

Aluminium (III)-Alginate Immobilized Yeasts to Enhance Anthocyanin Stability during Bayberry (*Myrica rubra*) Red Wine Fermentation

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Abstract : Bayberry (*Myrica rubra*) wine usually loses its red color, because its naturally-existing anthocyanins (CA) would be faded to a large extent during its free yeast cells fermentation (FCF). Anthocyanins are metabolically degraded by β -D-glucosidases that are widely expressed in fungi, yeasts and plants. To overcome this problem, aluminum (III) alginate-immobilized yeasts, instead of free ones, were utilized in the wine fermentation tests. Four commercially available *Saccharomyces cerevisiae* strains, Angel, Lalvin D254, Lalvin BM45, and Lalvin L2323 were evaluated. The CA stability in ICF (immobilized cells fermentation) of four yeast strains after 20 days was dramatically enhanced with the retaining rates as 50.0% (L.D254), 46.4% (Angel), 45.5% (L.L2323), and 42.7% (L.BM45), respectively, whereas, those corresponding values in FCF were only 22.7%, 20.9%, 24.5%, and 26.3%, respectively. The enhanced property of ICF on anthocyanins stability was due to the lower diffusion effect occurring in the compact outer layers of Al-alginate beads, the inhibition of β -D-glucosidases as ethanol to accumulate in medium, as well as the stabilization of the aluminum (III) in complex with anthocyanins. Moreover, during the early stage of the tests, ICF provided more stable fermentation rate as well as more slowly releasing of sulfur dioxide in medium, very important to the final retaining rate of CA.

Key words: Aluminum (III)-alginate; anthocyanin stability; β -D-glucosidase; bayberry (*Myrica rubra*) wine

海藻酸铝固定化酵母在杨梅果酒发酵中提高花色苷稳定性研究

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摘 要: 杨梅果酒采用游离酵母发酵时, 由于微生物所产生的 β -D-葡萄糖苷酶对果汁中的呈色花色苷有脱糖作用, 导致其失色或降解, 从而使杨梅果酒失去鲜艳的红色。为了解决这一问题, 本研究采用海藻酸铝固定化酿酒酵母(商品名分别为安琪, Lalvin D254, Lalvin BM45, and Lalvin L2323)代替游离酵母发酵杨梅果酒, 对四种固定化酿酒酵母的花色苷稳定作用进行了试验。结果表明四种酵母的海藻酸铝固定化发酵均表现出明显的护色效果, 经过20d发酵后, 呈色花色苷保存率分别为50%(D254)、46.4%(安琪)、45.5%(L2323)、42.7%(BM45), 而在相应品种的酿酒酵母的游离细胞发酵时, 保存率仅分别为22.7%、20.9%、24.5%、26.3%。这种保护作用主要来自于海藻酸铝固定化细胞珠致密外层的物质低扩散性、后期酒精对的抑制作用以及形成铝离子对花色苷的稳定作用。另外, 在发酵前期, 海藻酸铝固定化酵母的平稳发酵速度和慢速消耗酒醪中的二氧化硫对最后的呈色花色苷保存率也有重要意义。

关键词: 海藻酸铝; 花色苷稳定性; β -D-葡萄糖苷酶; 杨梅果酒; 酿酒酵母

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1 Introduction

The semitropical bayberry (*Myrica rubra*) grows widely in China, Korea and Japan (Tao et al., 2002). The berry, which abounds in anthocyanins(CA) such as cyanidin, pelargonidin and delphinidin (Ye et al., 1994), has been mainly used for semi-dry and dry styles of fruit wines in China since the last decade. However, the bayberry wine is easy to lose its CA through the extraction treatment during its fermentation. For example, in our previous work, the bayberry wine lost its CA to a great extent during the primary stage of FCF (free yeast cells fermentation), and the color quality in the resultant wine was greatly affected (Zhong et al., 2003).

