IMPACT OF TWO COMMERCIAL ENZYMES APPLICATION ON SOME PHYSICOCHEMICAL PROPERTIES OF RED PITAYA JUICES

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ABSTRACT

Application of enzyme in fruit juice clarification has been known widely in fruit processing industries. Clarification of red pitaya (*Hylocereus polyrhizus*) juice by enzymatic treatment has been done to obtain stable juice. The aim of this work was to observe the effectiveness of two commercial enzymes i.e. Pectinex CLEAR and Pectinex Ultra SP-L in clarifying red pitaya juices. The effects of both enzymes on some physicochemical properties of red pitaya juices were obtained. Enzymation leads to the juice with higher yield, higher acidity, and higher total soluble solids. It also increased the juice clarity and colour appearance. However, enzymation reduced the juice pH and viscosity. This study demonstrated the effect of pectinases application on several physicochemical properties of red pitaya juices with an attempt to obtain some interesting and valuable data for the fruit processing industries. The data obtained are helpful for the optimization of red pitaya juices production as a step towards promoting the potential of red pitaya juices as functional beverages.

Keywords: fruit juice clarification, red pitaya juice, *Hylocereus polyrhizus*, enzymatic treatment, physicochemical

1.0 INTRODUCTION

More than 500 edible fruits grow in the tropical and subtropical regions, but less than 15 are commercially processed. Only 15% of tropical fruits are used for juice production (Ramadan and Moersel, 2007). International markets for tropical fruits such as pineapple, mango, guava, banana and kiwi already exist. The most popular products made from tropical fruits are fruit juices, nectars, and drinks. The consumption of fruit juices from temperate zones (e.g. apple) is growing more slowly than that of citrus and tropical fruit juices altogether, which today account for about 70% of the fruit juice present in the market (Ramadan and Moersel, 2007). This trend has caused an upswing in the fruit juice industries of the fruit-growing countries, which endeavour to promote and improve production, to be competitive for both domestic demand and export markets (Askar, 1998).

Enzyme is an essential tool in juice processes, both in terms of quality improvement and cost saving (Ramadan and Moersel, 2007). Fruit and vegetable juice production is nowadays unthinkable without the use of enzymes (Baumann, 1981). The degradation of plant cell walls by exogenous enzymatic treatment results in easier release of the components contained in cells (Janser, 1997).

The cloudiness in the juices is mainly caused by the presence of polysaccharides such as pectin and starch. The pectin can be associated with plant polymers and the cell debris, which has a fiber-like molecular structure and makes the clarification process harder. Enzyme-catalyzed breakdown of the plant cell-wall matrix and middle lamella may first increase the immediate turbidity in the juice, which is generally assumed to be mainly due to the presence of pectin and other fractions of fruit cell-wall material (Grassin and Fauquembergue 1996). Therefore, enzymatic treatment by using pectinase is an effective way to reduce the pectin in the fruit juices because pectinase has the ability to hydrolyze pectin and cause pectin–protein complexes to flocculate (Rai *et al.* 2004; Lee *et al.* 2006; Liew Abdullah *et al.* 2006; Sin *et al.* 2006), which could be easily removed by filtration. A complete enzymatic breakdown of pectin is the key for producing clear and stable fruit juices. Though hard to believe, juice clarification is the oldest and still largest market for commercial pectinases (Baumann, 1981).

In fruit products, enzymes have been exploited for the following purposes: to break down all polymeric carbohydrates such as pectin, hemicelluloses and starches, thus increasing the yield of juice by enabling better pressing of the pulp; to improve the yield of substances contained in fruit, e.g., acids, coloring or aroma substances; to clarify juices; and to liquefy the entire fruit pulp for maximum yield (Gerhartz 1990). The resulting juice will have a much lower amount of pectin, lower viscosity and also lower turbidity, which is easier for filtration process (Isabella *et al.* 1995; Alvarez *et al.* 1998; Ceci and Lozano 1998; Rai *et al.* 2004; Lee *et al.* 2006; Sin *et al.* 2006).

