IMPACT OF COMMERCIAL PECTOLYTIC ENZYMES ON SELECTED PROPERTIES OF WHITE DRAGON FRUIT JUICE

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ABSTRACT

The aim of this work was to observe 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 white dragon fruit (Hylocereus undatus) juice. The differences of these properties in fresh fruit pulp and juices produced without enzymatic treatment were also observed. The dragon fruit juice produced after enzymation and pasteurisation has not shown significant changes in most of its major chemical parameters such as moisture, ash, fat, carbohydrate and calorie. However, enzymation using Pectinex CLEAR leads to the juice with higher yield of protein. The protein content after the treatment increased to 0.33% w/w from 0.17% w/w of the fresh juice. Phenolics amounts were slightly higher up to 15% in the enzyme samples which suggest that dragon fruit beverage is rich in antioxidant capacity than in the unprocessed fruit.

Keywords: *Chemical Composition, Commercial Enzyme, Dragon Fruit, Total Polyphenols, Vitamin C*

1.0 INTRODUCTION

Dragon fruit is also called pitahaya, strawberry pear, pitaya (in South East Asia), night-blooming cereus, and paniniokapunahou or papipi pua (in Hawaii). Dragon fruit is known to be highly nutritious. It has been called the most beautiful in the cactus family [1]. The dragon fruit flesh can be white, red or magenta depending on variety. It is rich in potassium, ferum, protein, fiber, vitamin C and minerals which are good for human health. This exotic fruit is now being converted into ready-to-drink beverages, jam and wine. Fruits have always played an important role in human nutrition [2]. The demand for fruit beverages is largely based on their nutritive value, flavour, aroma and colour.

Juice with low viscosity, high clarity and yet high in nutrition is more desirable by consumers. Enzymes can play a key role in improving juice clarity, stability as well as reducing the viscosity. The presence of polysaccharides such as pectin and starch has caused cloudiness in the juice. An effective way to reduce the pectin in the fruit juice is by enzymatic treatment using pectinase because of its ability to hydrolyse pectin and causes pectin-protein complexes to flocculate [3-6]. Pectinases are obtained from plants and fungi. However, the major commercial source of pectinases is *Aspergillus* sp.. Pectinases are used industrially to weaken plant cell walls and enhance extraction of fruit juice. They help to improve juice recovery, decrease viscosity and prevent gelation in the extracts. Easier release of the components contained in cells is due to the degradation of plant cell walls by exogenous enzymatic treatment [7].

In juice processing, enzyme is an essential tool in terms of quality improvement and cost saving [8]. Currently, the production of fruit and vegetable juices is unthinkable without the use of enzymes [9]. The key of producing clear and stable fruit juice is a complete enzymatic breakdown of pectin. Generally, fruit juice extraction involves maceration of the pulp followed by centrifugation and filtration, to separate the juice from the solids. The aim of mash treatment is to increase juice yield and to facilitate the extraction of the soluble substances from the cells [9]. Filtration is employed to remove insoluble plant material from the enzyme-treated juice.

For a few years ago, studies have been done to study on the color pigments of red pitaya [10-13]. Research on the cultivation of pitaya has also become appreciated [1]. However, not many studies have been done on white pitaya or dragon fruit. In this work, the chemical composition, vitamin C and total polyphenols contents of fresh and treated white dragon fruit juices are reported for the first time. The data found in this study are expected to be useful for the optimisation of dragon fruit juice production.

Table 1: Optimum processing condition for enzymatic clarification of white dragon fruit pulp

Types of enzymes	Enzyme concentration (%)	Incubation temperature (°C)	Incubation time (min)
Pectinex Ultra SP-L	0.06	49	40
Pectinex CLEAR	0.10	40	81