CA degradation is due to complicated causes in red fruits and wines. One of the reasons is that some of β -D-glucosidases may act as anthocyanin- β -D-glucosidases (or anthocyanases) by breaking the linkage between the glucose and the anthocyanidin moieties, therefore, the corresponding anthocyanidins will spontaneously be converted to brown or colorless compounds (Blom, 1983; Paloma et al., 2000). But β -D-glucosidases are usually used for enhancement of wine flavor by hydrolyzing of terpenyl- β -D-glucosides into terpenols (Marie-Paule et al., 1998). These enzymes are widely expressed in plants, fungi (such as *Aspergillus niger*) and yeasts (Zeng et al., 1998; Marie-Paule et al., 1998; Paloma et al., 2000). However, in red fruits and wines, fungal and yeast (especially *Candida* and *Hanseniaspora* genera) β -D-glucosidases as extracellular enzymes have been described as being responsible for inducing loss of colour (Manzanares et al., 1999; Sánchez-Torres et al., 1998). Although *Saccharomyces cerevisiae* strains are not considered as a good producer of extracellular β -D-glucosidases (Mateo et al., 1997; Paloma, 2000), they also cause most of the young bayberry wine CA discoloration at the first stage of free cell fermentation (FCF) due to the microbial metabolisms.

It has been reported that the immobilized cell systems can influence yeast metabolism (Melzoch et al., 1994; Russell, 1992; Rhiel et al., 2002). Alginate has often been investigated as a suitable immobilizing material that can restrict the diffusion of various solutes, such as proteins, sugars and salts, in and out of the gel (Amsden, 1999; Eric et al., 2001). Alginate-immobilized yeast can reduce the diffusion coefficients of both yeast enzymes and large molecular colorants out and into of the gel. This suggests that anthocyanins may be protected more or less due to the restriction caused by

immobilization.

In the present study, Al-alginate beads in good strength, high density and low friability were used to immobilize yeast cells during the first stage of bayberry wine fermentation. The CA stability of young bayberry wine inoculated by four commercially available yeasts of *Saccharomyces cerevisiae* strains was evaluated both in FCF and ICF tests.

2 Materials and Methods

2.1 Yeast strains

A total four active dry yeasts belonging to *Saccharomyces cerevisiae* strains were used. Lalvin D254, Lalvin BM45, and Lalvin L2323 were purchased from LALLEMAND S.A. (Shanghai, China), and Angel from Angel Yeast Co. (Hubei province, China), all yeast strains were prepared according to manufactures' recommendation.

2.2 Preparation of must

'Biji' bayberry must (pH3.5, containing 8.9mg/L free SO₂) was from Yongchen Bayberry winery (Zhoujiang province, China) in 2002. The must was used after being adjusted with sugar to 26.0%(W/V) total solid concentration and then sterilized at 85℃ for 15min.

2.3 Immobilization of cells

Each of the four active dry yeast strains (3g) was suspended in 50ml of a 4% sterile sugar solution and incubated at 28℃ for 1h, then the suspension was mixed with 250ml of a 4% sterile alginic acid sodium salt solution. The mixture was injected with syringes (#7) drop by drop into a 1000ml sterile 2% CaCl₂ solution for 2h while stirring continuously. The beads (2~3mm diameter) were washed with de-ionized water three times and then hardened in 1000ml sterile 1% Al₂(SO₄)₃ solution at 4℃ for 24h, eventually washed three times with de-ionized water again. The Al-alginate beads without yeast cells also were prepared according to the above procedure as control. The two kinds of beads were both divided in three batches and directly used for wine making.

2.4 Fermentation conditions

2.4.1 Free cells fermentation

The fermentation was carried out in triplicate with 4-litre hydroseal flasks for each yeast strain. Two litres of sterile bayberry juice and 80g Al-alginate beads without yeast cells were added into flask. The medium was inoculated with 1g dry yeast which was first activated at 28℃ for 1h in 30ml 4% sterile sugar solution. The flask was incubated at stationary 20℃.

2.4.2 Immobilized cells fermentation

In the case of immobilized cells fermentation, the conditions were same as those of free cells fermentation except for inoculum form. Each batch medium was inoculated with 100g of Al-alginate beads with entrapped cells of the four yeast strains.

