ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Saccharomyces cerevisiae Immobilisation in Sodium Alginate for Red Dragon Fruit Winemaking

N. P. Minh[1*], P. X. Mai[2], V. T. B. Lieu[2], D. T. Son[3]

[1.] *Faculty of Food Technology - Biotech, Dong A University, Da Nang City, Vietnam.*

[2.] *Can Tho University, Can Tho City, Vietnam.*

[3.] *Vinh Long University of Technology Education, Vinh Long Province, Vietnam.*

***Corresponding Author: N. P. Minh**

Abstract

Red dragon fruit is rich in sugar, organic acids and minerals and other nutrients. It has good processing properties; it is used in the production of fruit juice, jam, and preserved fruit. Its economic value in local market is quite low. Making wine is not only converting the fruit into wine, but also improves its economic value of dragon fruit. Objective of this study focused on the effect of encapsulation of *Saccharomyces cerevisiae* in sodium alginate complexed with calcium chloride in red dragon fruit wine fermentation. Different technical parameters such as sodium alginate concentration (2.0%, 4.0%, 6.0%, 8.0%, 10%), bead size (2mm, 4mm, 6mm, 8mm, 10mm), bead loading (5 g/100ml, 10 g/ 100ml, 15 g/100ml, 20 g/ 100ml, 25 g/ 100ml), initial total soluble solid (10, 12, 14, 16, 18°Brix), clarifying agent (gelatin, bentonite, wheat gluten, egg white, kieselsol and kaolin) in wine making. Results showed that the best wine quality based on ethanol content (19.38%v/v) and sensory score were recorded by encapsulation of *Saccharomyces cerevisiae* in 6% sodium alginate, bead size 6mm, bead loading 20 g/ 100ml, initial soluble solid 16%, gelatin as clarifying agent.

Keywords: *Dragon fruit, Sodium alginate, Calcium chloride, Saccharomyces cerevisiae, Encapsulation, gelatin, Wine.*

Introduction

Pitaya (*Hylocereus undatus*) is an exotic fruit also known as pitahaya, dragon fruit. Its production has become a lucrative industry in Vietnam in recent years. Pitaya (*Hylocereus undatus*) is an exotic non-climacteric fruit that reaches its best eating quality when harvested ripe, decreasing thereafter during storage [1]. *H. undatus* is a medium to large berry with red peel and green or red fleshy scales. The flesh is sweet and white with numerous tiny black seeds, and is consumed fresh or used for juice, jellies, marmalades, jams, jelly, wine, netar and beverages [2, 5].

Red dragon fruits contain high levels of health-promoting betalains [6, 7]. Red dragon fruit or pitaya is well known in the fruit market as an excellent source of antioxidants and for its high content of nutrients, particularly potassium, as well as dietary fibre. Fermented products are one of the popular functional food choices due to their good functional and nutritional properties [8]. The antioxidant potential and the chemical properties of pitaya fruit can contribute to maintaining a healthy diet [9]. Yeast immobilisation for the production of wine has some potential. A calcium alginate bead has been used to immobilize *S. cerevisiae* [10].

Calcium alginate is favored by its non-toxic characteristic. It involves a very simple gel entrapment method [11]. Some advantages of the yeast-immobilization systems include: high cell densities, product yield improvement, lowered risk of microbial contamination, better control and reproducibility of the processes, as well as reuse of the immobilization system for batch fermentations and continuous fermentation technologies [12]. Bottle-fermented sparkling wines were produced using *Saccharomyces* *cerevisiae* immobilized within double- layer calcium-alginate beads or strands, and some factors affecting the leakage of viable cells from the gel into the wine during fermentation and aging were investigated [13]. The wine yeast Saccharomyces cerevisiae was immobilized in sodium alginate beads as a biocatalyst in/for pomegranate wine making [14].

Saccharomyces cerevisiae was immobilized in sodium alginate beads to produce a biocatalyst for use in guava wine making [15]. One study prepared ionically cross-linked (with $CaCl_2$) gellan particles with immobilized yeast cells for their use in repeated fermentation cycles of glucose [16]. The immobilization of yeast inside calcium alginate and *κ*-carrageenan gels extended the time of preliminary fermentation and reduced the degree of fermentation in samples with calcium alginate [17].

