contain a significant amount of polyphenols in range from 335 to 1830 mgGAE/L for strawberry and black currant, respectively. In case of raspberry wine it is from 1050 to 1900mg GAE/L (Heinonen 1998, Đorđević 2012).

Also, there is a growing interest in using immobilized yeast cell systems for numerous applications in food technology and biotechnology. The success of such applications depends on achieving suitable conditions for cell growth inside microbeads. Ca-alginate hydrogel are still the most frequently used for immobilization of yeast cells (Nedovic 2001, 2011). The objective of this study was to investigate influence of yeast cell immobilization on kinetics of raspberry pulp and raspberry juice fermentation. Also, the total polyphenol content (TPC) of raspberry wine samples was investigated.

MATERIALS AND METHODS

Meeker variety raspberries were obtained from Aleksandrovac, Serbia. The selected yeast, *Saccharomyces cerevisiae bayanus* EC-1118 was purchased from Lallemand (Montreal, Canada). The alginate (ALGOGEL^{TM}3001, Cargill) was generously donated by PALCO (Šabac, Serbia).

Raspberry wine production: The pulp and juice of *Meeker*, was fermented at 15°C with and without selected yeast cells, and with immobilized yeast cells. The pulp and juice were added in the glass bioreactors (2L). Samples were fermented at 15°C: one sample of juice and one of pulp without selected yeast, with selected yeast and with immobilized yeast cells. Samples were prepared with addition of sulfur-dioxide in concentaration of 50mg/L_{medium}. Fermentation was monitored by measurement of Brix values daily. The initial Brix value was 10.50 and the pH was 3.20. At the end of fermentation, seeds and pulp residue were separated from the wine by pressing and wine was transferred to bottles the capacity of 330 mL and stored at 5°C for sedimentation of the biomass.

Anton Paar alcolyzer plus, DMA 4500 density meter was used for monitoring of sugar and alcohol content in the samples.

Folin-Ciocalteu method: TPC was determined according to the Folin Ciocalteau method (Singleton 1999). Absorbance of each sample in triplicate was measured at 765 nm using a spectrophotometer. The calibration curve was prepared with gallic acid (GA) solution and TPC was expressed as mg gallic acid equivalent per liter of sample (mg GAE/L).

Imobilization procedure: Calcium alginate beads entrapping the yeast cells (*Saccharomyces cerevisiae* var *bayanus* EC-1118) were produced by electrostatic extrusion technique (Nedovic 2001).

Alginate powder was dispersed in distilled water to produce solutions of 0.015g/mL. Freeze-dried yeast cells were added to alginate (~1g of dried cells/100mL alginate solution) after rehydration in 10mL of distilled water for 10 min. The alginate/cells suspension was mixed on magnetic mixer for 1h and extruded through 0.7mm blunt stainless still needle using a syringe pump (Pump 11, Harvard Apparatus, SAD) under constant flow rate of 70mL/h. The spherical droplets were formed by combined action of electrostatic force and gravity. Electrostatic potential (6.5kV) was formed by electrostatic encapsulation unit VAR V1 (Nisco Encapsulator, Switzerland). The collecting solution was calcium chloride (0.015 g/mL). After extrusion, the beads were left in the collecting solution for 45 min. After that, the beads were rinsed and left in physiological solution (9.0 g of NaCl per liter water) at 4°C. The beads with cells were cultivated in bioreactors, which contained 1L of medium and 100 g of beads.

RESULTS AND DISCUSSIONS

As shown in Tab1., before fermentation started, total polyphenol content in the sample was 1640.4 mg GAE/L (mg/L gallic acid equivalents). After 48 hours of fermentation, the sample fermented without selected yeast cells reached its maximum value of 2206.0 mg GAE/L, whereas the sample with immobilized yeast cells reached the value of 1835.0 mg GAE/L. The samples of juice had lower values then these three pulp samples. After 120 hours of fermentation, total polyphenol content was lower than after 48 hours in all samples.

