Wine Production with Immobilized Yeasts on Grape Pomace

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Abstract

The alcoholic fermentation is one of the most important stages in the winemaking process and contributes decisively for the quality of the final product, particularly aromatic characteristics. The immobilization of yeast cells in fermentation processes presents several technological and economic advantages when compared with free cells systems, such as increased productivity and greater tolerance of the cells to inhibitory substances.

In this context, the objective of this work consisted in using immobilized yeasts for driving and controlling the alcoholic fermentation process in winemaking. The immobilization of *Saccharomyces cerevisiae* took place on grape pomace by natural adsorption. The evolution of the alcoholic fermentation was followed daily by measuring must density until a value lower than 1000 kg/m^3 was reached. Physical-chemical and sensorial characterization of the wines, produced with free and immobilized cells, were carried out.

The immobilized yeasts were able to effectively conduct the alcoholic fermentation and therefore, to produce wine. Sensory analysis demonstrated the existence of perceptible olfactory differences in wines produced by free cells and immobilized cells. Moreover, the produced wines presented significant differences respecting color attributes.

Resumo

A fermentação alcoólica é uma das etapas mais importantes no processo de produção de vinho e contribui decisivamente para a qualidade final do produto, particularmente o aroma. A imobilização de células de levedura em processos fermentativos apresenta diversas vantagens tecnológicas e económicas quando comparada com sistemas de células livres, tais como incremento da produtividade e maior tolerância das células a substâncias inibitórias.

Neste contexto, o objectivo do trabalho consistiu na utilização de leveduras imobilizadas para para a condução e controlo do processo de fermentação alcoólica em vinificação. A imobilização de *Saccharomyces cerevisiae* foi realizada por adsorção natural num suporte natural constituído por bagaço de uva. A evolução da fermentação foi seguida diariamente pela medição da massa volúmica, sendo dada por terminada para valores inferiores a 1000 kg/m^3. Os vinhos produzidos, quer com células livres quer com células imobilizadas, foram avaliados por caracterização físico-química e por análise sensorial.

As leveduras imobilizadas foram capazes de conduzir eficazmente as fermentações alcoólicas e, por conseguinte, de produzir vinho. A análise sensorial dos vinhos demonstrou a existência de diferenças olfactivas perceptíveis nos vinhos produzidos a partir de células

livres e de células imobilizadas. Além disso, as análises de cor demonstraram que os vinhos produzidos apresentaram diferenças significativas.

Introduction

The cell immobilization in alcoholic fermentation presents several technological and economic advantages when compared with free cells systems, such as increased productivity, higher cell concentrations in the reactors, reuse of the biomass in batch processes, greater tolerance of the cells to inhibitory substances and the possibility to run the process in continuous mode of operation. The supports for cell immobilization in the alcoholic beverages industry should have a high structural resistance and stability and should be food-grade (Kourkoutas *et al*., 2004). The grape pomace is the most important solid waste product of the wine industry, being of interest to find an alternative use for this by-product of wine production.

The aim of the present study was to produce wine with *S. cerevisiae* immobilized by natural absorption on the grape pomace surface, and to compare the results with similar tests using free cells.

Materials and Methods

For cell immobilization, the carrier was washed with water and dried to constant weight. The immobilization of *S. cerevisiae* was carried out in 500 mL Erlenmeyer flasks containing 300 mL of white grape must and 50 g (dry weight) of material carrier at 25 ℃ for 24 h.

After immobilization the biocatalyst was washed with must and used in two consecutive batch fermentations for wine production (batch1 and batch2). Alcoholic fermentation was done in Erlenmeyer flasks containing 2.75 L of white grape must cv. *Azal* (initial sugar content of 220 g/L) and 400 g (wet weight) of grape pomace with immobilized cells. Additionally, experiments with free cells were carried out for comparison between samples.

The final products were submitted to chemical and sensory analysis in order to evaluate its quality. Glucose, fructose, ethanol and glycerol were determined by High Performance Liquid Chromatography (HPLC) in a Jasco chromatograph equipped with a refractive index detector (Jasco 830-RI), a ultraviolet detector (UV-Vis) and a column Varian Metacarb 67H column (300 mm $\times$ 6.5 mm).

The color of the wines was assayed by CIELab method. This analysis consisted of measuring the wavelengths between 380 nm and 770 nm (data pitch: 2 nm) using a spectrophotometer (Jasco 560). The recorded absorbance values were processed by an algorithm using the program Matlab version r2010a to calculate CIELab coordinates L^*, a^* and b^*. The saturation (C^*), the variation in saturation (ΔC^*) and the variation in lightness (ΔL^*) were determined according to Almela *et al.* (1995).

