

Effect of enzyme treatments on physical properties of durian aril and rind slurries

(Kesan pengolahan enzim terhadap sifat fizikal sluri pulpa dan kulit pangsa durian)

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Key words: pectinase, cellulase, amylase, durian rind, durian aril

Abstract

Durian (*Durio zibethinus*) is a tropical fruit native to Southeast Asia. The use of amylase, cellulase and pectinase to modify physical properties of durian aril (DA) and durian rind (DR) slurries was studied. Treatments of DR slurries with pectinase, combination of pectinase and cellulase, and combination of pectinase, cellulase and amylase produced slurries with lower pH, higher solubility and lower viscosity than DR slurries incubated without enzyme. The enzyme treatment, however, showed no such effect on the physical properties of DA slurries. Analysis of sugars and galacturonic acid produced in enzyme-treated DR slurries provided some indication of synergistic effects of pectinase and cellulase in disrupting the cell wall of DR. Overall results conclude that pectinase was the most effective in hydrolyzing polysaccharides in DR compared to cellulase and amylase. This preliminary result indicates the possibilities of modifying physical properties of DR slurries by using enzyme treatments.

Introduction

Amylase, pectinase and cellulase are the common enzymes used for food purposes. These enzymes account for approximately 20% of the world enzyme market (Mantyla et al. 1998). Pectinase is widely used in juice clarification as fruit juices contain colloids that are mainly polysaccharides such as pectin, cellulose, hemicellulose, lignin and starch. Several studies reported the use of pectinase for tangerine juice clarification (Chamchong and Noomhorm 1991), pineapple juice clarification (Carneiro et al. 2002) and apple juice clarification (Girard and Fukumoto 1999) and recently carambola juice clarification (Abdullah et al. 2007) and banana juice clarification (Lee et al. 2006). Cellulase on the other hand is widely used in beer and wine biotechnology.

Although beer brewing and wine making are old technologies and have an ancient history, cellulase enzyme technology plays a central role during the fermentation within the brewery (Galante et al. 1998). Usually a combination of cellulase and pectinase accelerates the rate of hydrolysis for achieving complete liquefaction of slurried products. Cellulase randomly splits cellulose chains into glucose whereas pectinase may degrade pectin into galacturonic acid. Amylase is one of the most important hydrolytic enzymes for starch-based industries. As microbial amylases have been replacing chemical hydrolysis in the starch processing industry, they could also be used in bio-processing industries that utilize agricultural waste.

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Durian (*Durio zibethinus*) is a tropical fruit native to Southeast Asia. It is one of the most highly valued and desired fruits among Southeast Asians and known as “King of the fruits” due to its distinct flavour and unique taste. The fruit is ovoid to nearly round shape with an average size weighing 2.0–4.5 kg depending on the varieties (Hokputsa et al. 2004).

The aril, the only edible portion of durian, constitutes about 30% of the fruit, while the seeds and rinds are treated as food waste (Amiza et al. 2007). Durian aril comprised mainly of starch and sugars such as sucrose, glucose, fructose and maltose, and organic acids such as malic, citric, tartaric and succinic acids (Voon et al. 2006). Therefore, the effect of the enzyme treatment on durian aril is anticipated to be minimum. Nevertheless, information generated from this comparison may be beneficial for product development that uses durian arils.

The durian rind has some potential applications in the pharmaceutical field and food processing industries particularly as a gelling agent (Pongsamart and Panmaung 1998), even though this has not been fully exploited. Polysaccharides from the durian rinds are composed of pectin as the main component followed by hemicellulose and starch as a contaminant (Hokputsa et al. 2004; Khurnpoon et al. 2007). It is possible to use pectinase, cellulase and amylase to hydrolyze pectin, cellulose and starch of the rind. The end products of the hydrolysis may include galacturonic acid, glucose, fructose and other sugars. Galacturonic acid and sugars from pectin hydrolysis can be fermented by bacteria such as *Escherichia coli* K011 to produce ethanol and acetic acid (Grohman et al. 1994).

The main purpose of this research work was to study the effect of enzyme treatments and their combinations on physical properties of durian aril (DA) and durian rind (DR) slurries. Development of alternative, higher valued products utilizing

durian rind waste would benefit durian orchards and processors.