Application of immobilised yeast cells on single-layer Ca-alginate or double-layer alginateechitosan for mead production was assessed [18]. A study was carried out on encapsulation of wine yeast (*Saccharomyces cerevisiae*) and its use in wine making compared to free yeast [19]. Influence of two yeast strains in free, bioimmobilized or immobilized with alginate forms on the aromatic profile of long aged sparkling wines was examined [20].

There was little report on production of dragon fruit wine. One research project was performed to produce dragon fruit wine [21]. There was not any research mentioned to the application of immoblized yeast in calcium-alginate bead for dragon fruit wine making. Therefore, objective of this study focused on the effectiveness of encapsulation of *Saccharomyces cerevisiae* in sodium alginate complexed with calcium chloride in red dragon fruit wine fermentation.

Materials and Method

Material

Dragon fruit were collected from Tien Giang province, Vietnam. After collecting, they must be conveyed to laboratory within 8 hours for experiments. They were washed under tap water to remove foreign matters. Fruits were sliced and peeled to collect pulp ready for juice extraction. Distilled water was added for juice preparation. Sucrose was added in different levels to achieve the right total soluble solid. 5% metabisulfite was added to the must to sterilize and prevent contamination. The fermentation was conducted in 250 ml flasks. Aseptic sampling for monitoring fermentation was performed using a syringe-type system. Besides dragon fruit we also used another material during the research such as sodium alginate, $CaCl_2$, *Saccharomyces cerevisiae,* potassium metabisulphate, sucrose, gelatin. Potassium metabisulphate was used in dragon fruit juice to sanitize before fermentation.

Researching Procedure

Effect of Alginate Concentration in Yeast Immobilization during Dragon Fruit Wine Making

Different alginate solutions (2.0%, 4.0%, 6.0%, 8.0%, 10%) were prepared. To each 50 ml alginate solution with different alginate concentration, 50 ml of cell suspension containing 2 g/L of yeast cells was added. The suspension was dropped by syringe into calcium chloride solution (5%), which resulted in Ca-alginate beads. Each cell bead has diameter 4mm. Anaerobic fermentation was carried with 15 gram of immobilized beads and 100 ml of dragon fruit must for winemaking. Total soluble solids (TSS, ^{o}Brix) and ethanol (%v/v) were observed during 15 days by 3 day-intervals.

Effect of Cell Bead Size in Yeast Immobilization during Dragon Fruit Wine Making

To each 50 ml alginate solution 6%, 50 ml of cell suspension containing 2 g/L of yeast cells was added. The suspension was dropped by syringe into calcium chloride solution (5%), which resulted in Ca-alginate beads. Different cell bead size (2mm, 4mm, 6mm, 8mm, 10mm) were examined. Anaerobic fermentation was carried with 15 gram of immobilized beads and 100 ml of dragon fruit must for winemaking. Total soluble solids (TSS, ^{o}Brix) and ethanol (%v/v) were observed during 15 days by 3 day-intervals.

Effect of Cell Bead Loading during Dragon Fruit Wine Making

Cell loading was varied between of 5 g/100ml to 25 g/mL with 6 mm bead diameter and 6 % sodium alginate concentration for fermentation. Immobilized alginate beads were washed three times with 100 ml of distilled water. Then 5 g, 10 g, 15 g, 20 g, 25 g of beads were mixed with 100 ml of dragon fruit juice and subjected to anaerobic fermentation. Total soluble solids (TSS, °Brix) and ethanol (%v/v) were observed during 15 days by 3 day-interval.

Effect of Initial Total Soluble Solid during Dragon Fruit Wine Making

Cell loading was 15 g/100ml with 6 mm bead diameter prepared from 6 % sodium alginate concentration for fermentation. Immobilized alginate beads were washed three times with 100 ml of distilled water. Different initial total soluble solid (10, 12, 14, 16, 18°Brix) were investigated. Total soluble solids (TSS, °Brix) and ethanol (%v/v) were observed during 15 days by 3 day-interval.

Effect Clarifying Agent in Wine Storage

After the final fermentation process, wines are fined to remove particles that may cause the wine to brown or lose color and heat-unstable proteins. Gelatin, bentonite, wheat gluten, egg white, kieselsol and kaolin slurry was prepared as clarifying agents by dispensing 25g of each clarifier (Gelatin, bentonite, wheat gluten, egg white, kieselsol and kaolin) in 475 ml of boiling water and allowed to mix for 3 minutes to form slurry separately such that, the resultant mixture was very smooth and free of lumps. They were then assessed individually as clarifiers by adding 10ml of each slurry to 1000 ml of the wine samples. Clarity of the wine samples was determined using sensory evaluation.