2.0 MATERIALS AND METHODS

2.1 Samples

Ripe white-fleshed dragon fruits were obtained from a local farm in Sepang (Selangor, Malaysia). Two commercial pectinase enzymes preparations were used which are Pectinex Ultra SP-L (from *Aspergillus niger*) and Pectinex CLEAR (from *Aspergillus aculeatus* and *Aspergillus niger*) that both obtained from Novo Nordisk Ferment Ltd., Dittigen, Switzerland were used for enzymatic treatment of white dragon fruit juice and stored at 4°C. Pectinex Ultra SP-L and Pectinex CLEAR are commercial enzymes used to clarify the fruit juice. Polygalacturonase, pectinesterase and pectin transeliminase are mainly contains in the commercial enzyme. The activity of Pectinex Ultra SP-L and Pectinex CLEAR enzymes are 26 000 PG per ml (polygalacturonase activity per ml). Such enzymatic preparations rupture the juice retaining particles of the pulp; the effect is due to action of polygalacturonase in splitting the peptic chain and the action of pectinesterase in hydrolysing the methyl ester groups of the pectin molecule [14, 15]. The ideal conditions for fruit processing are when the optimum enzyme reaction conditions are at pH 3.5 – 6.0 and temperature range below 50°C [16].

2.2 Production of Clarified White Dragon Fruit Juice at Laboratory Scale

Dragon fruits were peeled and a juice extractor (Philips Juicer Model: HR 1858155; Royal Philips Electronics, Eindhoven, The Netherlands) were used to extract and remove the seeds and coarse cloud particles. The dragon fruit pulp was added with two commercial enzyme preparations separately for each enzyme. The parameter for the clarification were enzyme concentration, time and temperature of enzymatic treatment for Pectinex Ultra SP-L enzyme and the parameter have been obtained from trials done by Nur 'Aliaa *et al*. [17]. For Pectinex CLEAR, the processing condition data for enzymatic clarification of white dragon fruit juice was also obtained from trials (data not published). Pectinex Ultra SP-L and Pectinex CLEAR were added separately to the pulp for each sample and incubated (Wise Cube Shaking Incubator Model: WIS–S10; DAIHAN Scientific, Co., Ltd., Seoul, Korea) with continuous shaking (250 rpm). The optimum conditions for enzymatic clarification of white dragon fruit pulp using both enzymes shown in Table 1.

Pasteurisation at 90°C for 5 min was done to the treated pulp in a hot waterbath (Laboratory Waterbath Model: LMWB-6PC; PLT Scientific Sdn. Bhd., Selangor, Malaysia) and then circulating waterbath (Model: 71; PolyScience, Illinois, USA) was used to cool it at 2°C. These steps were performed before filtration to inactivate the enzyme action. The treated white dragon fruit juice was centrifuged (Refrigerated Centrifuge 5800; Kubota Corporation, Fujioka, Japan) for 10 minutes at 3000g, and the supernatant was collected. A filter paper (Whatman No.1, Whatman International Ltd., Kent, England)

