INFLUENCE OF INITIAL TANNIN CONCENTRATION IN MUST ON THE KINETICS OF WINE FERMENTATION, USING YEAST IMMOBILIZED IN CALCIUM ALGINATE GEL

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ABSTRACT: *This study investigated the effects of five initial tannin contents (1.8, 2.8, 3.8, 9.8 and 17.8g of acid tannic/L) on the kinetics of red wine fermentation, using yeast immobilized in calcium alginate gel. Our experimental results showed that in the medium with high tannin content (9.8 and 17.8g of acid tannic/L), maximum cell density and maximum specific growth rate of the immobilized cells were higher than those of the free cells. In all cases, the glucose utilization rate and ethanol production rate of the immobilized yeast were always higher than those of the free yeast. Moreover, using immobilized yeast in wine fermentation decreased pH value and volatile acidity of the culture. Thus the colloidal and biological stability and the organoleptic characteristics of the final product were ameliorated.*

Keywords: *alginate, fermentation, immobilization, tannin, wine.*

1. INTRODUCTION

In the past few years there has been an upsurge of interest in immobilized cells due to attractive technical and economic advantages compared with the conventional free cell system [18]. Activity stability of yeast is prolonged because the immobilization support may act as a protective agent against physico-chemical effects of pH, temperature, solvents or even heavy metals [12]. Many reports have proposed various immobilization supports for wine making such as gamma alumina and kissiris [1,7,9], delignified cellulosic material and gluten [2,13,14], apple pieces [8,10,11], dried raisin berry [19], polyvinyl alcohol [15], alginate [4,17]... In this study, alginate was selected for yeast immobilization because of simple immobilization procedure, high cell density in the gel and non-toxic carrier [3].

Tannins are important compounds in must because they influence on wine taste and colour [16]. In addition, these compounds can affect microbial metabolism. The aim of this study was to investigate the influence of initial tannin concentration on the kinetics of wine fermentation, using yeast cells immobilized in calcium alginate gel.

2. MATERIALS AND METHODS

Yeast: a strain of *Saccharomyces cerevisiae* from Food Microbiology Laboratory (Ho Chi Minh city University of Technology) was used in this study. Grape juice was used for yeast multiplication. Preculture was prepared by two successive inoculations: 1) in 250mL erlenmeyer shake flask containing 100mL of grape juice for 24 hours, and 2) in a 2L erlenmeyer shake flask containing 500mL of grape juice. For both periods, the inoculum was grown at 28°C and 250rpm.

Alginate: Sodium alginate was supplied by Biotechnology Center, Nha Trang University of Fisheries. The viscosity (2% alginate solution, 25°C) was 423.6cp.

Must: Fermentation medium was prepared from *Red Cardinal* grape (Ninh Thuan province, Vietnam). The must was adjusted to 240g of glucose/L, 195ppm N, 112 ppm SO_2

and pH 4. The tannin concentration in must was alternatively adjusted to 1.8, 2.8, 3.8, 9.8 or 17.8g/L by tannic acid.

Yeast immobilization: The immobilization procedure was carried out by the traditional external gelation method [3]. Yeast concentration was $25x10^6$ cells/mL of gel bead.

Fermentation: The fermentation was conducted in an erlenmeyer containing 500 mL of must at 22-25°C. The inoculating rate was $5x10^6$ cells/mL. Free yeast cells were used in the control sample.

Analytical methods:

- Yeast cell number was quantified by haemocytometry, using Thoma counting chamber. For counting yeast cells inside the gel beads, the beads were dissolved in a 2%w/v Na-EDTA solution [3].
- Reducing sugar concentration was determined by spectrophotometric method, using 3,5-dinitrosalicylic reagent [6].
- Alcohol in the cultures was distilled and measured using hydrometer [6].
- Volatile acidity was estimated by titration of distillate that was obtained by steam distillation of wine sample, using 0.1M NaOH solution [22]

Statistical treatment

Each presented result was the average of three independent experiments. The statistical software package Statgraphics Plus v. 2, from STSC, Inc. (Rockville, MD) was used to perform a single analysis of variance (ANOVA) in order to study the difference between free and immobilized yeast cultures.

3. RESULTS AND DISCUSSION

Yeast growth

Figure 1 shows the maximum cell density and maximum specific growth rate of immobilized and free yeasts in the cultures with various initial tannin concentrations. Increase in tannin concentration of the juice from 1.8 g/L to 17.8 g/L decreased both maximum cell densities and maximum specific growth rates of the immobilized and free yeasts. It can be explained that tannin inhibited the growth of *Saccharomyces cerevisiae.* According to Wauters et al.[20] &[21], iron deprivation caused by tannic acid may thus affect the synthesis of functional respiratory chain as well as the synthesis of unsaturated fatty acids and sterol. Therefore, yeast growth decreased.

Besides, in the cultures with low initial tannin content (1.8 and 2.8g/L), the maximum cell density and maximum specific growth rate of the immobilized cells were lower than those of the free cells. On the contrary, in the cultures with high initial tannin content (9.8 and 17.8g/L), the maximum cell density and maximum specific growth rate of the immobilized yeast were similar or higher in comparison with those of the free yeast. So cell immobilization in calcium alginate gel improved the yeast growth in medium with high tannin content.