Physico-chemical, Sensory and Statistical Analysis

Total soluble solid (°Brix) was examined by refractometer. Ethanol (%v/v) content in wine was analyzed by HPLC system. Sensory score was evaluated by a group of panelist using 9 point-Hedonic scale. Clarity of the wine samples was determined at a seven-day interval for a period of 28 days using sensory evaluation. Twenty five (25) ml of each sample were dispensed without agitation of flasks to evaluate the clarity by sensory score. The experiments were run in triplicate with three different lots of samples. Statistical analysis was performed by the Stat graphics Centurion XVI.

Result & Discussion

Effect of Alginate Concentration in Yeast Immobilization during Dragon Fruit Wine Making

Na-alginate and CaCl2 reacts and forms the Ca-alginate gels. This gel involves formation of a porous structure in the beads. Application of immobilized yeast in wine production decreased the fermentation time in comparison with the free cell. The sugar uptake rate of the immobilized yeast was always higher than that of the free yeast [15]. In our present study, different alginate solutions (2.0%, 4.0%, 6.0%, 8.0%, 10%) were prepared. Our results were noted in Table 1.

Table 1: Effect of alginate concentration in yeast immobilization during dragon fruit wine making

Alginate concentration (%)	2	4	6	8	10
Ethanol content (%v/v)	6.75±0.04[c]	8.95±0.00[bc]	13.21±0.02[a]	11.43±0.04[ab]	10.78±0.01[b]
Sensory score	4.32±0.03[c]	5.72±0.03[bc]	6.14±0.01[a]	6.04±0.02[ab]	5.92±0.03[b]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Lower alginate concentration had the problem of cell leakage whiles the beads with higher alginate concentrations had the good mechanical properties as compared to the low concentrations. The increase in the Na-alginate concentration results in denser structure. Thus, higher alginate concentration decreases the pore size in the bead. It results in more retardation of substrate molecule i.e., increase in internal mass transfer resistance and decrease in effective diffusivity of the substrate molecule and thus resulting in lower rates of fermentation [15].

Effect of Cell Bead Size in Yeast Immobilization during Dragon Fruit Wine Making

As the diameter of the bead is increased, the substrate molecule has to travel more to reach the center of the bead [15]. In our present study, different bead size (2mm, 4mm, 6mm, 8mm, 10mm) were examined. Our results were noted in Table 2.

Table 2: Effect of of cell bead size (mm) in yeast immobilization during dragon fruit wine making

Bead size (mm)	2	4	6	8	10
Ethanol content (%v/v)	8.63±0.01[c]	13.21±0.02[bc]	16.05±0.03[a]	15.74±0.01[ab]	14.88±0.03[b]
Sensory score	5.91±0.00[c]	6.14±0.01[bc]	7.59±0.02[a]	6.97±0.05[ab]	7.31±0.02[b]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Effect of Cell Bead Loading during Dragon Fruit Wine Making

The bioactive compounds (fatty acids, phytosterols, betacyanins and acetic acid) and other aspects (physical, microbiological, chemical and nutritional properties) of fermented liquid dragon fruit (*Hylocereus polyrhizus*) without and with pasteurization were examined. Heat treatment (75°C for 15 s) may have exerted a favourable effect on the concentration of these bioactive compounds [22]. In our present study, different bead loading (5, 10, 15, 20, 25 g/100ml) were examined. Our results were noted in Table 3.

Table 3: Effect of cell bead loading during dragon fruit wine making

Bead loading (g/ 100ml)	5	10	15	20	25
Ethanol content (%v/v)	13.74±0.01[c]	14.83±0.00[bc]	16.05±0.03[b]	17.42±0.03[a]	17.50±0.03[a]
Sensory score	7.06±0.03[c]	7.22±0.02[bc]	7.59±0.02[b]	8.04±0.00[a]	8.09±0.04[a]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Effect of Initial Total Soluble Solid during Dragon Fruit Wine Making

Sugar is an important nutrient and influence the flavor of wine, it has a direct relationship of the degree of fermentation and is an important precursor of wine aroma, directly affect the taste and quality of fruit wine [23]. In our present study, different initial total soluble solid (10, 12, 14, 16, 18 °Brix) were examined. Our results were noted in Table 4.