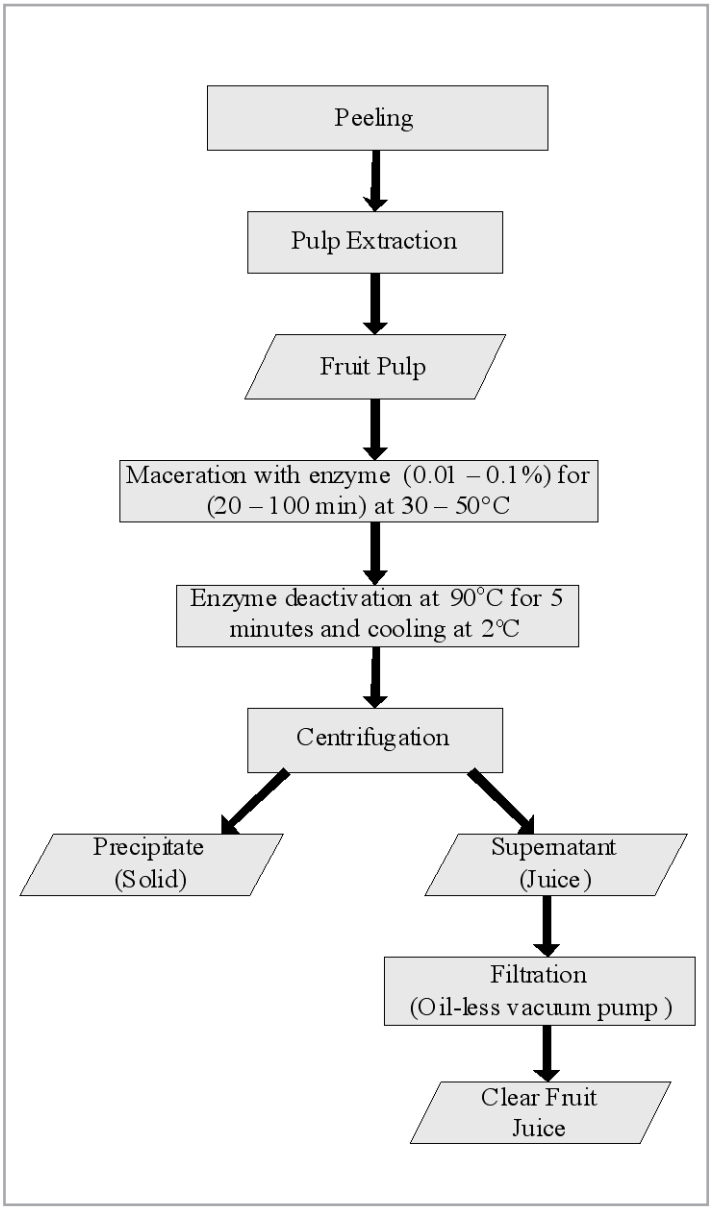

Figure 1: Steps for extraction and subsequent clarification by enzymatic treatment of white dragon fruit juice

Table 2: Chemical composition of white dragon fruit juice

Treatment	Protein (%w/w)	Fat (%w/w)	Moisture (%w/w)	Ash (%w/w)	Carbohydrate[a] (%w/w)	Calorie[b] (Kcal/100g)
Fresh fruit pulp (untreated pulp)	0.17±0.06	0.00	87.73±0.06	0.40±0.00	11.70±0.00	47.67±0.58
Control sample (pasteurized only)	0.23±0.06	0.00	87.33±0.06	0.47±0.06	11.97±0.12	48.67±0.58
Juice treated with Pectinex Ultra SP-L	0.13±0.06	0.00	87.40±0.00	0.40±0.00	12.07±0.06	49.00±0.00
Juice treated with Pectinex CLEAR	0.33±0.06	0.00	87.43±0.06	0.40±0.00	11.83±0.06	48.67±0.58

Values given are the mean of three replicates ± standard deviation

[a] *% Carbohydrate = 100 – (% Protein + % Fat + % Moisture + % Ash)*

[b] *Calorie (Kcal) = 4 (% Carbohydrate) + 4 (% Protein) + 9 (% Fat)*

was used to filter the juice, which has the pore size of 11 μm using oil-less vacuum pump (Model: Rocker 300; Rocker Scientific Co., Ltd., Taipei County, Taiwan). In order to avoids the costly pressing steps, the separation of the pulp from the juice is filter on a rotary vacuum filter and the juice obtained is clear [9]. Figure 1 shows the step for the production of clarified white dragon fruit juice at laboratory scales. A control sample without enzymatic treatment step was produced and pasteurised at 90°C for 5 min followed by cooling at 2°C.

2.3 Determination of Chemical Parameters, Vitamin C and Total Polyphenols Contents of White Dragon Fruit Juice

Protein, fat, carbohydrate, moisture, ash and calorie were determined according to AOAC [18]. Ascorbic acid was determined volumetrically with 2-6 dichlorophenol-indophenol reagent [18]. Total polyphenol was measured by modified Folin-Ciocalteu method using gallic acid as a standard. 5g of juice was extracted with 20ml mixture of methanol-water and with 30ml mixture of acetone-water separately under stirring for 1 hour. Then the supernatant were combined in volumetric flask and made up to 50ml with distilled water and is called extract. 0.5ml of extract is added with 0.5ml Folin-Ciocalteu reagent and allowed to react for 3 min under continuous stirring. After that, 10ml of sodium bicarbonate (75 g L^{-1}) was added and the mixture was diluted to 25ml with distilled water. The solution was kept at room temperature for 2 hour in the dark; the absorbance was then measured at 750nm using UV-Vis Spectrophotometer (Model UV-260; Shimadzu Corporation, Tokyo, Japan). The mean value of total polyphenols was obtained from triplicate experiments.

