THE EFFECT OF IMMOBILIZED YEAST CELLS IN CA-ALGINATE ON ETHANOL FERMENTATION OF DRAGON FRUIT (HYLOCEREUS COSTARICENSIS)

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Abstract

Dragon fruit-based wine is a value-added product that enhances the value of domestic agricultural products, especially for those facing challenges in raw form export. In this study, Saccharomyces cerevisiae yeast cells were immobilized using the Ca-alginate carrier for assessing the influence of Na-alginate and CaCl2 concentrations on the quality of immobilized Ca-alginate beads during wine fermentation. A repeated fermentation study was conducted to determine the efficiency and stability of immobilized beads in dragon fruit-based wine fermentation. The results indicated that the immobilized Ca-alginate beads exhibited good fermentation efficiency with 3% Na-alginate and 2% CaCl2 concentrations. Moreover, the fermentation efficiency was maintained through at least four fermentation cycles. The immobilized yeast cells contributed to the production of wine with favorable qualities in terms of color and taste, meeting the standards in laboratory-scale TCVN 3215-79. These findings underscore the potential of cell immobilization technology using Ca-alginate carriers in the fermentation process of dragon fruit- based wine. This technology significantly enhances the value and diversifies the range of Vietnamese agricultural products, mainly dragon fruit.

Keywords: cell immobilization, dragon fruit juice, ethanol, fermentation, saccharomyces cerevisiae

1. Introduction

Dragon fruit (*Hylocereus spp.*) is an agricultural product that provides significant economic benefits to farmers, especially in regions with arid climates in central Vietnam. This fruit, introduced to Vietnam a century ago, contains higher nutrients and minerals than fruits such as mangoes, custard apples, and pineapples (Phuong & Viet, 2015). Vietnam's dragon fruit cultivation area is estimated at over 28 thousand hectares, mainly concentrated in Binh Thuan, Tien Giang, and Vinh Long provinces. White-fleshed dragon fruit accounts for 92% of the total cultivated area and is predominantly geared towards the export market. Although the national output of dragon fruit has significantly increased in recent years, product quality still needs to meet market demands consistently. Moreover, food safety and branding barriers continue to impact the value of dragon fruit

(Huyen et al., 2020). To ensure sustainable development and enhance the value of dragon fruit, converting fresh dragon fruit into processed foods and beverages is a viable solution to elevate its worth in the future.

Alcoholic beverages containing ethanol offer an effective way to transform fresh dragon fruit products. Dragon fruit can be used as a substrate for fermentation, similar to grape must, to create a novel and promising beverage. Applying science and technology to enhance and improve the fermentation process of various agricultural products, including dragon fruit, is a highly applicable research area. Cell immobilization technology has long been considered a practical solution to improve the efficiency of the fermentation process. Cell immobilization involves encapsulating or attaching yeast cells onto a carrier through various methods, such as entrapment in gel, adsorption, ion exchange, or atomic bonding, without compromising the biological activity of the cells (Bayrock & Michael Ingledew, 2001; Huong et al., 2012). Presently, the most widely and commonly applied immobilization method involves encapsulating cells in Ca-alginate and k-carrageenan gels, both natural polymers, non-toxic to cells, and rapidly formed gels (Mater et al., 1995). In this study, dragon fruit extract will be fermented using yeast cells immobilized in Ca-alginate gel to evaluate the factors influencing the fermentation efficiency and the quality of the dragon fruit- based wine.

2. Materials and methods

2.1. Materials

The research utilized a strain of *Saccharomyces cerevisiae* yeast (AB Mauri Vietnam Company) and fresh dragon fruit sourced from a local market in Thu Dau Mot City, Binh Duong Province.

2.2. Methods

Cultivating and immobilizing yeast cells

The yeast cell immobilization method, as described by Yu et al. (2007), involved the following steps: 0.5g of yeast cells were cultivated in a medium containing 60g/l glucose, 3g/l $(NH_4)_2SO_4$, 0.5g/l $MgSO_4.7H_2O$, 0.6g/l K_2HPO_4, and 5g/l yeast extract at room temperature for 48 hours. Subsequently, the yeast biomass was centrifuged, harvested, and rinsed in 0.85% physiological saline solution for 10 seconds. The entire yeast cell biomass was then mixed in a Na-Alginate gel at concentrations of 2.5%, 3%, and 3.5% until fully dissolved and dropped into a $CaCl_2$ solution at concentrations of 1%, 2%, and 3% for 30 minutes. The immobilized cell-containing gel beads were recovered and stored in a 0.5% $CaCl_2$ solution at 8°C.