Table 4: Effect of initial total soluble solid during dragon fruit wine making

Initial total soluble solid (°Brix)	10	12	14	16	18
Ethanol content (%v/v)	17.42±0.03[c]	18.42±0.01[b]	18.95±0.01[ab]	19.38±0.04[a]	17.75±0.02[bc]
Sensory score	8.04±0.00[c]	8.27±0.03[b]	8.45±0.02[ab]	8.69±0.00[a]	8.12±0.03[bc]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Effect of Clarifying Agent

After ethanol fermentation, wine becomes a colloidal solution and suspension. Natural sedimentation, centrifugation, and clarification with continuous alluviation filtration do not protect wines against colloidal haze. Clarifying wine is an essential quality attribute that is recognized and valued by consumers. Fining can improve wine stability in a number of ways and improve wine from an organoleptic point of view. Besides having a clarifying effect, fining leads to changes in the polyphenolic structure of wines. In our present study, different clarifying agents (Gelatin, bentonite, wheat gluten, egg white, kieselsol and kaolin) were examined. Our results were noted in Table 5. From table 5, we could clearly see that gelatin would be the best option for wine fining.

Table 5: Effect of clarifying agent during dragon fruit wine fining

Clarifying agent	gelatin	bentonite	wheat gluten	egg white	kieselsol	kaolin
Sensory score	8.69±0.00[a]	8.29±0.01[bc]	8.37±0.00[b]	8.19±0.02[c]	8.20±0.03[c]	8.50±0.04[ab]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Wheat gluten was used as clarifying agent of musts and white wines [24]. One study compared the effectiveness of gelatin and kaolin in clarifying wine variously produced from locally available fruits (pawpaw, pineapple, cashew and banana). Gelatin was a better clarifier than kaolin [25].

Conclusion

Red pitaya (*Hylocereus polyrhizus*) is a member of the family Cactaceae. The ripened fruit has an attractive purple-red peel and flesh which is delicate and juicy with small black seeds well-dispersed. Red pitaya flesh was rich in fiber, vitamins, calcium, phosphorus, magnesium, phytochemicals, polyphenols and antioxidants exhibiting higher antioxidant activities. We have successfully investigated several technical parameters in dragon fruit wine making by yeast immobilization. From this approach, the added value of this fruit could be improved. Vietnamese farmers would have better chance to increase their income from this product.

References

1. Sergio Tonetto de Freitas, Elizabeth Jeanne Mitcham (2013) Quality of pitaya fruit (Hylocereus undatus) as influenced by storage temperature and packaging. Sci. Agric., 70(4): 257-262.
2. Wybraniec S, Mizrahi Y (2002) Fruit flesh betacyanin pigments in Hylocereus cacti. Journal of Agricultural and Food Chemistry, 50: 6086-6089.
3. Chuah TG, Ling HL, Chin NL, Choong TSY, Fakhru'lRazi A (2008) Effects of temperatures on rheological behavior of dragon fruit (Hylocereus sp.) juice. International Journal of Food Engineering, 4: 1-28.
4. MZ Islam, MTH Khan, MM Hoque, MM Rahman (2012) Studies on the processing and preservation of dragon fruit (Hylocereus undatus) jelly. The Agriculturists, 10(2): 29-35.
5. Nguyen Phuoc Minh, Nguyen Phu Thuong Nhan, Diep Thi Tha, Le Kim Thuy, Lam Quoc Khai, Luong Ngoc Tu (2019) Different Aspects Affecting To Production Of Dragon Fruit (Hylocereus Undatus) Nectar. Journal of Pharmaceutical Science and Research, 11(3): 1040-1043.
6. OPS Rebecca, AN Boyce, S Chandran (2010) Pigment identification and antioxidant properties of red dragon fruit (Hylocereus polyrhizus). African Journal of Biotechnology, 9(10): 1450-1454.
7. Choo KY, Kho C, Ong YY, Thoo YY, Lim LH, Tan CP, Ho CW (2018) Fermentation of red dragon fruit (Hylocereus polyrhizus) for betalains concentration. International Food Research Journal, 25(6): 2539-2546.
8. Nguyen Phuoc Minh (2014) Various factors influencing to red dragon fruit (Hylocereus polyrhizus) wine fermentation. International Journal of Multidisciplinary Research and Development, 1(5): 94-98.
9. Michelle Cristina Jerônimo, Joice Vinhal Costa Orsine, Karine Kristie Borges, Maria Rita Carvalho Garbi Novaes (2015) Chemical and physical-chemical properties, antioxidant activity and fatty acids profile of red pitaya [Hylocereus undatus (haw.) britton & rose] grown in Brazil. Journal of Drug Metabolism and Toxicology, 6(4): 1-6.
10. Colagrande O, Silva A, Fumi MD (1994) Recent applications of biotechnology in wine production. Rev. Biotechnology Progress, 10(1): 2-18.
11. Blandino A, Macias M, Cantero D (1999) Formation of calcium alginate gel capsules: influence of sodium alginate and $CaCl_2$ concentration on gelation kinetics. Journal of Bioscience and Bioengineering, 688-696.
12. Jaime Moreno-García, Teresa Garcia Martinez, Juan C Mauricio, Juan Moreno (2018) Yeast immobilization systems for alcoholic wine fermentations: actual trends and future perspectives. Front Microbiol., 9: 241.
13. Koki Yokotsuka, Mizuo Yajima, Toshihide Matsudo (1997) Production of bottle-fermented sparkling wine using yeast immobilized in double-layer gel beads or strands. Am J. Enol. Vitic., 48: 471-481.
14. Sevda SB, Rodrigues L (2011) The making of pomegranate wine using yeast immobilized on sodium alginate. African Journal of Food Science, 5(5): 299-304.
15. SurajbhanSevda, Lambert Rodrigues (2014) Preparation of guava wine using immobilized yeast. J. Biochem. Tech., 5(4): 819-822.