3.0 RESULTS AND DISCUSSION

A general practice in juice processing is the treatment of fruit pulp with appropriate enzyme preparations [8]. The cloudy appearance of the ripe fruit juice extracted was due to the existing of substantial amount of pectins and starches which can be removed by enzymatic depectinization. Depectinization affected the composition of juice in relation to the type of juice, the type of enzyme and the time considered [19].

In this study, ripe white dragon fruits 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 juice freshness and quality that is crucial to the quality of the final product. Two pectinase enzymes application (Pectinex Ultra SP-L and Pectinex CLEAR) were used to demonstrate the effects of pectinases on the quality of fruit juice.

3.1 Influence of Enzyme Application on Chemical Composition of White Dragon Fruit Juice

The impact of different enzyme preparations on chemical parameters of white dragon fruit juice is presented in Table 2. By enzymatic treatment, not only the yield of juice increased, but also the macro- and micro-components contents of the product [20]. The white dragon fruit juice produced after enzymation and pasteurisation has not shown marked changes in the most of its major chemical parameters such as moisture, ash, fat, carbohydrate and calorie. From the study done by Ramadan and Moersel [8] on goldenberry juice, they found out that the effect of enzymation on these macro- and micro-constituents seems to be insignificant. It can be seen that fat levels remained zero in fresh pulp and even in pasteurised and enzyme-treated white dragon fruit juices. An

