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RESEARCH ARTICLE

Optimization Parameters for Enzymatic Clarification of Dragon Fruit Juice

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Abstract

Fresh dragon fruit juice has high viscosity and turbidity. Fruit juice clarification by ultrafiltration only does not remove active haze precursors, allowing haze formation during storage. Pectinase treatment can resolve this matter. Pectinase can break down complex polysaccharides of plant tissues into simpler molecules like galacturonic acids. Pitaya or dragon fruit can be utilized to produce fruit juice as functional beverage. In our research, pectinase was used in different parameters of enzyme concentration (0.02, 0.04, 0.06, 0.08, 0.10%), temperature (30, 35, 40, 45, 50^{o}C), time (45, 60, 75, 90, 105 min) and pH (4.2, 4.4, 4.6, 4.8, 5.0). Total soluble solid (^{o}Brix), total phenolic (g/L), turbidity (NTU) and viscosity (cP) were important indicators to identify the optimal treatment. Our results showed dragon fruit juice would have the best total soluble solid, total phenolic, clarity as well as viscosity by treatment of pectinase concentration 0.08%, temperature 40^{o}C in 90 min at pH 4.6. Our finding concluded that pectinase treatment would be a potential efficient approach of cloud removal in dragon fruit juice production

Keywords: *Pectinase, Dragon fruit, Total soluble solid, Phenolic, Turbidity, Viscosity.*

Introduction

Cloudy juice is a colloidal suspension where the continuous medium is a solution of protein, pectin, sugar, tannin, metal and organic acid, and the dispersed matter is mainly created by cellular tissue. The high concentration of pectin leads to colloid formation, which constitutes one of the main problems during the processing of clear fruit juices [1]. To obtain a clear juice, these suspended particles have to be removed. Suspension must be unstabilized to remove active haze precursors, prevent the probability of haze formation during storage.

Membrane clarification procedures have been applied as an innovative strategy to decrease the turbidity of fruit juices [2, 3], however this application is limited because of its high cost. Enzymatic clarification appears to be a useful approach to allow effective usage of clarifying agents to assist with cloud removal. Pectinases degrade pectin hence resulting in viscosity reduction and cluster formation, which facilitates separation through centrifugation or filtration, the press ability of the pulp improves, the jelly structure disintegrates and the fruit juice is easily obtained and with higher yields [1, 4]. The juice shows higher clarity, more concentrated flavour and colour [5]. The dragon fruit is considered as a health benefit fruit that is high in antioxidants, fiber, and low in calories. It is a good source of vitamin, minerals, especially calcium and phosporus [6].

The pulp and peel of the dragon fruit are rich in polyphenols and betacyanin (Jamilah et al., 2011). Dragon fruit aids digestion, reduces cholesterol levels and high blood pressure, prevents diabetes and colon cancer, as well as combats against cough and asthma [7]. Juice processing from dragon fruit is one of efficient strategies to produce value added products to increase its commercial value in agriculture. There were several studies mentioned to utilization of pectinase for clarification of dragon fruit juice. The effects of commercial enzymes, i.e., Pectinex Ultra SP-L and Pectinex CLEAR on the chemical composition, vitamin C and total polyphenols contents of red pitaya (*Hylocereus polyrhizus*) juice were examined [8].

One research improved the extraction efficiency and quality of the juice extracted from red dragon fruit (*Hylocereus polyrhizus*), by using commercial hydrolytic enzymes, namely Pectinex Ultra SP-L and Viscozyme L.[9].Fresh dragon fruit is very perishable. It can only last a maximum of 14 days at 10^{o}C and 5 days at room temperature [10]. Objective of our study focused on the effectiveness of different processing variables such as pectinase concentration, temperature, time and pH during incubation of dragon fruit juice in respect of total soluble solid (^{o}Brix), total phenolic (g/L), turbidity (NTU) and viscosity (cP).

Materials and Method

Material

Dragon fruits were collected from Tien Giang province, Vietnam. After collecting, they must be conveyed to laboratory for experiments. They were subjected to washing and treatment. The fruits were peeled and pulped by a juice extractor and seeds were discarded. Pulp (30 g) was mixed with 60 mL distilled water and pectinase enzyme in a 100 mL Erlenmeyer flask, placed in an incubator with shaking at 250 rpm. Different parameters of pectinase enzyme concentration (0.02, 0.04, 0.06, 0.08, 0.10%), temperature (30, 35, 40, 45, 50^{o}C), time (45, 60, 75, 90, 105 min) and pH (4.2, 4.4, 4.6, 4.8, 5.0) were examined. At the end each treatment, pectinase enzyme were deactivated at 95^{0}C in 3 minutes. Clear fruit juice was collected by filtration and ready for testing.