The pitaya or dragon fruit is also known as pitahaya, strawberry pear, nanettikafruit or thanh long. It is a cactus species of the genus *Hylocereus* and *Stenocereus*. Generally, pitaya comes in three types: *Hylocereus undatus* (white flesh with pink skin), *Hylocereus polyrhizus* (red flesh with pink skin) and *Seleicereus megalanthus* (white flesh with yellow skin) (Wikipedia, 2007). Pitaya originated in Mexico and Central America and subsequently, the plant has been widely cultivated in countries such as Vietnam, Taiwan and Malaysia. Pitaya is mildly sweet and is low in calories, has an attractive flesh and is juicy with a subtle fruity flavor (melon-like flavor). Pitaya is round or oval, the skin is fuchsia pink or yellow depending on the species, leathery and slightly leafy, and the fruit usually weighs about 200–700 g. It is rich in fiber, vitamin C and minerals. Pitaya may be converted into juice, jam and wine. Pitaya juices are turbid, very viscous and contain colloidal suspension. Therefore, the juices need to be clarified in order to be commercialized. The use of pitaya as food colorant has also been reported (Stintzing *et al.* 2002; Mobhammer *et al.* 2005; Herbach *et al.* 2006; Esquivel *et al.* 2007).

Since no data have been available for enzymatic clarification of pitaya juices, thus in this work, some physicochemical parameters of red pitaya juices are reported for the first time. The data obtained are helpful for the optimization of red pitaya juices production as a step towards promoting the potential of pitaya juices as functional beverages.

2.0 Materials and Methods

2.1 Materials

Ripe red-fleshed pitayas were obtained from a local farm in Sepang (Selangor, Malaysia). Two commercial pectinase enzymes preparations were applied: Pectinex Ultra SP-L (from *Aspergillus niger*) and Pectinex CLEAR (from *Aspergillus aculeatus* and *Aspergillus niger*) both obtained from Novo Nordisk Ferment Ltd., Dittigen, Switzerland were used for enzymatic treatment of red pitaya juices and stored at $4^{o}C$. Pectinex Ultra SP-L and Pectinex CLEAR are commercial enzymes used to produce clear and stable fruit juices. The commercial enzyme contains mainly polygalacturonase, pectinesterase and pectin transeliminase. The activity of Pectinex Ultra SP-L and Pectinex CLEAR enzymes are 26 000 PG per ml (polygalacturonase activity per ml). It appears that such enzymatic preparations rupture the juice retaining particles of the pulp, such effect being apparently due in part to action of polygalacturonase in splitting the peptic chain and the action of pectinesterase in hydrolyzing the methyl ester groups of the pectin molecule (Wieland, 1972). The optimum enzyme reaction conditions are at pH 3.5 – 6.0 and temperature range below 50°C (Kashyap et al., 2001). These are ideal conditions for fruit processing.

2.2 Production of Clarified Red Pitaya Juices at Laboratory Scale

Pitayas were peeled and extracted using a juice extractor (Philips Juicer Model: HR 1858155; Royal Philips Electronics, Eindhoven, Netherlands) to remove the seeds and coarse cloud particles. Two commercial enzyme preparations were added separately to the pitaya pulp. The enzyme concentration, time and temperature of enzymatic treatment for Pectinex CLEAR enzyme have been obtained from trials done by Siti Mazlina *et al*. (2007). For Pectinex Ultra SP-L, the processing condition data for enzymatic clarification of red pitaya juice was also obtained from trials (data not published). For each sample, Pectinex Ultra SP-L and Pectinex CLEAR were added separately to the pulp and incubated (Wise Cube Shaking Incubator Model: WIS–S10; DAIHAN Scientific, Co., Ltd., Seoul, Korea) with continuous shaking (250 rpm). Table 2.1 shows the optimum conditions for enzymatic clarification of red pitaya pulp using both enzymes respectively.

Table 2.1 Optimum processing conditions for enzymatic clarification of red pitaya pulp

Types of enzymes	**Enzyme concentration (%)**	**Incubation temperature (°C)**	**Incubation time (min)**
Pectinex Ultra SP-L	0.10	40	45
Pectinex CLEAR	0.09	46	82

The treated pulp was pasteurized at 90°C for 5 min in a hot waterbath (Laboratory Waterbath Model: LMWB-6PC; PLT Scientific Sdn. Bhd., Selangor, Malaysia) and then cooled at 2°C using circulating waterbath (Model: 71; PolyScience, Illinois, USA). These steps were performed to the samples to inactivate the enzyme action before filtration. The treated red pitaya juices were centrifuged (Refrigerated Centrifuge 5800; Kubota Corporation, Fujioka, Japan) at 3000*g* for 10 minutes, and the supernatant was collected. The juice was filtered through a filter paper (Whatman No.1, Whatman International Ltd., Kent, England) using oil-less vacuum pump (Model: Rocker 300; Rocker Scientific Co., Ltd., Taipei County, Taiwan). The separation of the pulp from the juice by filtering the liquid pulp on a rotary vacuum filter avoids the costly pressing step and the juice obtained is clear (Baumann, 1971). Figure 2.1 illustrates the step for the production of clarified red pitaya juices at laboratory scales. A control sample was produced without enzymatic treatment step but was pasteurized at 90°C for 5 min followed by cooling at 2°C.

