ISSN: 2338-1345 –Vol. 4. (1): 1- 4 2016 Journal online http://journal.bakrie.ac.id/index.php/APJSAFE

Asia Pacific Journal of Sustainable Agriculture Food and Energy (APJSAFE)

Application of Hydrolytic Enzymes for Improvement of Red Dragon Fruit Juice Processing

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Abstract— The objective of this study was to improve 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. The two enzymes were applied in constant total rate (0.1%), but at different combination ratios, temperatures and enzymatic treatment durations. By the effect of the enzymes, the extraction yield was significantly increased while the juice quality was obviously improved. High extraction yield (86.35%) and reducing sugar content in the juice (28.87 mg/mL) were obtained at temperature 40^{o}C, in 120 min and with a combination of Pectinex Ultra SP-L and Viscozyme L (70/30). Meanwhile, extraction without enzyme treatment resulted in significantly lower yield (54.04%) and less reducing sugar content in juice (20.63 mg/mL). Also, by application of the enzymes at optimal conditions, 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.

Keywords— **Cellulase, dragon fruit, fruit juice, hemicellulase, pectinase**

INTRODUCTION

Red dragon fruits (*Hylocereus polyrhizus)* are of large oblong shape, dark red peel with large scales, red pulp with little black seeds inside. Originated from Central America, the variety is nowdays cultivated in many Asian counties including Malaysia, Thailand, the Philippines and Vietnam **(**Ortiz-Hernández & Car, 2012). The 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 C, minerals, especially calcium and phosporus (Stintzing et al., 2002). In general, pulp and seeds make up 70-80% of fresh dragon fruits. Both the flesh and peel of the fruit are rich in polyphnols (Jamilah et al., 2011). Juice production from red dragon fruit is an efficient way for producing value-added products and increasing economics of the fruit sector. Enzyme is an important tool in juice processing, both in terms of quality and cost saving. Commercial pectinolytic enzymes have been applied in red dragon fruit juice processing. By using enzyme Pectinex CLEAR lead to the juice with higher protein content and the phenolics amounts were increaed by up to 15% (Nur'Aliaa, 2010). In another study where Pectinex® Ultra SP-L was used (Kunnika & Pranee, 2011), the juice was higher in total phenolic, total flavonoid and soluble dietary fiber content. However, no study on using cellulolytic enzymes was available for such kind of juice. The objective of this study was to apply in combination of hydrolytic enzymes (Pectinex Ultra SP-L and Viscozyme L) to improve red dragon fruit juice processing.

MATERIALS AND METHODS

Materials and chemicals

Red dragon fruits were bought from a local market in Binh Thuan province. Two kinds of enzymes, namely Pectinex Ultra SP-L and Viscozyme L, were from Novozymes (Denmark). Chemicals and solvents for analytical tests such as Folin –Ciocalteu reagent, Gallic acid, 3,5-dinitrosalicylic acid, ascorbic acid and methanol were purchased from local agents of Merck or Sigma or of analytical grade. The solutions were prepared freshly before testing.

Experimental procedures

The fruits were peeled and pulped by a juice extractor and seeds were discarded. Pulp (20 g) was mixed with 40 mL distilled water and 0.1% enzyme (of the fresh flesh) in a 100 mL Erlenmeyer flask, placed in an incubator with shaking at 150 rpm while pH adjusted to 4.5. Enzyme combination (Pectinex Ultra SP-L & Viscozyme L) was investigated in 5 w/w ratios (10:0; 7:3; 5:5; 3:7 and 0:10) as compared to the control treatment without any enzyme addition. Temperature and duration of pulp maceration were from 30 to 60°C (4 levels) and from 90 to 120 min (3 levels), respectively. At the end each treatment, enzymes were deactivated at 90°C for 5 minutes. Clear juice was collected via filtration.

Analytical methods

Extraction yield was defined as a ratio of total soluble solids (TSS) in juice to the dry matter in pulp (%). The TSS content was measured by using a digital refractometer (Atago RX-5000a, Japan). Moisture and dry matter was measured by using an oven drying method. Relative viscosity of the juice was measured by using a capillary viscometer (Oswald type) at room temperature by comparison of flowing time with distilled water. Total acidity (TA) was measured by a titration method against 0.1M NaOH solution and expressed as equivalent gram of citric acid. Reducing sugar (RS) was determined spectrophotometrically by using 3,5-DNS reagent. Vitamin C was determined by using the iodine titration method (Choo & Yong, 2011). Total phenolic content (TPC) was determined by a spectrophotometry method (Genesys 10S UV-Vis, USA), using Folin –Ciocalteu reagent and Gallic acid as a standard. The absorbance was measured at 750 nm (Kunnika & Pranee, 2011).