2.5 Analytical assays

At 24h intervals, proper quantities of fermentation fluid samples were removed from the flasks and analyzed. Absorption spectra were recorded with an UV- spectrophotometer (916, GBC Co., Australia) fitted with quartz cells. Every sample was filtered with an injector microfilter (0.22 μ m pore-size cellulose acetate membrane) prior to absorption spectra assay. The CA measurements imitated the well-established spectrophotometric methodology for making wine as described by Arnous (Arnous et al., 2002). The bayberry wine sample was placed in a 0.2cm path-length quartz cuvette, and the absorbance was measured at 520nm (A_{520}). Following this, 0.02ml of a 20% sodium metabisulphite solution was added, the sample was mixed well and after 1min the absorbance was read at 520nm ($A_{520} \text{ SO}_2$). Wine (0.02ml) was mixed with 0.98ml 1 N HCl solution (dilution 1:50) in a 1.5ml Eppendorf tube, vortexed and allowed to stand for 180 min at room temperature. The absorbance was read at 520nm ($A_{520} \text{ HCl}$), using a 1.0cm path-length cuvette. For the blank, 0.02ml of a 12% ethanolic solution was used instead of wine. Absorbance readings were corrected according to the dilution factor. The total anthocyanins and colored anthocyanins were calculated according to the following formula:

Total anthocyanins (TA) (mg/L) = $20 \times [A_{520} \text{ HCl} - (5/3) \times A_{520} \text{ SO}_2]$

Coloured (ionised) anthocyanins (CA) (mg/L) = $20 \times (A_{520} - A_{520} \text{ SO}_2)$.

Acidity was determined by using acid-base titration method (as citric acid). Alcohol concentrations were determined by using a Gay-Lussac Alcoholmeter. Because samples ethanol was not extracted through distillation, apparent residual soluble solid concentration (SSC) was determined by a hand-refractometer (ATAGO N-1 α , Japan). All analyses were run in duplicate, unless specified, and values averaged.

3 Results and Discussion

3.1 Fermentation parameters of young bayberry wine

In ICF of four yeast strains, Al-alginate beads would decrease SSC of bayberry must by 2% due to the dilution effect of beads free water. In order to compensate for the free

water effect between FCF and ICF of each yeast strain, proper amount of Al-alginate beads without yeast cells was added into each FCF medium. For both FCF and ICF processes, the fermentation time lasted for 20 days in order to investigate the changes of CA efficiently.

The results were summarized in Table 1. Generally, the total acidity of bayberry must was higher than that of grape must, so this resulted in decreasing the fermentation rate of young bayberry wine. There was no obvious change between the total acidities of FCF and ICF samples in both processes, but the ethanol concentrations of ICF were slightly lower than those of FCF for all strains. The λ_{\max} of all samples brought hypsochromic effect from 4 to 15nm and the shift number of FCF samples was larger than that of ICF (as also shown in figure 1). The hypsochromic shift of λ_{\max} indicated that anthocyanins with longer λ_{\max} were faded a lot, or anthocyanin-anthocyanin complexes were destructed. Furthermore, the absorbance values at λ_{\max} decreased dramatically by more than 70% for all young bayberry wine of FCF. Because the TA and CA of bayberry must were just about 20mg/L and 12 mg/L, respectively, which is much less than those of aged grape wine (TA \approx 165.7mg/L and CA \approx 13.4 mg/L, on average respectively) (Arnous et al., 2002), the large loss of CA in young bayberry wine directly led to low color quality of end products.

Due to their high reactivity, anthocyanins readily degrade and form colourless or undesirable brown-coloured compounds. Many exterior factors affected the stability of anthocyanins during wine making, including temperature, light, pH, oxygen, and enzymes (Mazza & Miniati, 1993; Bolivar et al., 2004). The effect caused by the former two factors was negligible during bayberry must fermentation because of the incubation media located in low temperature and dark conditions. Oxidations on CA generally caused brown polymeric colorants formation (usually showed at absorbance 420nm) in wine aging stage (Gómez, 1995). However, as shown in table 1, most of CA were authentically faded according to the λ_{\max} and absorbance values of young bayberry wine, which indicated that oxidation was also not the main destructive factor. pH was an important factor which affected the color change of anthocyanins (Brouillard et al., 1997), but the pH of bayberry must during fermentation steadily fixed in 2.9~3.1, a range which kept the light red color of bayberry juice as control.