Fermentation of dragon fruit juice

Dragon fruit underwent processing for juice extraction, solid separation, and sugar content adjusted to 20°Bx. Fermentation was carried out in 500ml conical flasks containing 100ml of sterilized dragon fruit juice, with each experiment repeated three times. The anaerobic fermentation process was initiated by adding 10g of immobilized yeast cells into the dragon fruit juice flasks, maintained at 28-30°C for ten days, including an initial 24-hour aerobic activation period. Ethanol concentration was measured using a refractometer to determine alcohol levels. The refractometer was standardized before and after fermentation to minimize errors caused by the color of the dragon fruit- based wine.

Sensory evaluation of dragon fruit- based wine quality

Sensory evaluation was conducted using a scoring system based on the TCVN 3215-79 standard by a panel of 15 trained sensory assessors. The assessment criteria included flavor, taste, and product clarity.

Statistical analysis

The collected data were statistically processed using GraphPad Prism version 8.636 (GraphPad Software, Inc., San Diego, CA).

3. Results and discussion

3.1. Effect of Na-alginate concentration on fermentation performance of immobilized cells

The results showed that Na-alginate concentration in cell immobilization significantly influences dragon fruit–based wine fermentation efficiency. Despite variations in the fermentation efficiency of immobilized yeast cells at different Na-alginate concentrations, cells immobilized at 3% Na-alginate showed the best performance across all evaluation criteria (Figure 1). The ethanol concentration obtained in dragon fruit-based wine fermentation using cells immobilized in 3% Na-alginate averaged 10% v/v, 2.7 times higher than cells immobilized at 2.5% (3.7% v/v) and 1.58 times higher than cells immobilized at 3.5% (6.33% v/v). The highest volume of wine produced was achieved when fermenting with cells immobilized at 3%, approximately 1.4% more than that produced with cells immobilized at 3.5% and 12% more than that produced with cells immobilized at 2.5% (Figure 1). However, a significant difference in wine volume was found only between the 2.5% concentration and the others, with no notable difference between the 3% and 3.5% concentrations.. This could be explained by the impact of the gel pore size on yeast-cell-carrier substance contact, leading to enhanced fermentation, producing secondary products other than ethanol within the cycle (Gemeiner et al., 1996).

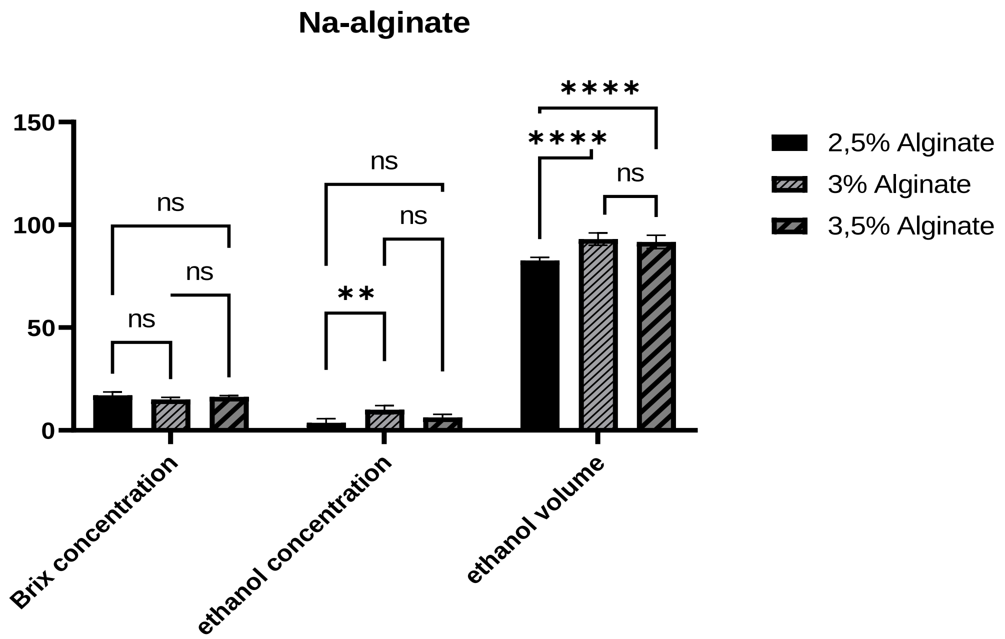

Figure 1. The effect of Na - Alginate concentration on fermentation parameters.
(*: p<0.05; **: p<0.01 ns: Not significant)