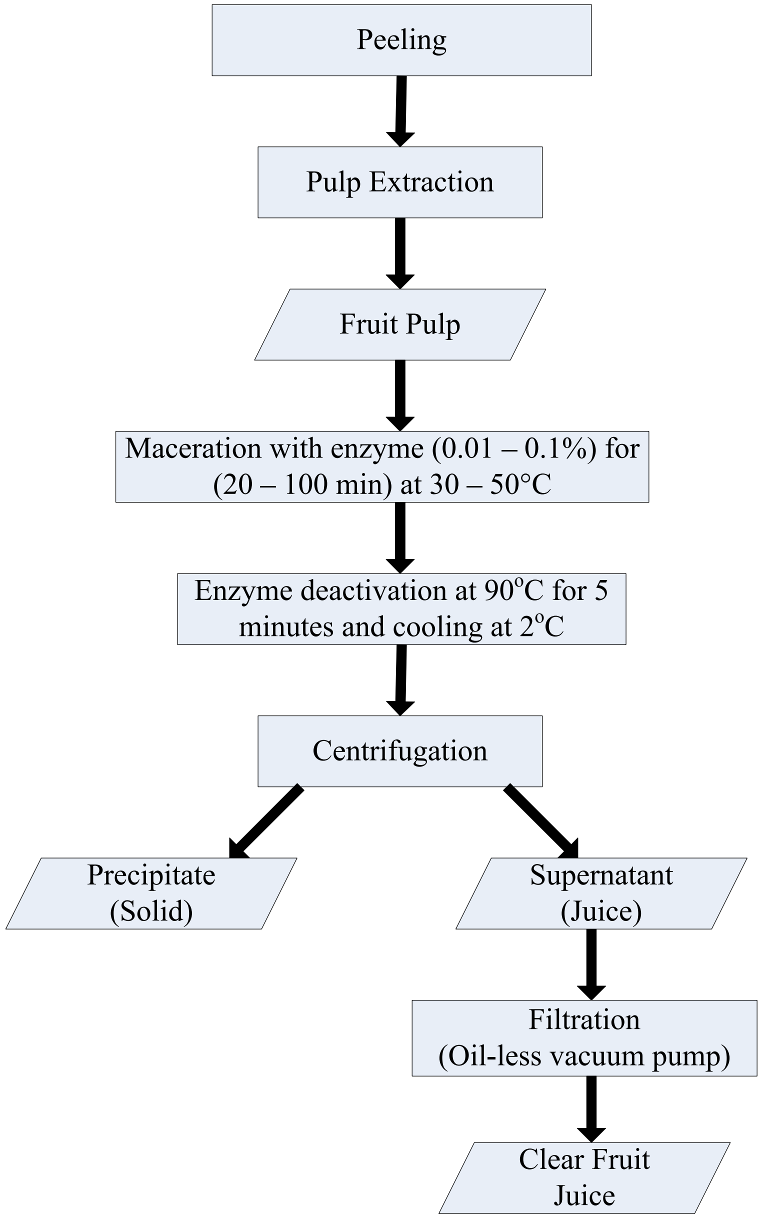

Figure 2.1: Steps for extraction and subsequent clarification by enzymatic treatment of red pitaya juice

2.3 Determination of some physicochemical properties of red pitaya juices

Total soluble solids (TSS) were determined using Atago Hand Refractometer (Model: Master-α; ATAGO Co., Ltd., Tokyo, Japan) and pH values were measured using Microprocessor-based Bench pH (Model: pH 211; Hanna Instruments (M) Sdn. Bhd., Selangor, Malaysia). Juice yield, viscosity, clarity and colour measurement were obtained according to the methods reported by Nur 'Aliaa *et. al*. (2008).

3.0 Results and Discussion

Treatment of fruit pulp with appropriate enzyme preparations is a general practice in juice processing (Ramadan and Moersel, 2007). Enzymes are used in the juice industry to aid in the separation of juice from the fruit cells and to clarify the juice by the removal of pectin and naturally occurring starches that contribute to undesired viscosity, poor filtration, and a cloudy appearance (Enzyme Technical Association, 2002). Since juices extracted from ripe fruit contain substantial amount of pectins and starches, thus they lead to a cloudy appearance to the juices, making the juices less appealing by consumers due to the appearance and mouth-feel of the juices. The cloudiness that pectin causes is difficult to remove unless by enzymatic depectinization.