Therefore, the most important reason that the bayberry CA was faded greatly within several days of incubation was

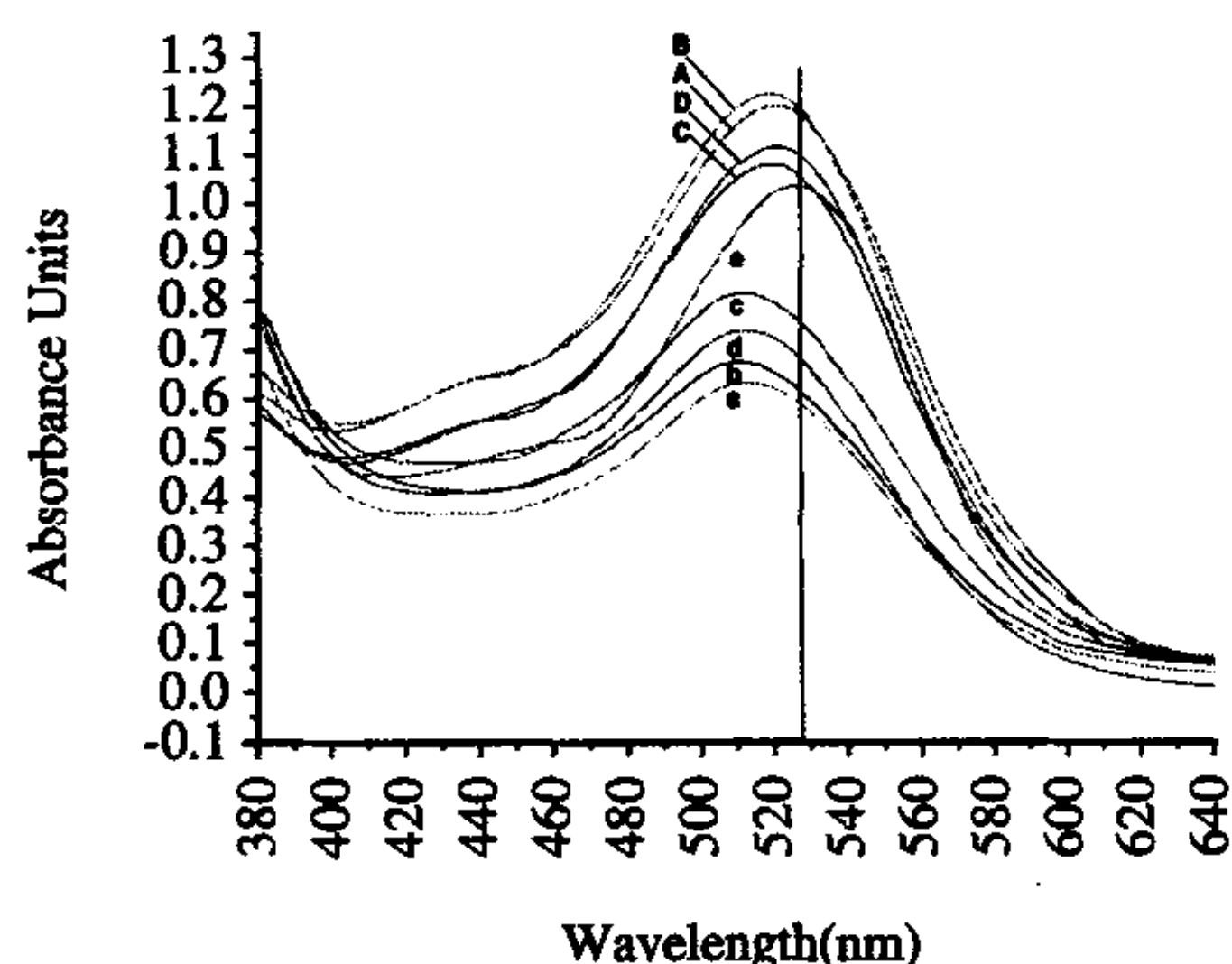
Table 1 Fermentation parameters obtained in young bayberry wine fermentations with four yeast strains