The concentration of the carrier substance is a critical factor influencing the stability of immobilized cell beads. Lower concentrations improve cell retention within the matrix, while excessively high concentrations may hinder cell-matrix interactions, which could disrupt the structure due to free Na^+ ions. A 3% Na-Alginate concentration has also shown high efficiency in immobilizing cells for fermenting sugar beet molasses (Huong et al., 2012). This suggests that a 3% Na-alginate concentration in cell immobilization shows great potential for alcoholic fermentation from various raw materials, including sugar beet molasses, dragon fruit, and possibly other fruits.

3.2. Effect of $CaCl_2$ concentration on fermentation performance of immobilized cells

The concentration of $CaCl_2$ in cell immobilization influenced the wine yield from dragon fruit fermentation. Cells immobilized at 2% demonstrated stable efficiency compared to other concentrations. At a concentration of 2% (9.33% v/v), the alcohol levels in the wine products were 1.75 and 1.22 times higher than those at concentrations of 1% (5.33% v/v) and 3% (7.67% v/v), respectively (Figure 2). Furthermore, the wine volume obtained was 1%, and the 2% remained high. Significant differences were compared to the 3% concentration, where the alcohol volume was notably reduced (Figure 2).

$CaCl_2$ concentration has also been shown to affect cell immobilization efficiency. Ca^{2+} ions in solution cross-link with alginate, forming a strong carrier network that shapes immobilized particles (Cheetham et al., 1979). At low Ca^{2+} concentrations, cell particles struggle to form a stable structure, making them prone to breakage, and the large gel pore size leads to cell loss into the surrounding environment. Conversely, high Ca^{2+} concentrations can cause cell death within the immobilized beads by altering cell membrane permeability (Huong et al., 2012; Cheetham et al., 1979).

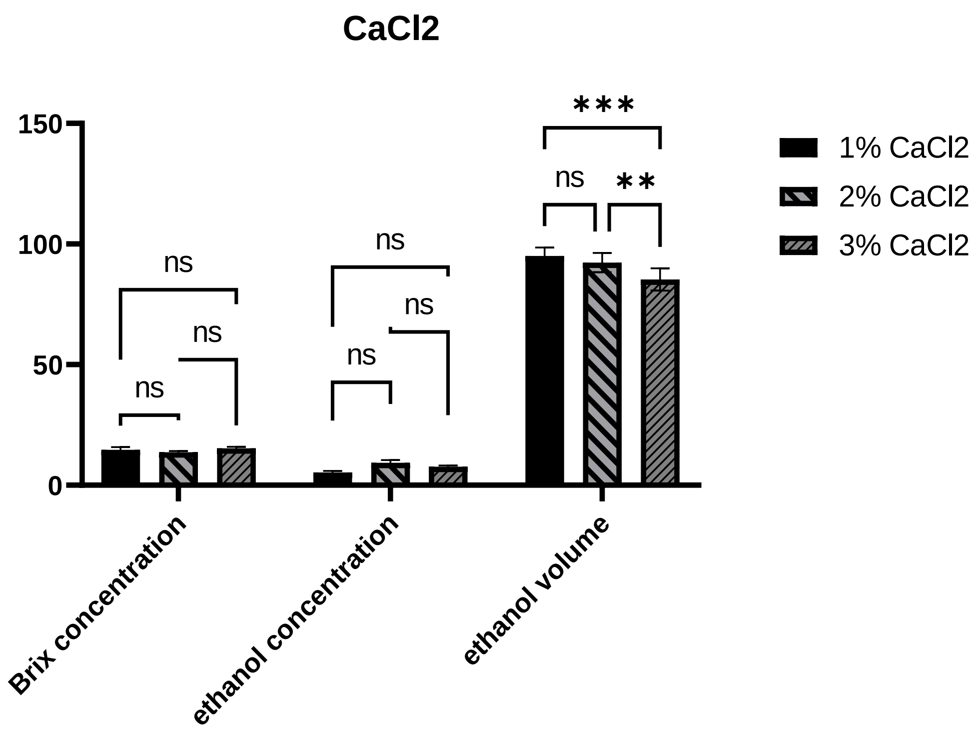

Figure 2. The effect of $CaCl_2$ concentration on the fermentation efficiency of immobilized cells (*: $p<0.05$; **: $p<0.01$ ns: Not significant).