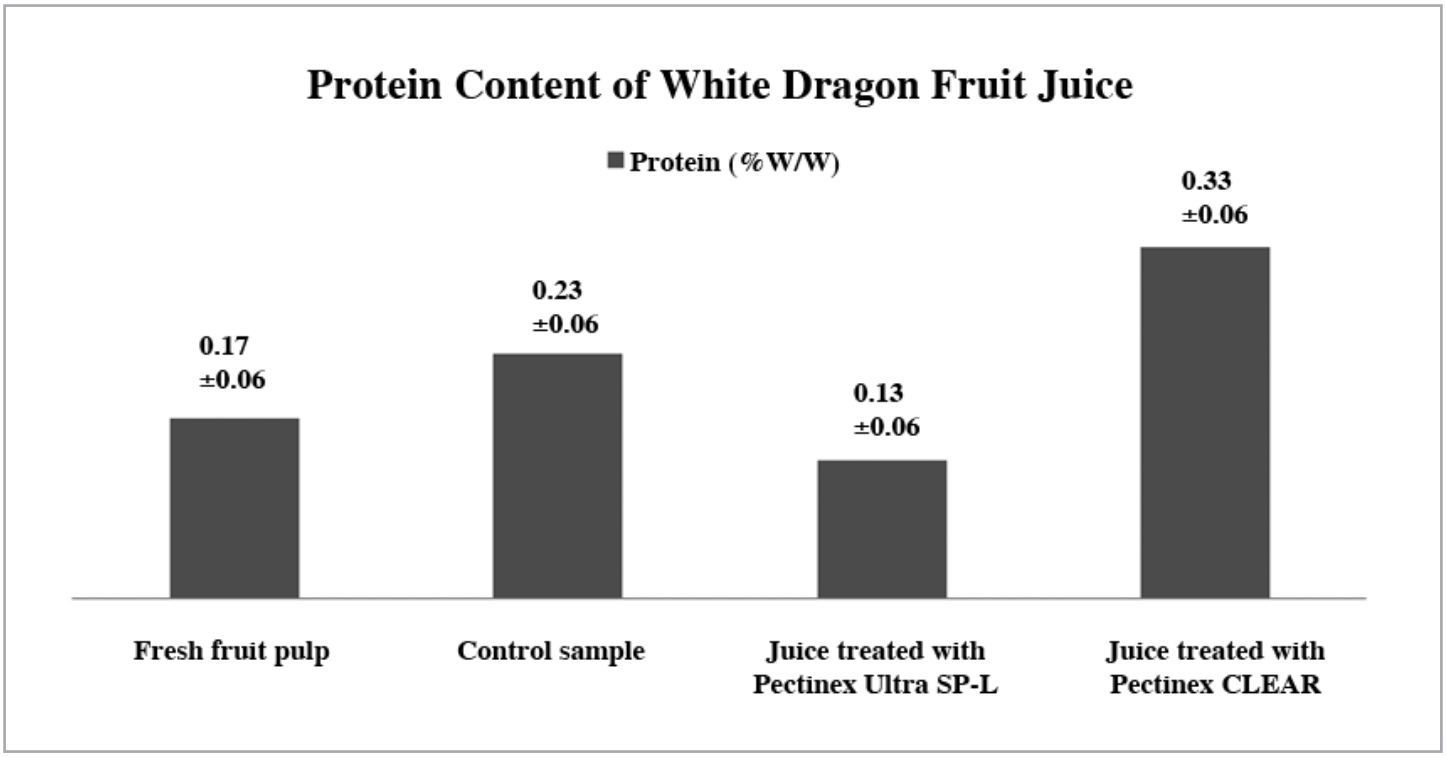

Figure 2: Protein content of white dragon fruit juice

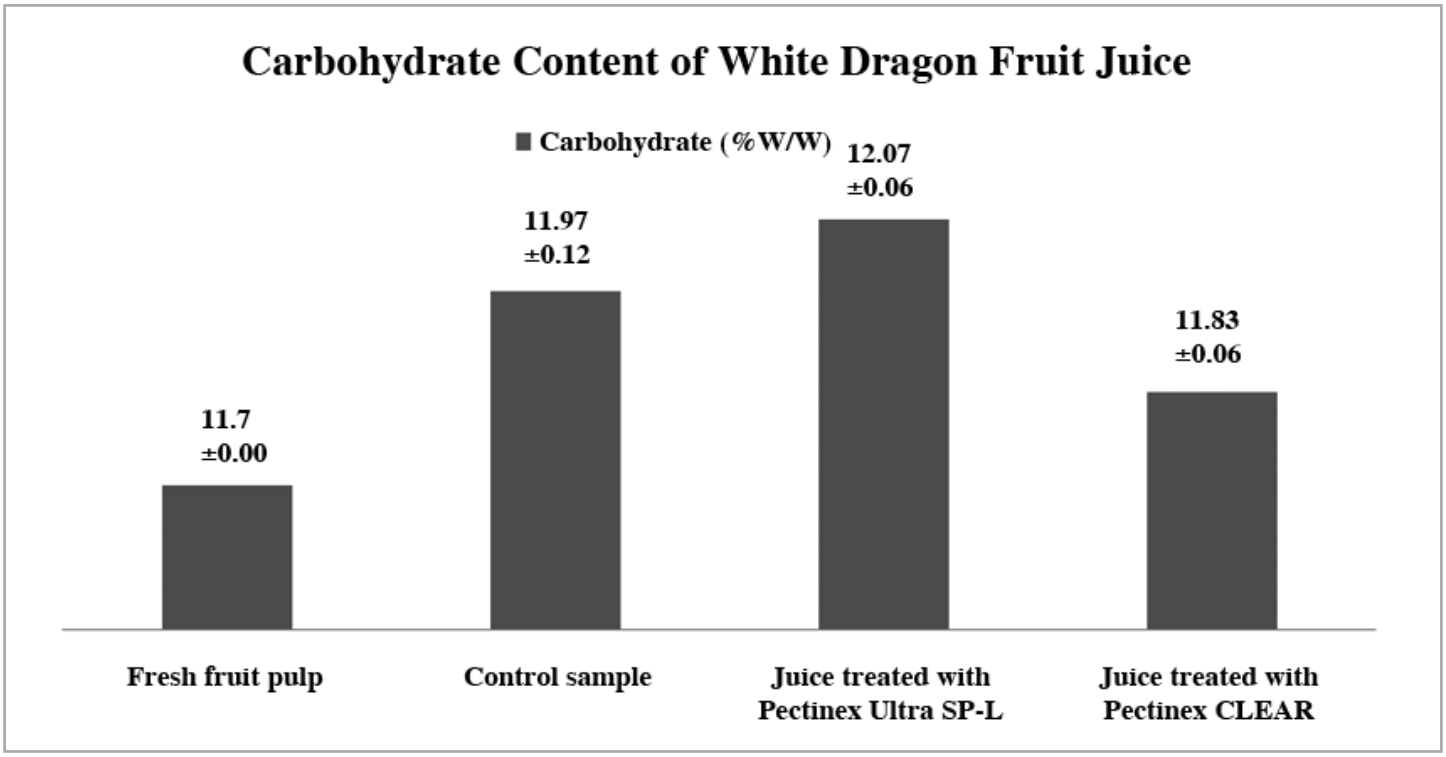

Figure 3: Carbohydrate content of white dragon fruit juice

interesting result is that the juice treated with Pectinex CLEAR enzyme preparation resulted in markedly higher yield of protein as shown by the chart in Figure 2. The same trend was also observed for the protein content in red dragon fruit juice [21].