Sample	Total acidity (g of citric acid L ⁻¹)	Ethanol concn. (% V/V)	λ_{max} (nm)	Absorbance(λ_{max})	Absorbance(420nm)
Bayberry must	9.3	—	526	2.86	1.10
Angel (FCF)	9.4	11.9	511	0.63	0.37
Angel (ICF)	9.4	11.0	521	1.20	0.58
L. D254 (FCF)	9.6	12.4	512	0.68	0.43
L. D254 (ICF)	9.4	11.6	522	1.23	0.58
L. BM45 (FCF)	9.8	12.6	512	0.82	0.47
L. BM45 (ICF)	9.3	11.6	520	1.08	0.51
L. L2323 (FCF)	9.5	11.4	513	0.74	0.41
L. L2323 (ICF)	9.3	11.1	521	1.12	0.50

likely related to the metabolism of yeasts. Paloma et al. (2000) reported that many wine yeasts expressed anthocyanin- β -D-glucosidase activity during fermentation, and only yeast species belonging to the genera *Dekkera*, *Rhodotorula* and *Schizosaccharomyces* did not produce the enzyme. Although *Saccharomyces cerevisiae* strains were not recognized as being a good producer of extracellular β -D-glucosidases (Paloma et al., 2000), the obvious force of destruction of CA from yeast metabolism at the first stage of bayberry wine making was very strong.

3.2 The effect of color preservation in ICF

The change patterns of fermentation rate of CA both in FCF and ICF for the four yeast strains were shown in Figure 2. In ICF processes, all four yeast strains except for L.BM45 started fermentation on the sixth day, about two to four days later than FCF processes. This suggested that the restriction effect on metabolism of yeasts caused by lower diffusion coefficients of solutes in Al-alginate beads was also strong. It was well known that sulfur dioxide in must would depress the activity of yeasts in the first several days of brewing (Iconomopoulou et al., 2002; Kourkoutas et al., 2003). When compared with the changed amplitude of CA both in ICF and FCF media, the immobilized yeast cells slowly depleted sulfur dioxide but efficiently reproduced CA from colorless bisulfite-adducts. In the case of FCF, both sulfur dioxide and released CA were demolished violently within the next six days. As fermentation promoting, the released CA in ICF media also faded sharply at the accelerated stage of fermentation. Along with ethanol accumulation, metabolism of yeast was depressed gradually and the CA increased slightly due to transformation from colorless bisulfite-adducts. McMahon et al (1999) stated that grape β -glucosidases could exhibit a 60% loss of activity at ethanol concentrations of 3.5%. Mateo and Stefano (1997) also reported that *Saccharomyces* β -glucosidase was inhibited by about 50% with 5% ethanol in the medium. The CA in bayberry wine stopped to be faded at final