3.3. Effect of immobilized cells on dragon fruit-based wine fermentation

Yeast performance in fermenting dragon fruit- based wine differs between immobilized and free cells. Immobilized cells produce wine with an ethanol concentration 38.4% higher than free cells. The residual reducing sugar content is 9.67°Bx, which is 2.67°Bx higher than free cells (*table 1*). Statistical analysis confirms the significance of these differences (Figure 3). This result is consistent with previous studies demonstrating the enhanced efficiency of immobilized cells in converting sugar into ethanol (Shen et al., 2003). Despite higher ethanol concentration, immobilized yeast cells produce lower wine volume than free cells. Wine volume from immobilized yeast cells is 95ml, 4.2% lower than that of free yeast cells (*table 1*), possibly due to water absorption by the gel beads, which caused partial swelling after fermentation.

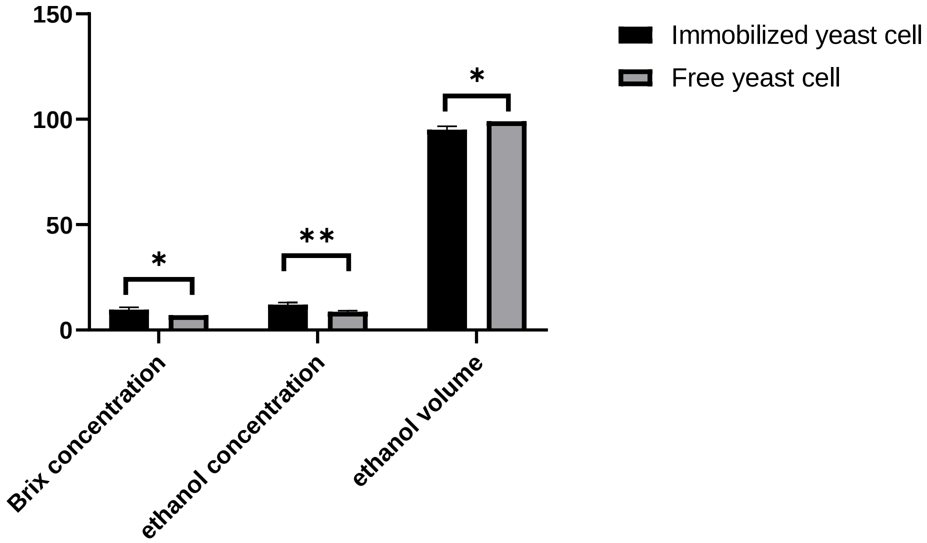

Figure 3. The influence of immobilized yeast and free yeast in dragon fruit- based wine fermentation (*: p<0.05; ns: Not significant)

*TABLE 1***.** Results of evaluating the influence between immobilized yeast and free yeast on dragon fruit- based wine fermentation

Variables	**Immobilized yeast**		**free yeast**					
	Mean	**SD**	**Mean**	**SD**	**N**	**Summary**	**P value**	**Q value**
Brix concentration (°Bx)	9.67	1.15	7.00	0.00	3.0	Yes	0.0161	0.032
Ethanol concentration (v/v)	12.00	1.00	8.67	0.58	3.00	Yes	0.0075	0.022
Ethanol volume (ml)	95.00	1.73	99.00	0.00	3.00	Yes	0.0161	0.032

3.4. The Stability of immobilized cell beads in dragon fruit-based wine fermentation

The assessment of immobilized cell fermentation performance shows that immobilized cells maintain consistent fermentation efficiency over four consecutive cycles. Throughout these cycles, ethanol concentration in dragon fruit- based wine ranges from 8% to 12% (v/v), peaking at 12% in the first cycle. Similarly, the residual sugar concentration stabilizes after an initial decrease in the second cycle (Figure 4). The wine volume obtained after fermentation follows a similar pattern, decreasing slightly in the second cycle before stabilizing in the later cycles. This may be due to the yeast cells in the immobilized beads not being fully integrated into the carrier matrix during the first cycle, leading to some cell loss and a slight decrease in fermentation efficiency (Duarte et al., 2013). However, the cells mature and stabilize after three cycles, improving overall efficiency. Despite minor variations across cycles, statistical analysis confirms these differences are insignificant, with fermentation efficiency remaining broadly consistent across all cycles. This result is also consistent with other studies demonstrating that yeast cells can be effectively reused in fermentation (El-Dalatony et al., 2016; Liang et al., 2020).