Researching Procedure

Effect of Pectinase Concentration during Enzymatic Treatment to Juice Clarification

Raw dragon fruit juice samples were treated with different pectinase concentration (0.02, 0.04, 0.06, 0.08, 0.10%), at 30^{o}C within 45 min at pH 4.2. At the end of treatment, all samples were pasteurized at 95^{0}C in 3 minutes to deactivate pectinase. Clear fruit juice was collected by filtration and ready for testing. Total soluble solid (^{o}Brix), total phenolic (g/L), turbidity (NTU) and viscosity (cP) were important indicators so they were chosen to identify the optimal treatment.

Effect of Incubation Temperature during Enzymatic Treatment to Juice Clarification

Raw dragon fruit juice samples were incubated with different temperature (30, 35, 40, 45, 50^{o}C) with 0.08% pectinase within 45 min at pH 4.2. At the end of treatment, all samples were pasteurized at 95^{0}C in 3 minutes to deactivate pectinase. Clear fruit juice was collected by filtration and ready for testing. Total soluble solid (^{o}Brix), total phenolic (g/L), turbidity (NTU) and viscosity (cP) were important indicators so they were chosen to identify the optimal treatment.

Effect of Incubation Time during Enzymatic Treatment to Juice Clarification

Raw dragon fruit juice samples were incubated in different duration (45, 60, 75, 90, 105 min) with 0.08% pectinase in 40^{o}C at pH 4.2. At the end of treatment, all samples were pasteurized at 95^{0}C in 3 minutes to deactivate pectinase. Clear fruit juice was collected by filtration and ready for testing. Total soluble solid (^{o}Brix), total phenolic (g/L), turbidity (NTU) and viscosity (cP) were important indicators so they were chosen to identify the optimal treatment.

Effect of pH during Enzymatic Treatment to Juice Clarification

Raw dragon fruit juice samples were incubated in different pH values (4.2, 4.4, 4.6, 4.8, 5.0) with 0.08% pectinase at 40^{o}C within 90 min. At the end of treatment, all samples were pasteurized at 95^{0}C in 3 minutes to deactivate pectinase. Clear fruit juice was collected by filtration and ready for testing. Total soluble solid (^{o}Brix), total phenolic (g/L), turbidity (NTU) and viscosity (cP) were important indicators so they were chosen to identify the optimal treatment.

Physico-chemical, Sensory and Statistical Analysis

Soluble soluble solids were measured with a hand-held refractometer at 20^{o}C.

Total phenolic (g/L) was quantified using the Folin-Ciocalteu method. Turbidity (expressed as Nephelometric Turbidity Units, NTU) was measured using a turbidimeter. Viscosity (cP) was analyzed by viscometer. The experiments were run in triplicate with three different lots of samples. Statistical analysis was performed by the Statgraphics Centurion XVI.

Result & Discussion

Effect of Pectinase Concentration in Enzymatic Treatment of Dragon Fruit Juice

Turbidity test is a measure of clarification efficiency. There are many factors affecting the turbidity of beverages, the most frequent cause is protein–polyphenol interaction [11]. In our research, raw dragon fruit juice was treated by different pectinase concentration (0.02, 0.04, 0.06, 0.08, 0.10%). Our result showed that the optimal pectinase concentration should be 0.08% to achieve the best quality of dragon fruit juice.

Table 1: Effect of pectinase concentration (%) to quality of dragon fruit juice

Pectinase concentration	0.02	0.04	0.06	0.08	0.10
Total soluble solid (°Brix)	16.29±0.02[b]	16.83±0.01[ab]	17.14±0.03[ab]	17.85±0.00[a]	17.90±0.02[a]
Total phenolic (g/L)	4.55±0.04[c]	4.68±0.00[bc]	4.76±0.03[b]	4.92±0.02[ab]	4.95±0.00[a]
Turbidity (NTU)	1.89±0.01[a]	1.73±0.00[ab]	1.62±0.01[b]	1.47±0.01[bc]	1.45±0.01[c]
Viscosity (cP)	2.24±0.03[a]	2.14±0.04[ab]	2.03±0.02[b]	1.91±0.03[bc]	1.87±0.02[c]

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%)

The pectinase concentration has a important effect on the linear and the quadratic effects of clarity because it is dependent on the enzyme concentration [12]. One research improved the extraction efficiency and quality of the juice extracted from red dragon fruit (*Hylocereus polyrhizus*), by using commercial hydrolytic enzymes, namely Pectinex Ultra SP-L and Viscozyme L.