Table 1. Total polyphenol content of raspberry wine during fermentation

t (h)	KMM S*[a]	KM MI[a]	KM M[a]	TMM S[a]	TM MI[a]	TM M[a]
0	1640	1640	1640	1640	1640	1640
24	1722	1659	1764	1638	1505	1692
48	2206	1835	1872	1630	1573	1640
72	2033	1704	1754	1625	1419	1651
96	1895	1985	1363	1621	1310	1415
120	1792	1904	1360	1602	1302	1414

[a]- Results are given in mg GAE/L
*KMMS-sample of pulp without selected yeast; KMMI-sample of pulp with immobilized yeast; KMM-sample of pulp with selected yeast; TMMS-sample of juice without selected yeast; TMMI- sample of juice with immobilized yeast, TMM- sample of juice with selected yeast.

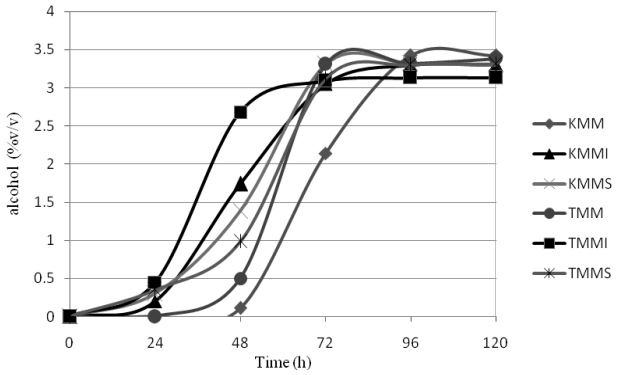

Figure1. Fermentation kinetics (production of alcohol)

Fig 1. shows change in amount of alcohol during 120 h, daily. The highest values are very similar in all samples, but in samples with immobilized yeast cells that values are reached earlier, after 48h in pulp sample.

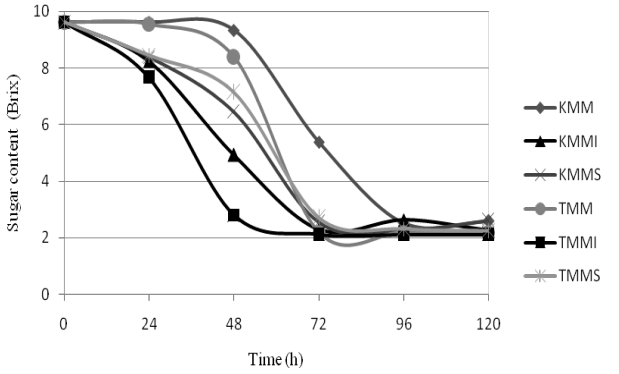

Figure 2. Fermentation kinetics (consumption of sugar)

In Fig.2 is shown decrease of sugar content in all six samples. As in previous graph, final content is almost same in all of them. However, samples of pulp and juice with immobilized yeast cells were the first two samples which reached the lowest point of sugar content.

CONCLUSIONS

The obtained results indicate that addition of immobilized yeast cells has significantly influence the extraction of phenolic compounds from raspberry pulp, while the addition of selected yeast cells has no significant influence. Samples without selected yeast have shown higher values, comparing to previous two. One of the reasons could be adsorption of polyphenols on surface of selected yeast. For example, values of TPC in the sample without selected yeast exceed 2000 mgGAE/L, after 48 and 72 hours. During fermentation, TPC in all of samples constantly increased to 48 hours where reached its maximum except in case of juice fermented spontaneously. However, the highest final results of TPC had sample of pulp fermented with immobilized yeast cells.

Also, the results of this study show that fermentation medium (pulp or juice) had strongly influence on TPC content of wine. When fermentation was finished all samples had decrease in TPC values, especially in case of samples where juice was medium. As expected, those final values were lower comparing to starting point and pulp samples.

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