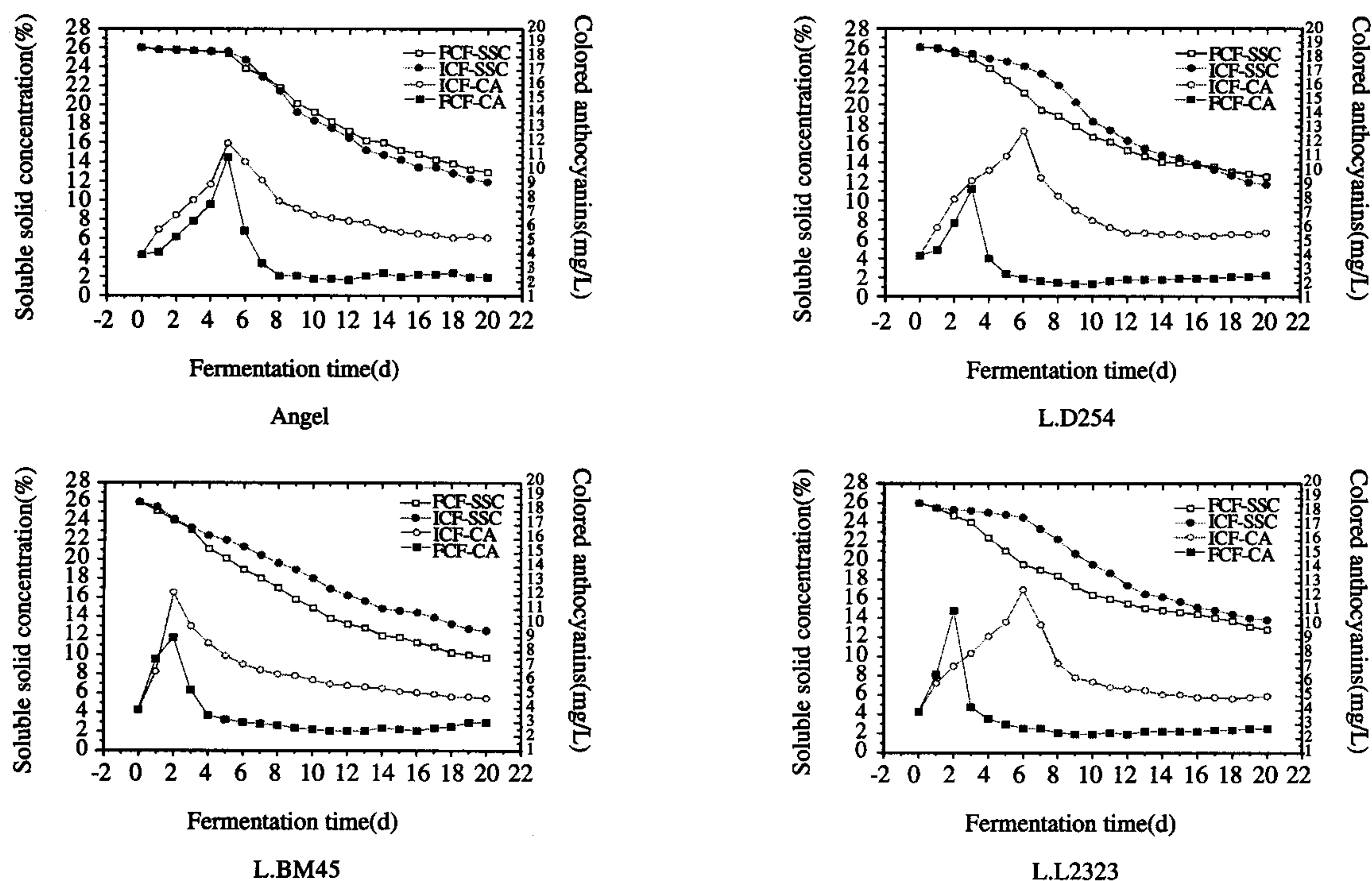


a, b, c and d referred to the wine samples of free cells fermentation, and A, B, C and D referred to the wine samples of immobilized cells fermentation. a and A for Angel strain; b and B for L.D254 strain; c and C for L.L2323 strain; d and D for L.BM45 strain; and E for bayberry must diluted with de-ioned water before fermentation.

Fig.1 UV-VIS spectra of bayberry must and young bayberry wine samples both in FCF and ICF for the four yeast strains

stage of fermentation should be partly benefited from this inhibition. The CA stability in the four Al-alginate immobilized yeast strains fermentation after 20 days was dramatically enhanced with the residual rates of 50.0% (D254), 46.4% (Angel), 45.5% (L2323), and 42.7% (BM45), respectively, whereas, those corresponding values in the free cells fermentation were only 22.7%, 20.9%, 24.5%, and 26.3%, respectively. Anyway, the metabolism of yeast cells immobilized in Al-alginate was different from that of free yeast cells.

The enhanced property of Al-alginate immobilized yeast on anthocyanins stability also was related to the protective effect from the Al^{3+} in gel beads. Elhabiri et al (1997) reported that the ability of natural anthocyanins to form stable complexes with small highly charged metal ions such as Al^{3+} and Fe^{3+} , and these metalloanthocyanin complexes could strengthen the pigment-copigment interaction leading to bathochromic shift and higher stability. In order to investigate whether possible free Al^{3+} diffused from gel beads contributed to such stable effect on anthocyanins, all young



FCF-SSC and ICF-SSC refer to apparent soluble solid concentration for free cells and immobilized cells fermentation; FCF-CA and ICF-CA refer to the colored anthocyanins in samples for free cells and immobilized cells fermentation.

Fig.2 The change pattern of fermentation rate CA both in FCF and ICF for the four yeast strains

bayberry wine samples were scanned with UV- spectrophotometer from 380 to 650nm. Figure 2 showed that all λ_{max} of samples did not bring bathochromic shifts. But the Al^{3+} cations bonded with the alginate gel likely relocated and yielded aluminum (III)-anthocyanin complex because the beads discovered were being red dyed after inoculation.