Color of the final juice was measured in the CIE L*a*b* color scale by using a colorimeter (Konica Minolta 10PB, USA), and compared to that of the non-enzyme-assisted juice product at the same maceration conditions.

Statistical analysis

Each presented treatment was done triplicate and the obtained results were subjected to analysis of variance (ANOVA), using standard software SPSS, version 22.

RESULTS AND DISCUSSIONS

Effect of enzyme concentration on extraction yield and juice quality

At a fixed level of total enzyme concentration of 0.1%, the extraction yield of juice reached 65.69 to 82.46%, significantly higher than that without enzyme treatment (54.04%). A combination of Pectinex Ultra SP-L and Viscozyme L in a ratio 7:3 resulted in highest extraction yield of juice (82.46%), significantly higher than treatments with a single enzyme, either Pectinex Ultra SP-L or Viscozyme L (Table 1). The two enzymes supported each other in hydrolyzing plant cell walls. Hydrolytic enzymes facilitated pulp pressing, increased juice yield (Kashyap et al., 2001).

Table 1. Effect of enzyme concentration on extraction yield and juice quality

Enzymes' combination (Pectinex : Viscozyme)	(10:0)	(7:3)	(5:5)	(3:7)	(0:10)	Control
Extraction yield (%)	73.40^{b}	82.46^{a}	71.91^{b}	71.81^{b}	65.70^{c}	54.04^{d}
Reducing sugar (mg/mL)	28.60^{b}	30.70^{a}	28.52^{b}	28.20^{c}	23.96^{d}	20.63^{e}
Relative viscosity	1.24^{d}	1.19^{e}	1.26^{cd}	1.28^{c}	1.34^{b}	1.42^{a}
TTA (g citric acid/100mL)	0.58^{b}	0.66^{a}	0.64^{a}	0.59^{b}	0.52^{c}	0.47^{d}
TPC (mgGAE/100g puree)	16.75^{b}	17.70^{a}	16.61^{b}	16.04^{c}	15.42^{d}	13.68^{e}
Vitamin C (mg/100mL)	34.28^{b}	36.63^{a}	34.05^{b}	31.47^{c}	29.82^{d}	27.94^{e}

Different letters in row indicated significant differences at the level $p<0.05$

Similar trend was observed for the RS content, TA, vitamin C and TPC, while the change in viscosity was in an opposite pattern. The highest RS content, TA, vitamin C content and TPC was 30.70 mg/mL, 0.66 g/100mL, 36.63 mg/100g and 17.70 mgGAE/100g, respectively, achieved in the juice obtained by the combination in 7:3 ratio of Pectinex Ultra SP-L to Viscozyme L. Treatment without enzyme resulted in significantly lower level of RS content, total acidity, vitamin C content and TPC and higher value of the juice viscosity.

The combination of Pectinex Ultra SP-L and Viscozyme L resulted in degrading the glycosyl bonds of pectin, cellulose and hemicellulose in the cell wall of fruit tissues, led to release out more cell components such as reducing sugars, organic acids, vitamin C and phenolic compounds (Mutlu et al., 1999). Enzymatic digestion helped to reduce pulp viscosity (Schweiggert et al., 2009) and hemicellulase might have a synergetic effect to pectinase in viscosity reduction. Dragon fruits are a good source of vitamin C and antioxidants: the vitamin C content (32.65±1.59 mg/100g) and the TPC (17.70 mgGAE/100g juice) found in this study were close to that in previous studies (Choo & Yong, 2011; Nurliyana et al., 2010).

Effect of maceration temperature on extraction yield and juice quality

At fixed time (120 min), enzyme concentration (0.07% Pectinex Ultra SP-L and 0.03% Viscozyme L) and pH (4.5), the effect of temperature showed the general trend that the extraction yield decreased as temperature raised above 40°C (Table 2). At 40°C the extraction yield reached highest (84.56 %).

The highest RS content, TA, Vitamin C and TPC in the juice was achieved at 40°C, while the juice viscosity was lowest. At 60°C, reducing sugar content was lowest (25.49 mg/mL), showing in-activation of enzymes at higher temperature. Too low temperature could not provide enough energy to support the works of enzymes, while higher temperatures may deactivate enzymes (Schweiggert et al., 2009). Vitamin C and phenolic compounds are sensitive to temperature, at 60°C the vitamin C content was sharply decreased to 1.76 mg/100ml and the TPC was lowest.

The results provided evidence for choosing 40°C as the most appropriate for applications of dual enzymes such as Pectinex Ultra SP-L and Viscozyme L in processing of dragon fruit juice.