Fermentation time

In this experiment, the fermentation was considered as completed when the fermentation productivity reached approximately 97%. Fermentation productivity was the ratio between the reducing sugar content consumed by yeast during the fermentation and the initial reducing sugar content in the medium. Table 1 presents the fermentation time in the immobilized and free yeast cultures. It can be noted that the fermentation time of the immobilized cells was 1.2

– 1.3 times shorter than that of the free cells. This phenomenon permitted to reduce the production cost and augment the winery productivity.

In addition, increase in tannin content in the medium augmented the fermentation time in both immobilized and free yeast cultures. The higher the initial tannin concentration in must, the longer the fermentation time.

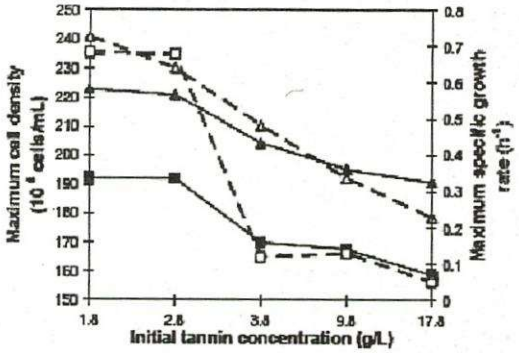

Figure 1 Effect of initial tannin concentration on maximum cell density (▲△) and maximum specific growth rate (■□) of immobilized and free cells in wine fermentation. Filled and open symbols indicate immobilized and free cells respectively.

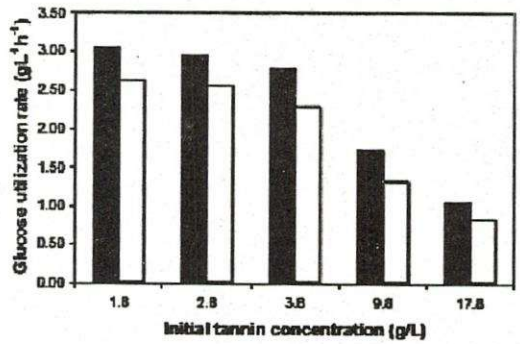

Figure 2 Effect of initial tannin concentration on average glucose utilization rate of immobilized and free cells in wine fermentation. Black and white columns indicate immobilized and free cells respectively.

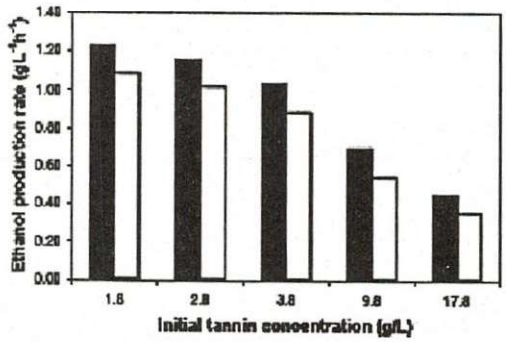

Figure 3 Effect of initial tannin concentration on average ethanol production rate of immobilized and free cells in wine fermentation. Black and white columns indicate immobilized and free cells respectively.

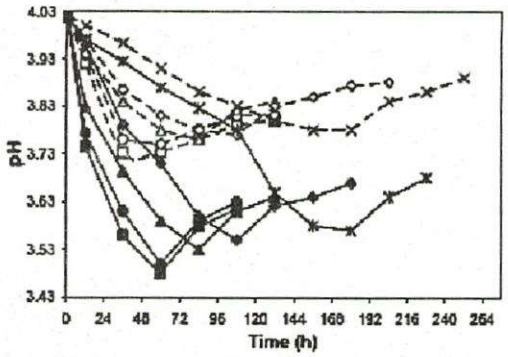

Figure 4 Change in pH value of media during wine fermentation using immobilized and free cells with various initial tannin concentrations: ■□, 1.8 g/L; ●○, 2.8 g/L; ▲△, 3.8 g/L; ◆◇, 9.8 g/L; ✱×, 17.8g/L. Continuous and dashed lines indicate immobilized and free cells respectively.

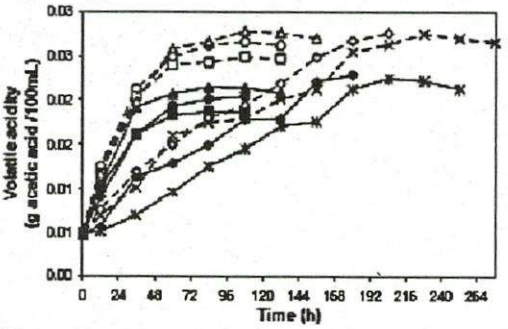

Figure 5 Change in volatile acidity during wine fermentation using immobilized and free cells with various initial tannin concentrations: ■□, 1.8 g/L; ●○, 2.8 g/L; ▲△, 3.8 g/L; ◆◇, 9.8 g/L; ✱×, 17.8g/L. Continuous and dashed lines indicate immobilized and free cells respectively.