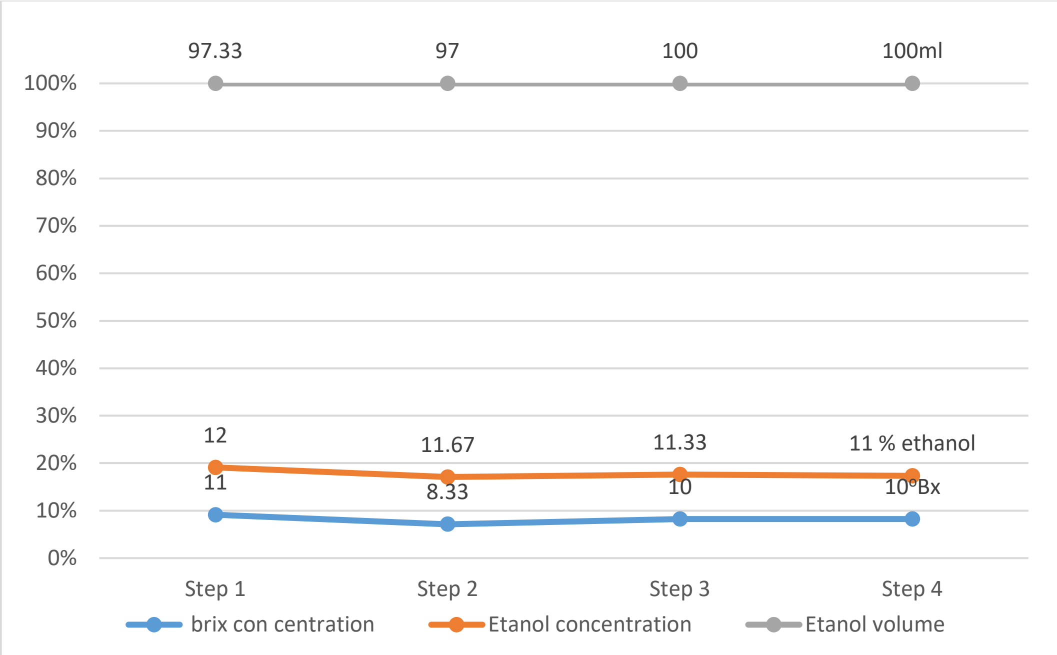

Figure 4. The stability of immobilized cell beads in dragon fruit- based wine fermentation after four fermentation cycles.

3.5 Effect of immobilized cells on dragon fruit-based wine quality

The wine produced using immobilized yeast cells exhibits superior quality to that of free yeast cells. Specifically, the sensory evaluation score for flavor and taste of wine fermented with immobilized cells is approximately 1.8 times higher than that of wine fermented with free yeast cells (*table 2*). The immobilized cell wine has a pleasant flavor, a well-balanced taste, and an alcohol concentration that aligns better with consumer preferences. Additionally, quality assessments indicate that using immobilized yeast beads does not affect the clarity of the dragon fruit- based wine (*table 2*). Based on user satisfaction evaluations, these results suggest that using immobilized cell pellets positively impacts the overall quality of the wine, achieving a total score of 13.4/15 points (*table 2*). Therefore, immobilized yeast cells could serve as an effective solution to enhance the quality of wine products for consumers.

TABLE 2. Statistical analysis of sensory evaluation of post-fermentation wine

Variables	**P value**	**Mean of immobilized yeast**	**The mean of free yeast**	**Difference**	**SD**	**t ratio**	**df**	**Q value**
Clarity	0.087	4.47	4.07	0.4	0.23	5.602	28	0.09
Flavor	<0.0001	4.60	2.80	1.8	0.29	4.234	28	2E-06
Taste	<0.0001	4.33	2.27	2.1	0.33	5.134	28	2E-06

4. Conclusion

Immobilizing yeast cells offers an effective solution to enhance both the cost-efficiency and quality of the fermentation process. Under 3% Na-alginate and 2% $CaCl_2$ conditions, yeast cells exhibit high and consistent fermentation performance across four cycles. The resulting dragon fruit- based wine maintains excellent clarity and flavor after fermentation. Utilizing cell immobilization technology to convert dragon fruit into alcoholic beverages presents considerable potential for future applications.

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