The three wines produced in the fermentations were also subjected to a sensory analysis, in dark glasses, using the triangular test (ASTM E1885-04 norm). The tests were conducted by 35 panelists, selected randomly at the laboratory. The panelists were also asked to name a preference in each series of three wines.

The determination of the immobilized biomass was done at the end of the immobilization, by washing the biocatalyst with a 30 g/L NaOH solution, according to Genisheva *et al.* (2011).

Results and Discussion

The quantity of the immobilized cells, X_{im}, in grape must medium was 30.0 mg/g (cells/dry support). This amount is slightly higher than the results published by Genisheva *et al*. (2011), 25.1 mg/g, where *S. cerevisiae* was immobilized on grape skin in a synthetic medium.

Fermentation assays of white grape variety cv. *Azal* were conducted with immobilized cells of *S. cerevisiae*. Fermentations with immobilized cells are known to have higher productivities than fermentations with free cells. This fact was confirmed in our results. In fact, batch1 and batch2 fermentations, with immobilized cells, demonstrated ethanol yields of 0.33 g/g and 0.44 g/g, respectively, compared with 0.27 g/g for the free cell fermentation. The highest efficiency of fermentation, relatively to the maximum possible yield, was found for batch2 (86 %) followed by batch1 (65 %); the lowest efficiency was registered in the free cell fermentation (53 %).

The concentrations of glucose, fructose, glycerol and ethanol were determined by HPLC and are depicted in Table 1.

Table 1. Concentrations (*C*) of the compounds analyzed by HPLC for the tree wines

Compound	*C*/(g/L)		
	free cells	batch1	batch2
glucose	0.08	0.23	0.57
fructose	1.16	2.43	8.87
glycerol	7.04	4.82	6.94
ethanol	60.21	71.53	96.16

In the fermentations with free cells the glycerol concentration was slightly higher. With regard to ethanol, the fermentation with immobilized cells showed higher concentrations compared with fermentation with free cells. Since the tolerance of immobilized cells to ethanol is higher, they can keep their fermentation activity even when the alcohol content is high.

Sensory analysis was performed through olfactory test, which compare the three wines from the fermentations (free cells, batch1 and batch2). The triangular test is used to evaluate possible differences between two products, based on the analysis of three samples in which the taster has to decide which of the three samples is different or what are the two most similar. According to ASTM E1885-04 standard, considering a confidence level of 95 %, the number of correct answers should be greater than 31 in a total of 70 possible answers. This rule was observed, stating that there were differences between the wines in terms of

olfactory testing. Wine batch 2 has recorded the greatest number of preferences from the tasters. As evidenced by the sensory analyses, the technique of cell immobilization applied to wine production promoted the quality of the final product.

The color of wines was carried out by the CIELAB method, by determining the coordinates L^*, a^* and b^*. Moreover, to compare the wines, the differences of color *i.e.* the variation in lightness ΔL^* and in saturation ΔC^*, were also calculated; as standard values, the coordinates L^* and C^* of wines produced with free cells, were considered (Table 2).

Table 2. Mean values and confidence limits ($p<0.05$) for L^*, a^* and b^*, obtained from the CIELab method, and for the calculated values for C^*, ΔL^* and ΔC^*

Wine	L^*	±	a^*	±	b^*	±	C^*	±	ΔL^*	ΔC^*
free cells	96.18	0.21	-0.45	0.04	0.07	0.18	1.69	0.17	0.00	0.00
batch1	94.94	0.33	-1.48	0.02	0.06	0.15	6.63	0.14	-1.24	4.94
batch2	95.43	0.73	-1.06	0.14	0.27	0.66	4.99	0.62	-0.75	3.29

Wines produced with immobilized cells (batch1 and batch2) presented the lower values of L^* (lower brightness and higher opacity), which means that these wines have a higher color intensity. These results suggest that the support used in the fermentation process directly influences the intensity of the color of wines. However, if the support will be used in successive batch fermentations, this problem may become innocuous. Furthermore the parameter C^* was higher for wines batch1 and batch2, indicating a higher saturation of color.

Conclusions

The results showed that grape pomace is an efficient carrier for yeast immobilization in alcoholic fermentation for wine production. The application of immobilized cells in the alcoholic fermentation was adequate and an effective process for winemaking. The produced wines demonstrated significant differences in colour and olfactory characteristics. Moreover, in sensory evaluation, the preference of panelists was inclined for a wine produced with immobilized cells.

Acknowledgments

Zlatina Genisheva gratefully acknowledges FCT (Contract/grant number: SFRH/BD/48186/2009) for financial support of this work.

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