Table 1 Effect of initial tannin concentration on fermentation time of wine fermentation time using immobilized and free cells

Initial tannin concentration (g/L)	Fermentation time (h)	
	Immobilized cell	Free cell
1.8	76.8 ± 2.0^{a}	88.8 ± 2.4^{c}
2.8	79.2 ± 3.3^{a}	91.2 ± 2.4^{c}
3.8	84.0 ± 2.6^{b}	102.0 ± 4.0^{d}
9.8	134.4 ± 3.8^{e}	175.2 ± 5.8^{f}
17.8	218.4 ± 6.9^{g}	276.0 ± 8.6^{h}

Each values represents mean and standard deviation of 3 independent samples.

Different letters mean significant differences ($P < 0.05$).

Glucose utilization rate

Figure 2 indicates the glucose utilization rate of the immobilized and free yeasts. In all cases, the immobilized cells utilized glucose faster than the free cells. Besides, when initial tannin content in the medium increased from 1.8 to 17.8 g/L, the glucose uptake rate of the immobilized and free cells fell 2.8 and 3.1 times, respectively. In a study of ethanol production, Galazzo et al., (1990) also concluded that the immobilized cells consumed glucose twice as fast as the free cells [5].

It can be confirmed that cell immobilization in calcium alginate gel improved the substrate uptake rate of the yeast. It was due to protective role of the carrier for yeast cells. According to Wauters (2001), in medium with high tannin content, tannins may be adsorbed onto the surface of the free cells and this phenomenon inhibited glucose consumption of the yeast [20,21].

Ethanol production rate

Figure 3 presents the average ethanol production rate in the cultures with different tannin contents. It can be noted that ethanol biosynthesis by the immobilized cells was faster in comparison with that by the free cells. In addition, when the initial tannin content augmented from 1.8 to 17.8 g/L, the ethanol production rate of the immobilized and free yeasts decreased from 1.22 to 0.44 and from 1.09 to 0.35 ($gL^{-1}h^{-1}$) respectively.

Acidity

The evolution of pH value and volatile acidity during the fermentation are showed in Figure 4 and 5. From the obtained results, it can be affirmed that wine produced by the immobilized yeast had lower pH value and volatile acidity content than that produced by the free yeast. The same result was also observed by Tsakiris et al.[19]. In wine fermentation by yeast immobilized on dried raisin berries, these researchers also concluded that the wine produced by immobilized yeast had lower volatile acidity in comparison with the control sample fermented by free yeast.

Some volatile acids such as acetic acid, butyric acid... influence negatively on the product flavour. The higher the content of volatile acidity in wine, the lower the sensory quality of the produced wine. The application of immobilized yeast in wine fermentation decreased the volatile acidity in wine and improved the product sensory quality. Moreover, the pH value of the immobilized yeast culture was also lower than that of the free yeast culture. Thus the colloidal stability and biological stability of the produced wine were ameliorated.

4. CONCLUSION

Increase in tannin content in must decreased the yeast growth, glucose utilization rate and ethanol production rate but increased the fermentation time and volatile acidity. In comparison with the free yeast, the immobilized yeast in calcium alginate gel had higher glucose utilization rate and ethanol production rate. So the fermentation time of the immobilized cells was always shorter than that of the free cells. Besides, the colloidal stability, biological stability and organoleptic characteristics of the produced wine fermented by the immobilized yeast were improved.

It can be concluded that the yeast immobilized in calcium alginate gel was more tolerant to high content of tannin than the free yeast. So, using immobilized yeast can overcome disadvantage effects of tannin on metabolic activities of *Saccharomyces cerevisiae*.

ẢNH HƯỞNG HÀM LƯỢNG TANNIN BAN ĐẦU TRONG DỊCH NHO ĐẾN ĐỘNG HỌC QUÁ TRÌNH LÊN MEN RƯỢU VANG, SỬ DỤNG NẤM MEN CỐ ĐỊNH TRONG GEL ALGINATE

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TÓM TẮT: *Bài báo này khảo sát sự ảnh hưởng của nồng độ tannin ban đầu trong dịch nho (1.8, 2.8, 3.8, 9.8 và 17.8g acid tannic/L) đến động học quá trình lên men rượu vang đỏ, sử dụng nấm men cố định trong gel alginate calcium. Kết quả thực nghiệm cho thấy trong môi trường với hàm lượng tannin cao (9.8 và 17.8g acid tannic/L), giá trị mật độ tế bào cực đại và tốc độ sinh trưởng riêng cực đại của nấm men cố định cao hơn so với nấm men tự do. Trong tất cả các trường hợp khảo sát, tốc độ sử dụng đường và tốc độ sinh tổng hợp ethanol của nấm men cố định luôn cao hơn so với nấm men tự do. Ngoài ra, việc sử dụng nấm men cố định còn làm giảm giá trị pH và hàm lượng các acid dễ bay hơi trong rượu vang. Do đó, độ bền keo, độ bền sinh học và giá trị cảm quan của rượu vang thành phẩm sẽ được cải thiện.*

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