Carbohydrate contents in enzyme-treated white dragon fruit juice were found to be higher than in the untreated pulp and control samples. Treatment with Pectinex Ultra SP-L increased the level up to 12.07% as shown in Figure 3. The effect of Pectinex Ultra SP-L was different than Pectinex CLEAR because pectinases act in different ways on the pectins. Carbohydrate content in other fruits was reported to be as follows: strawberry and watermelon (3%); apricot, guava, lemon, orange, papaya, peach and plum (6%); apple, grape, mango and pineapple (15%); and banana and puree (20+%) [22]. Since the range of carbohydrate content in

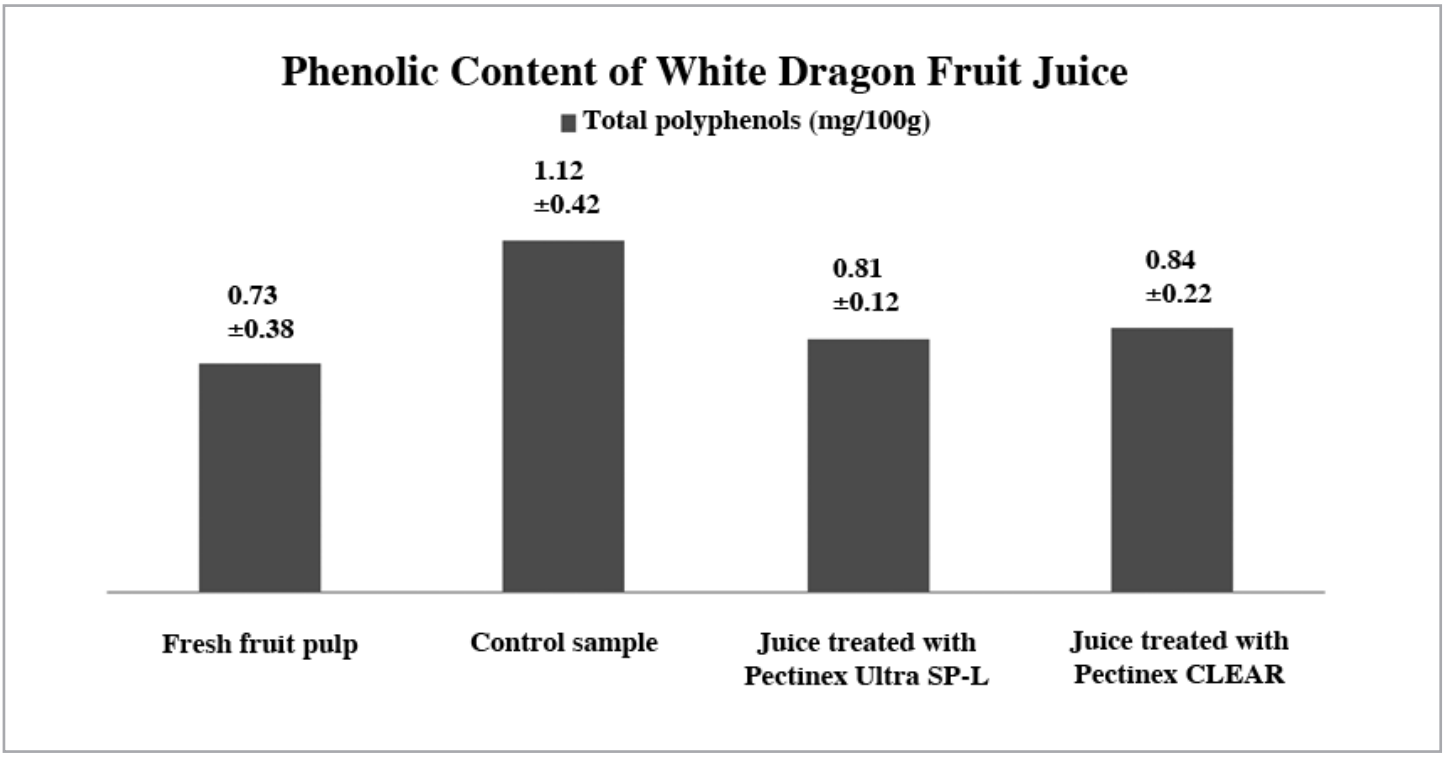

Figure 4: Phenolic content of white dragon fruit juice

white dragon fruit juice was 11.70% - 12.07%, it can be concluded that carbohydrate in dragon fruit juice was higher than that of other tropical fruits such as guava and papaya, and lower than that of mango, pineapple and banana. The calories in fresh and processed dragon fruit juices are less than 100 kcal and can be considered as low. Thus, dragon fruit juice can be classified as a healthy beverage.

3.2 Effect of Enzymatic Treatment on Vitamin C and Total Polyphenols Contents of White Dragon Fruit Juice

Table 3 shows the data obtained for vitamin C and total polyphenols content in white dragon fruit juice. The untreated pulp had slightly higher vitamin C content than enzyme-treated and control samples. The slight reduction was due to the effects of the heat applied during the treatment. Miller and Rice-Evans [23] suggested that the phenolic antioxidants of fruit juices protect the vitamin C content from oxidative degradation. Phenolics are important because of their contribution to the sensory quality of fruits (colour, astringency, bitterness and flavour), which may be affected during the technological processes used for obtaining the juices and other transformation products [8]. Fair amounts of polyphenols were found in white dragon fruit juices which were 0.73 to 1.12 mg/100g juice as gallic acid equivalents (GAE). Higher amounts of phenolics were found in processed pitaya juice as shown by the chart in Figure 4 which suggests that dragon fruit beverages are rich in antioxidants capacity than in the unprocessed mash. The presence of phenolics components play a significant contributory role as antioxidant; which is important in the maintenance of health and protection from coronary heart disease and certain cancers [23].