16. Camelia Elena Iurciuc, Catalina Peptu, Alexandru Savin, Leonard Ionut Atanase, Kaies Souidi, Grahame Mackenzie, Patrick Martin, Gerard Riess, Marcel Popa (2017) Microencapsulation of baker's yeast in gellan gum beads used in repeated cycles of glucose fermentation. International Journal of Polymer Science, Article ID 7610420, 15.
17. Paweł Sroka, Paweł Satora, Tomasz Tarko, Aleksandra Duda-Chodak (2017) The influence of yeast immobilization on selected parameters of young meads. Journal of the Institute of Brewing, 123(2): 289-295.
18. AP Pereira, A Mendes-Ferreira, JM Oliveira, LM Estevinho, A Mendes-Faia (2014) Effect of Saccharomyces cerevisiae cells immobilisation on mead production. LWT - Food Science and Technology, 56: 21-30.
19. Huma Bokkhim, Praksha Neupane, Smita Gurung, Rojeena Shrestha (2018) Encapsulation of Saccharomyces cerevisiae in alginate beads and its application for wine making. Journal of Food Science & Technology Nepal, 10: 18-23.
20. NievesLópez de Lerma, Rafael A Peinado, AnnaPuig-Pujol, Juan C Mauricio, Juan Moreno, Teresa Garcia Martinez (2018) Influence of two yeast strains in free, bioimmobilized or immobilized with alginate forms on the aromatic profile of long aged sparkling wines. Food Chemistry, 250 (1): 22-29.
21. Fe N Dimero, Teddy F Tepora (2018) Processing and development of dragon fruit wine. International Journal of Environment, Agriculture and Biotechnology, 3(5): 1943-1947.
22. Foong JH, Hon WM, Ho CW (2012) Bioactive compounds determination in fermented liquid dragon fruit (Hylocereus Polyrhizus). Borneo Science, 31: 38-56.
23. Xiao Gong, Lina Ma, Junwei Yin, Mao Lin, Jihua Li (2017) Change in soluble sugar and organic acids during fermentation of dragon fruit wine. 2nd International Conference on Materials Science, Machinery and Energy Engineering, 123: 1284-1288.
24. Richard Marchal, Laurence Marchal-Delahaut, Franck Michels, Maryline Parmentier, Armelle Lallement, Philippe Jeandet (2002) Use of wheat gluten as clarifying agent of musts and white wines. Am. J. Enol. Vitic., 53(4): 308-314.
25. Awe S (2018) Effect of clarifying agents (gelatin and kaolin) on fruit wine production. International Journal of Agriculture Innovations and Research, 6(4): 130-132.