High extraction yield (86.35%) and reducing sugar content in the juice (28.87 mg/mL) were obtained at temperature 40o C, in 120 min and with a combination of Pectinex Ultra SP-L and Viscozyme L (70/30). The juice relative viscosity was reduced from 1.42 to 1.09, while the total acidity increased from 0.47 to 0.75 (g/100 mL), the TPC from 13.68 to 14.16 (mgGAE/100g puree) and the vitamin C content from 27.94 to 32.29 (mg/100g puree) as compared to the treatment without enzymes [9].

Effect of Temperature in Enzymatic Treatment of Dragon Fruit Juice

In our research, raw dragon fruit juice was treated by pectinase concentration 0.08% in different temperature (30, 35, 40, 45, 50°C). Our result showed that the optimal pectinase treatment temperature should be conducted at 40°C to achieve the best quality of dragon fruit juice.

Table 2: Effect of temperature (°C) in enzymatic entreatment to quality of dragon fruit juice

Temperature (°C)	30	35	40	45	50
Total soluble solid (°Brix)	17.85±0.00[c]	17.96±0.03[ab]	18.33±0.00[a]	18.04±0.03[b]	17.90±0.01[bc]
Total phenolic (g/L)	4.92±0.02[bc]	5.06±0.02[ab]	5.12±0.04[a]	5.03±0.01[b]	4.81±0.04[c]
Turbidity (NTU)	1.47±0.01[a]	1.32±0.01[b]	1.24±0.02[c]	1.31±0.00[bc]	1.40±0.00[ab]
Viscosity (cP)	1.91±0.03[a]	1.71±0.02[ab]	1.45±0.01[c]	1.57±0.02[bc]	1.64±0.01[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%)

Enzymatic reaction rate is increased by the temperature, as well as the clarification rate, as for the enzyme until it is below the denaturation temperature [12].

Effect of Duration in Enzymatic Treatment of Dragon Fruit Juice

In our research, raw dragon fruit juice was treated by pectinase concentration 0.08% at temperature 40°C by different time (45, 60, 75, 90, 105 min). Our result showed that the optimal pectinase treatment duration should be last for 90 min to achieve the best quality of dragon fruit juice.

Table 3: Effect of time (min) in enzymatic entreatment to quality of dragon fruit juice

Time of treatment	45	60	75	90	105
Total soluble solid (°Brix)	18.33±0.00^{c}	18.49±0.00^{bc}	18.85±0.02^{b}	18.97±0.01^{ab}	19.00±0.02^{a}
Total phenolic (g/L)	5.12±0.04^{b}	5.27±0.01^{ab}	5.39±0.01^{ab}	5.55±0.03^{a}	5.57±0.00^{a}
Turbidity (NTU)	1.24±0.02^{a}	1.14±0.00^{ab}	1.03±0.03^{ab}	1.01±0.03^{ab}	1.01±0.00^{b}
Viscosity (cP)	1.45±0.01^{a}	1.41±0.04^{ab}	1.32±0.04^{b}	1.25±0.00^{bc}	1.23±0.03^{c}

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%)

When the incubation time is at its lowest, the filterability is found to increase along with the enzyme concentration [12]. Incubation time had a direct impact on the activity of enzyme.

Effect of pH in Enzymatic Treatment of Dragon Fruit Juice

In our research, raw dragon fruit juice was treated by pectinase concentration 0.08% at temperature 40°C within 90 min by different pH values (4.2, 4.4, 4.6, 4.8, 5.0). Our result showed that the optimal pectinase treatment duration should be emphazied at pH 4.6 to achieve the best quality of dragon fruit juice.