4.0 CONCLUSION

Recently, dragon fruits have attracted great interest from the fruit growers to the fruit processing industries due to their nutritional and health benefits. Processing of dragon fruit juice using enzyme is important to produce high quality fruit juice that meets the consumer interest. Application of enzyme proved to increase the juice recovery as well as the juice physicochemical characteristics. It also increased the nutritional composition in the juice such as protein, carbohydrate and total polyphenols. The protein content after enzymatic treatment using Pectinex CLEAR increased to 0.33% w/w from 0.17% w/w of the raw juice. Carbohydrate content also increased up to 3% from its initial value after treatment using Pectinex Ultra SP-L enzyme. Total polyphenols contents were slightly increased after treatment using both enzymes.

Degradation mechanism of pectin depends on the types of enzymes used. This explains the differences in chemical compositions, physicochemical parameters, vitamin C and total polyphenols between enzyme-treated juice and untreated juice. From the results, it was found that the effects of Pectinex Ultra SP-L and Pectinex CLEAR were different on white pitaya juice due to the different ways pectinases act on the pectins. This study showed the effect of pectinases application on the chemical composition, vitamin C and total polyphenols of white pitaya juice. The use of pectinase as a processing aid in the production of pitaya juice proved to be an important step in order to enhance the efficiency of the whole system [24].

Table 3: Vitamin C and total polyphenols contents of white dragon fruit juice

Treatment	Vitamin C (mg/100g)	Total Polyphenols (mg/100g)
Fresh fruit pulp (untreated pulp)	2.05±0.03	0.73±0.38
Control sample (pasteurized only)	2.09±0.01	1.12±0.42
Juice treated with Pectinex Ultra SP-L	1.56±0.006	0.81±0.12
Juice treated with Pectinex CLEAR	1.57±0.006	0.84±0.22

Values given are the mean of three replicates ± standard deviation

5.0 ACKNOWLEDGEMENTS

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PROFILE

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