3.3 Conclusions

Compared to ICF process, the residual CA of young bayberry wine samples inoculated with four *Saccharomyces cerevisiae* strains decreased dramatically by more than 70% in FCF processes, and that directly led to low color quality of the end products. Although *Saccharomyces cerevisiae* strains were not recognized as good producers of extracellular β -D-glucosidases, many other fungal and yeast β -glucosidases were not inhibited by the concentrations of ethanol in table wine (McMahon et al., 1999). Among the documented species showing ethanol-stable glycosidases were *Hanseniaspora vineae* (Vasserot et al., 1989), *Dekkera intermedia* (Blondin et al., 1983), and *Candida molischiana* (Gonde et al., 1985). In this research, the CA degradation rate from the yeast metabolism at the first stage of bayberry wine making in FCF was very rapid.

However, In ICF processes, immobilized yeast cells

slowly depleted sulfur dioxide, but efficiently reproduced CA, which once was bleached by sulfur dioxide. This indicated that the restriction effect caused by lower diffusion coefficients of solutes in aluminum (III) alginate beads affected the metabolism of yeast cells. The CA stability in the four aluminum (III) alginate-immobilized yeast strains fermentation after 20 days was greatly enhanced with the residual rates of 50.0% (D254), 46.4% (Angel), 45.5% (L2323), and 42.7% (BM45), respectively, whereas, those corresponding values in the free cells fermentation were less than 30% for all strains.

The enhanced property of Al-alginate immobilized yeast on anthocyanins stability was due to the lower diffusion of the compact outer layers of Al-alginate beads, the inhibition on β -glucosidases as ethanol accumulation in medium, and the more stable complex of aluminum-anthocyanins. Moreover, compared to FCF, ICF could provide more stable fermentation rate during the early stage of fermentation. It also retarded the releasing of sulfur dioxide in medium, which was very important for the final residual of CA. However, we hoped that this work served as a motivation for seeking for exploring the details of mechanism about rapid discoloration during the wine making for some sorts of specific red fruits abounded in anthocyanins, and eventually for

improving process and sensory quality of products. The further work on ICF effect was undertaken in our lab.

References:

- [1] Amsden, Diffusion characteristics of calcium alginate gels[J]. *Biotechnology and Bioengineering*, 1999, 65(5): 605-610.
- [2] Arnous A M, K Panagiotis. Anthocyanin composition and colour characteristics of selected aged wines produced in greece[J]. *Journal of Wine Research*, 2002, 13(1): 23-34.
- [3] Blom H. Partial characterization of a thermostable anthocyanin-b-glycosidase from *Aspergillus niger*[J]. *Food Chemistry*, 1983, 12: 197-204.
- [4] Blondin B, R Ratomahenina, A Arnaud, et al. Purification and properties of the β -glucosidase of a yeast capable of fermenting cellobiose to ethanol[J]. *Microbiol Biotechnol*, 1983, 17: 1-6.
- [5] Bolívar A C, C Luis. Stability of anthocyanin-based aqueous extracts of Andean purple corn and red sweet potato compared to synthetic and natural colorants[J]. *Food Chemistry*, 2004, 86: 69-77.
- [6] Brouillard R, P Figueiredo, M Elhabiri, et al. Phytochemistry of fruit and vegetables[M]. Oxford Science Publications, 1997.
- [7] Elhabiri M, P Figueiredo, K Toki, et al. Anthocyanin-aluminium and -gallium complexes in aqueous solution[J]. *Chem Soc*, 1997, (2): 355-362.
- [8] Eric F, L Michèle, Laurent A, et al. Diffusion of polyethyleneglycols in calcium alginate hydrogels[J]. *Physicochemical and Engineering Aspects*, 2001, 194: 197-206.
- [9] Gonde P, R Ratomahenina, A Arnaud, et al. Purification and properties of the exocellular β -glucosidase of *Candida molischiana* (Zikes) Meyer and Yarrow capable of hydrolyzing soluble cellodextrins[J]. *Can J Biochem Cell Biol*, 1985, 63: 1160-1166.
- [10] Gómez E, A Martínez, J Laencina. Prevention of oxidative browning during wine storage[J]. *Food Research International*, 1995, 28(3): 213-217.
- [11] Ionomopoulou M, K Psarianos, M Kanellaki, et al. Low temperature and ambient temperature wine making using freeze dried immobilized cells on gluten pellets[J]. *Process Biochemistry*, 2002, 37: 707-717.
- [12] Kourkoutas Y, M Komaitis, A A Koutinas, et al. Wine production using yeast immobilized on quince biocatalyst at temperatures between 30 and 0°C[J]. *Food Chemistry*. 2003, 82: 353-360.
- [13] Manzanares P, D Ramón, A Querol. Screening of non-*Saccharomyces* wine yeasts for the production of β -Dxylosidase activity[J]. *International Journal of Food Microbiology*, 1999, 46: 105-112.
- [14] Marie-Paule L, P Patrice. Purification and characterization of two β -D-Glucosidases from an *Aspergillus niger* enzyme preparation: affinity and specificity toward glucosylated compounds characteristic of the processing of fruits[J]. *Enzyme Microbial Technology*, 1998, 22: 374-382.
- [15] Mateo J J, R D Stefano. Description of the b-glucosidase activity of wine yeasts[J]. *Food Microbiology*, 1997, 14: 583-591.
- [16] Mazza G, E Miniati. 1993. Anthocyanins in fruits, vegetables and grains[M]. Boca Raton: CRC Press Inc, 1993.
- [17] Melzoch K, M Rychtera, V Habova. Effects of immobilization upon the properties and behavior of *Saccharomyces cerevisiae* cells[J]. *Journal of Biotechnology*, 1994, 32: 59-65.
- [18] McMahon H, B W Zoecklein, K Fugelsang, et al. Jasinski. Quantification of glycosidase activities in selected yeasts and lactic acid bacteria[J]. *Journal of Industrial Microbiology & Biotechnology*, 1999, 23: 198-203.
- [19] Paloma M, R Virginia, G Salvador, et al. A preliminary search for anthocyanin- β -D-glucosidase activity in non-*Saccharomyces* wine yeasts[J]. *International Journal of Food Science and Technology*, 2000, 35: 95-103.
- [20] Rhiel M, P Ducommun, I Bolzonella, et al. Real-time in situ monitoring of freely suspended and immobilized cell cultures based on mid-infrared spectroscopic measurements[J]. *Biotechnology and Bioengineering*, 2002, 77(2): 174-185.
- [21] Russell I, G G Stewart. Contribution of yeast and immobilization technology to flavor development in fermented beverages[J]. *Food Technology*, 1992, 46 (11): 146-150.
- [22] Sánchez-Torres P, L González-Candelas, D Ramón. Heterologous expression of a *Candida molischiana* anthocyanin-b-glucosidase in a wine yeast strain[J]. *Journal of Agricultural and Food Chemistry*, 1998, 46: 354-360.
- [23] Tao J, T Morikawa, I Toguchida, et al. Inhibitors of nitric oxide production from the bark of *Myrica rubra*: structures of new biphenyl type diarylheptanoid glycosides and taraxerane type triterpene[J]. *Bioorganic & Medicinal Chemistry*, 2002, (10): 4005-4012.
- [24] Vasserot Y, H Christiaens, P Chemardin, et al. Purification and properties of a β -glucosidase of *Hanseniaspora vineae* Vander Walt and Tscheushner with the view to its utilization in fruit aroma liberation[J]. *J Appl Bacteriol*, 1989, 66: 271-279.
- [25] Ye X Q, J C Chen, P Shu. Identification of the constituent of anthocyanin in Yang-Mei (*Myrica rubra* cv. Boqi) (in Chinese) [J]. *Journal of Zhejiang Agriculture University*, 1994, 20(2): 184-190.
- [26] Zeng Y-C, A D Elbein. Purification to Homogeneity and Properties of Plant Glucosidase[J]. *Archives of Biochemistry and Biophysics*, 1998, 355(1): 26-34.
- [27] Zhong R M, B Zhen. Semi-dry style bayberry (*Myrica rubra*) wine using multiple yeast fermentation (in Chinese)[J]. *Shipin Gongye Keji*, 2003, 24(4): 55-57.