Table 4: Effect of pH in enzymatic entreatment to quality of dragon fruit juice

pH of treatment	4.2	4.4	4.6	4.8	5.0
Total soluble solid (°Brix)	18.97±0.01^{b}	19.04±0.03^{ab}	19.11±0.00^{a}	18.92±0.02^{bc}	18.76±0.03^{c}
Total phenolic (g/L)	5.55±0.03^{c}	5.61±0.00^{b}	5.73±0.03^{a}	5.64±0.02^{ab}	5.59±0.02^{bc}
Turbidity (NTU)	1.01±0.03^{b}	0.95±0.02^{bc}	0.91±0.01^{c}	1.05±0.00^{ab}	1.14±0.03^{a}
Viscosity (cP)	1.25±0.00^{ab}	1.12±0.01^{bc}	1.01±0.02^{c}	1.15±0.01^{b}	1.29±0.01^{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%)

In the juice the viscosity was reduced due to the enzyme treatment which leads to the water holding capacity's reduction because of the pectin degradation. In one report, the optimum conditions for the clarification of banana juice are 0.055% concentration of enzyme at 40°C for 75 mins having 4.7 pH [12].

Conclusion

In the dragon fruit juice, the polysaccharides such as starch and pectin are the major cause for the viscosity and turbidity. The flocculation of pectin-protein complexes are caused by hydrolization of pectinase in pectin. The resulting juice facilitates the further filtration process, as it has a lower viscosity. Enzymatic treatment makes the juice clarity by breaking down the pectin and allowing the suspended particles to settle down, moreover undesirable changes in colour and stability can be avoided. Pectinase is utilized in fruit juice processing to increase the juice yield and to improve clarification by reducing turbidity and viscosity.

We have successfully examined different technical variables influencing to the pectinase treatment of dragon fruit juice. This finding can be considered suitable and very beneficial for the processing of dragon fruit juice as a step toward promoting a functional beverage.

References

1. Ivana Greice Sandri, Roselei Claudete Fontana, Débora Menim Barfknecht, Mauricio Moura da Silveira (2011) Clarification of fruit juices by fungal pectinases. LWT - Food Science and Technology, 44: 2217-2222.
2. Mirsaeedghazi H, Emam-Djomeh Z, Mousavi SM, Aroujalian A, Navidbakhsh M (2009) Changes in blocking mechanisms during membrane processing of pomegranate juice. Int. J. Food Sci. Technol., 44: 2135-2141.
3. Mirsaeedghazi H, Emam-Djomeh Z, Mousavi SM, Ahmadkhaniha R, Shafiee A (2010) Effect of membrane clarification on the physcochemical properties of pomegranate juice. Int. J. Food Sci. Technol., 45: 1457-1463.
4. Tapre AR, Jain RK (2014) Pectinases: Enzymes for fruit processing industry. International Food Research Journal, 21(2): 447-453.
5. Abdullah LAG, Sulaiman NM, Aroua MK, Noor MMMJ (2007) Response surface

optimization of conditions for clarification of carambola fruit juice using a commercial enzyme. Journal of Food Engineering, 81: 65-71.

6. Lee-Fong Siow, Yen-Ming Wong (2017) Effect of juice concentration on storage stability, betacyanin degradation kinetics, and sensory acceptance of red-fleshed dragon fruit (Hylocereus polyrhizus) juice. International Journal of Food Properties, 20: 623-632.
7. Jaafar RA, Abdul Rahman, ARB Mahmod, NZC Vasudevan R (2009) Proximate analysis of dragon fruit (Hylocereus Polyrhizus). American Journal of Applied Sciences, 6: 1341-1346.
8. AR Nur Aliaa, MK Siti Mazlina, FS Taip (2011) Effects of commercial pectinases application on selected properties of red pitaya juice. Journal of Food Process Engineering, 34: 1523-1534.
9. NM Phuong Truong, Q Tuan Dang (2016) Application of hydrolytic enzymes for improvement of red dragon fruit juice processing. Asia Pacific Journal of Sustainable Agriculture Food and Energy, 4: 1-4.
10. Nguyen Phuoc Minh (2014) Various factors influencing to red dragon fruit (Hylocereus polyrhizus) wine fermentation. International Journal of Multidisciplinary Research and Development, 1: 94-98.
11. Hatice R Oziyci, Mustafa Karhan, Nedim Tetik, Irfan Turhan (2013) Effects of processing method and storage temperature on clear pomegranate juice turbidity and color. Journal of Food Processing and Preservation, 37: 899-906.
12. Dillwyn S, S Elizabeth Amudhini Stephen, C Bazil Wilfred (2019) Use of a Commercial Enzyme for the Clarification of Banana Juice-Optimization Conditions. International Journal of Engineering and Advanced Technology, 8: 246-250.