Materials and methods

Durian fruits cultivar D24 used in this study were obtained from a fruit orchard in Relau, Pulau Pinang, in mid July 2007. Ripened fruits that dropped naturally were collected and transported within 3 h on the same morning (at 30 ± 2 °C). Fruits were selected for uniformity of size (with average weight of 2.0 ± 0.5 kg per fruit), colour and free from defects. A total of 25 fruits were selected for the study. The fruits were divided into three replicates of 8–9 fruits per group. Unless otherwise stated, all data reported in this study are the mean values of the three replicates.

The α -amylase from *Bacillus* species, cellulase from *Aspergillus niger*, pectinase from *A. niger*, D-(+)- glucose (99.5%) and D-(+)- fructose (99.5%) were supplied by Sigma Aldrich, USA. The D-(+)- galacturonic acid ($\geq 97.0\%$), Amberlite IRC – 50 and ethylenediaminetetraacetic acid calcium disodium salt hydrate were supplied by Fluka, United States. Acetic acid – glacial and sodium acetate anhydrous were obtained from System Chemar. Ion exchanger III was supplied from Merck, Germany, cyclohexamide (96%) was supplied from Acros Organics, and chloramphenicol was obtained from BDH Limited Poole, England.

Sample preparation

Durians were cut open with a sharp knife. Durian aril (DA) was separated from the durian rind (DR) and seed manually. The rinds were cut as to eliminate the hard and thorny parts. Later, the rinds were cut into small pieces (about 0.5 cm thick) to facilitate the drying and grinding process. The DA and DR were placed on separate aluminium foils and dried in a hot air oven (AFOS Oven, Hull England) at 60 °C to 70 °C for 48 h and 24 h respectively. The dried DA and DR were ground into powder by using a grinder (Micro Universal Bench

Top Grinder, Retsch ZM 100, Germany) and later packed separately into a sealable plastic bag and stored in desiccators. The DA and DR powder were used for enzymatic treatment then followed by physical analysis and HPLC analysis.

Preparation of slurries

Slurries were prepared according to the methods described by Grohmann et al. (1994). Slurries were prepared by mixing 3 g sample powder with 250 ml of 50 mM sodium acetate buffer at pH 4.8, which was within the optimal pH range (pH = 4.5–5.0) for the enzymes to produce 100 ml of powder-water slurry. Chloramphenicol and cyclohexamide were added at the level of 30 µg/ml each to protect the hydrolysates from microbial contamination (Grohmann and Baldwin 1992).

Enzymatic hydrolysis and sample designation

The DA and DR slurries were hydrolysed by α -amylase, cellulase, or pectinase, combination of α -amylase, cellulase and pectinase (P+C+A) and combination of cellulase and pectinase (P+C). Both slurries were hydrolysed according to the method described by Wilkins et al. (2007). Each of these enzymes was added to the slurries at 25 U activities and hydrolyses were carried out for 24 h at 45 °C in glass bottles rotated at 10–12 rpm in an incubator (Wilkins et al. 2007) (Orbital Shaker Incubator, LM – 570R). After hydrolysis was completed, hydrolysates were placed in an oven (Memmert Oven Model UL40) at 105 °C for 15 min to inactivate enzymes, and then stored at 3 °C. Hydrolysates were homogenized with a high shear mixer (Ultra – Turrax Homogenizer C/W ACCS) to produce uniform slurries for analysis. The DA and DR slurries incubated without enzyme were used as control samples. DA and DR hydrolysates were designated as DAH and DRH respectively.

Physical analysis

Viscosity of the slurries was determined by using the methods described by Al-Hooti et al. (2002). The viscosity was measured at 27 °C \pm 1 °C using Brookfield Viscometer (Model DV-E, Brookfield Eng. Lab., USA) equipped with No. 3 spindle. Shear rate of 100 rpm was used. Viscosity in centipoises (cps) was reported as the average of three replicates. The pH values of the slurries were measured with a pH meter (Mettler Toledo Delta 320). Colour of slurries was measured by using a colorimeter (Minolta Spectrophotometer CM 3500d) according to the method adapted from Al-Hooti et al. (2002). Colour was recorded using CIE – L* a* b* uniform colour space, where L* indicates lightness, a* indicates hue on green (–) to red (+) axis, and b* indicates hue on a blue (–) to yellow (+) axis.