In this study, ripe red pitayas were selected from a local farm in Malaysia with considerably higher quality which is reflected by the fruit larger size, well-developed colour and very juicy pulp. Ripe fruits lead to juices freshness and quality which is crucial to the quality of the final product. Two pectinase enzymes application which were Pectinex Ultra SP-L and Pectinex CLEAR were examined to demonstrate the effects of pectinases on the quality of fruit juices.

3.1 Effect of enzyme application on some physicochemical properties of red pitaya juices

Table 3.1 presents some physicochemical properties of red pitaya juices. The yield of juice produced without enzymatic treatment is 78.53%. A dramatic increase can be observed in the recovery of juice produced by enzymatic treatments which were 83.2% for treatment using Pectinex Ultra SP-L and 92.38% using Pectinex CLEAR. Pectinases break down the fruit pulp resulting in increased yields of juices. It was observed that as the heating and enzymation continues, the pulp becomes markedly more fluid and the freeing of the pulp fragments is readily apparent (Wieland, 1972). This elevation might be of importance for industrial and commercial applications under large-scale juice processing (Ramadan and Moersel, 2007).

Table 3.1 Some physicochemical properties of red pitaya juices

Treatment	Yield (%v/v)	Viscosity (cps)	Absorbance value (abs)	Colour (L*)	Total acidity (meq/kg)	pH	TSS[a] (%Brix)
Fresh fruit pulp	-	7.23± 0.23	1.957± 0.07	2.93± 0.006	0.58± 0.03	4.8± 0.01	12.0± 0.00
Control juice (pasteurized)	78.53± 1.93	3.9± 0.00	0.179± 0.009	12.46± 0.02	0.70± 0.006	4.55± 0.01	12.0± 0.00
Juice treated with Pectinex Ultra SP-L	83.2± 1.04	3.0± 0.00	0.152± 0.03	22.4± 0.006	0.77± 0.01	4.41± 0.01	12.0± 0.00
Juice treated with Pectinex CLEAR	92.38± 2.26	3.0± 0.00	0.062± 0.000	24.76± 0.02	0.75± 0.06	4.45± 0.06	12.1± 0.06

Values given are the mean of three replicates ± standard deviation
[a]Total soluble solids

Application of enzymes reduces juices viscosity and improves the juices clarity. In the case of red pitaya juices, such enzymatic treatment is effective to reduce the relative viscosity of the pulp to less than half of its initial value. The absorbance values markedly decreased for juices treated with enzymes. This characteristic is more preferred by consumers when compared to the cloudy appearance showed by freshly extracted fruit pulps. L* values also markedly increased for enzyme-treated juices when compared to untreated samples. The total titratable acidity also increased in the juices treated with enzymes. This increase might be due to the formation of galacturonic acid by enzymatic breakdown of pectin (Acar, 1999). The total titratable acidity in red pitaya is in the range of 0.58-0.77%. Total acidity for other fruit juices such as goldenberry is 1.0% (Ramadan and Moersel, 2007), pear 0.3%, orange 0.8%, apple 0.9%, strawberry 0.9%, pineapple 1.1%, plum 2.2% and apricot 2.4% (Belitz, 1999).

Since the total acid content in the juice was slightly high, thus the pH of the juices was low, with values within 4.41-4.8. The decrease of pH values may be due to the release of carboxyl groups and galacturonic acid from pectin (Ramadan and Moersel, 2007). For red pitaya juices, there was no significant increase in TSS levels even after enzymatic treatment and the values remained at 12.0%Brix.

4.0 Conclusions

The differences between untreated juices when compared to enzyme-treated juices show that the mechanism of pectin degradation depends upon the types of enzymes used. Variety of enzymes available in fruit and vegetable processing contribute to some degree to the conversions, degradations and breakdown reactions desired (Wieland, 1972). From the study done, it can be seen that the effects of Pectinex Ultra SP-L and Pectinex CLEAR were different on red pitaya juices because pectinases act in different ways on the pectins. Pectins are situated in the primary cell walls and in the middle lamella. The mechanism of pectin degradation depends upon the pectic composition of the substrate. This study demonstrated the effect of pectinases application on several physicochemical properties of red pitaya juices. Moreover, this study attempted to obtain some data from the processing of fruit juices especially pitaya juices and to provide valuable and interesting information that would be useful for fruit processing industries. Utilization of pectinase as a processing aid in the production of pitaya juices proved to be a crucial step in order to enhance the efficiency of the whole system.

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