Effect of maceration period on extraction yield and juice quality

Increase in treatment time led to the increase in extraction yield, the RS content and TA, meanwhile the decrease in relative viscosity, vitamin C content and TPC in the juice (Table 3). However after 120 min the change was not significant.

For 120 min of enzyme treatment, the extraction yield reached 86% while the reducing sugar content was nearly 29 mg/mL. The juice viscosity was about 10% larger than that of pure water. The prolonged time process facilitates enzyme activities, as more pectin compounds are degraded and more plant cell walls are hydrolyzed, the juice viscosity will be reduced.

Table 2. Effect of temperature for enzyme treatment on juice yield and quality

Temp. (^{o}C)	Ex. Yield (%)	RS content (mg/ml)	Rel. Viscosity	TA (g Citric acid/100 g)	Vit. C (mg/100g)	TPC (mgGAE/100g)
30	81.48±0.26^{a}	26.56±0.11^{b}	1.19±0.01^{b}	0.59±0.19^{a}	34.05±1.02^{bc}	13.13±0.16^{b}
40	84.56±0.31^{b}	30.31±0.04^{d}	1.11±0.00^{a}	0.73±0.19^{c}	35.22±1.68^{c}	15.35±0.22^{d}
50	83.29±0.30^{c}	29.85±0.15^{c}	1.12±0.00^{a}	0.69±0.19^{b}	33.46±1.37^{b}	14.04±0.30^{c}
60	81.69±0.24^{a}	25.49±0.09^{a}	1.20±0.01^{b}	0.59±0.19^{a}	1.76±0.15^{a}	12.56±0.11^{a}

Different letters in column indicated significant difference at $p<0.05$

Table 3. Effect of duration for enzyme treatment on juice yield and quality

Duration (min)	Ex. Yield (%)	RS content (mg/ml)	Rel. Viscosity	TA (g Citric acid/100 g)	Vit. C (mg/100g)	TPC (mgGAE/100g)
90	84.14±0.28^{a}	25.06± 0.48^{a}	1.11± 0.00^{b}	0.64± 0.00^{a}	35.22±0.05^{b}	15.42±0.18^{b}
120	86.35± 0.06^{b}	28.87± 1.27^{b}	1.10± 0.01^{a}	0.75± 0.02^{b}	32.29±1.01^{a}	14.16±0.21^{a}
150	86.75± 0.25^{b}	30.05± 0.12^{b}	1.10± 0.00^{a}	0.77± 0.00^{b}	31.11±1.02^{a}	13.80±0.14^{a}

Different letters in column indicated significant difference at $p <0.05$

The trend of decrease in TPC implied that phenolic compounds might be destroyed or altered as the process time was prolonged. Betacyanins form an important group in total polyphenols of red dragon fruit. Some authors reported that lower betacyanins concentration was observed for longer time of processing (Lim et al., 2011), so the decrease in betacyanins content leads to TPC decrease.

Similarly, vitamin C content showed the trend of decrease when the processing time was increased. Again, ascorbic acid was a sensitive chemical, thus it was easily destroyed not only by temperature but exposure time and light.

Evaluation of juice color for final product

The L*, a*, b* values for the juice samples were measured and presented in Table 4. At the same extraction conditions (40°C, 120 min, 0.1% enzymes), a* value which was determined as of reddish color, of the enzymatic-assisted juice (6.56) was higher than the non-enzymatic-assisted juice (5.65). Other findings which were done on flesh and peel of dragon fruit, were in agreement with this report (Kunnika & Pranee, 2011).

The color difference was calculated as ΔE value, by the formula:

$$\Delta E = \sqrt{(\Delta L^{*})^{2} + (\Delta a^{*})^{2} + (\Delta b^{*})^{2}}$$

By the way, the value ΔE equal to 1.39 showed an insignificant difference in color between the enzyme-assisted and non-enzyme-assisted juice samples. Visually, the difference in color is recognized only to a trained eye.

Table 4. Red dragon fruit juice color measurement

Experiment	L*	a*	b*
Enzyme-assisted samples	23.50±0.10	6.56±0.11	1.97±0.11
Non-enzyme-assisted samples	23.47±1.18	5.65±0.12	0.95±0.05

CONCLUSIONS

By the effect of the hydrolytic enzymes, the extraction yield was significantly increased while the juice quality was obviously improved in terms of viscosity reduction and increase in total phenolic, vitamin C and reducing sugar content. At the total enzyme inclusion rate of 0.1%, the best combination of Pectinex Ultra SP-L and Viscozyme L was in a ratio of 7:3. Additionally, the best temperature and maceration time for red dragon juice processing in this study was found to be 40°C and 120 min, respectively. Finally, the extraction yield of juice was increased from 54.04 to 86.35% when using enzymes at optimal conditions as compared to result from no-enzyme treatment.

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