Solubility of slurries before and after enzymatic treatment was determined by using the method described by Anema et al. (2006). Slurries were transferred into centrifuge tube and centrifuged at 3,500 rpm for 10 min by using a Bench Top Centrifuge (Kubota 5100, Fujioka, Japan). The supernatant was placed in a pre-weighed moisture dish and weighed. The moisture dish was dried overnight (about 18 h) in an oven (Memmert Oven Model UL40) at 105 °C until it reached a constant weight. The moisture dish was cooled in desiccators (to avoid any condensation affecting the results) and then reweighed. The amount of soluble material, σ , in the sample was calculated as:

$$\sigma = \frac{\text{Weight of dry material after drying}}{\text{Weight of sample powder}} \times 100\% \quad (1)$$

High performance liquid chromatography–refractive index (HPLC-RI) conditions and quantification

The types and concentration of sugars in the slurries were analysed by high performance liquid chromatographic (HPLC) method as described by Wight and Niekerk (1983).

The HPLC instrument used for sugar analysis was Water 515 HPLC Pump with a refractive index detector (Water 2414 Refractive Index Detector).

For sample preparation, 10 ml of sample was mixed with 2 g of anion exchanger resin (Ion exchanger III, Merck, Germany) for 15 min. The anion exchanger resin was filtered out with muslin cloth. The sample was mixed with another 2 g of cation exchanger resin (Amberlite IRC-50 as cation, Fluka) and allowed to stand for another 15 min. The deionised sample was filtered with Sep-Pak C₁₈ disposable cartridge which has been activated with HPLC grade methanol. The sample was then filtered with 0.45 µm membrane filter. For standard solutions preparation, glucose, fructose and galacturonic acid were used. A series of concentrations 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml were prepared for each sugar standard solution. The standard solutions were prepared with distilled deionised water and then filtered with 0.45 µm membrane filter before analysis. Distilled deionised water was prepared by using water purification system (Model Elga UHQ-II-MK3).

The Water Sugar-Pak I column was flushed overnight with 50 ppm Ca-EDTA at 0.2 ml/min. The mobile phase was prepared by adding 0.05 g of Ca-EDTA into 1,000 ml of distilled deionised water in a volumetric flask. The mobile phase was filtered with 0.45 µm membrane filter paper with the aid of vacuum pump and followed by sonication process by using an ultrasonic bath (ELMA Model T700 HW/ACC).

For detection and quantitation, the following conditions were used for analysis: flow rate mobile phase, 0.5 ml/min; temperature 90 °C; and the analysis running time was fixed to 20 min. The deionised samples 20 µl of was injected into analytical column. For each analyte, the retention time and peak area were recorded.

Statistical analysis

The experimental data were analysed statistically for analysis of variance (ANOVA) and mean values were evaluated by using Duncan new multiple range tests (SPSS 12.0 software program, Chicago, IL). The *p* values <0.05 were regarded as significant. The relationship between the peak area and the concentration of the standard solutions for HPLC analysis were analysed by using linear regression.

Results and discussion

Physical analysis was performed to obtain more information about the effect of enzymatic treatment towards changes in physical properties of DA and DR slurries. Minolta Spectrophotometer was used to measure the colour difference of the hydrolysates. The CIE L* a* b* colour values of the slurries are presented in *Table 1*. Under the tristimulus colour coordinate system, the L* value is a measure of lightness and varies from 0 (black) to 100 (white); the a* value varies from -100 (green) to +100 (red); and the b* value varies from -100 (blue) to +100 (yellow). As the values of a* and b* rise, the colour becomes more saturated or chromatic, but these values would approach zero for neutral colours (white, grey or black) (Al-Hooti et al. 2002).

Overall, the lightness of the DA slurries increased after enzyme hydrolysis. Of all durian aril hydrolysates (DAH) only that hydrolysed with cellulase showed significantly (*p* <0.05) lighter colour than DA slurries. This trend was almost similar to that of date syrup after hydrolysis with pectinase and cellulase enzymes (Al-Hooti et al. 2002). No significant difference (*p* >0.05) was seen in a* and b* values of DAH with DA slurries. The L* values of all durian rind hydrolysates (DRH) slurries were not significantly different (*p* <0.05) from DR slurry.

The a* values of all DRH slurries were significantly (*p* <0.05) higher (more positive) than the control DR slurry, and

Table 1. Effect of enzyme treatment on colour values of durian aril and durian rind slurries

	No enzyme		A	C	P	P+C+A	P+C
Durian aril slurries							
L* values	64.9a ± 0.38	66.75abc ± 0.92	67.00bc ± 1.33	66.94abc ± 1.45	66.61abc ± 1.10	66.38abc ± 1.28	66.61abc ± 1.10
a* values	-0.57ab ± 0.05	-0.27b ± 0.16	-0.27b ± 0.08	-0.29b ± 0.12	-0.34b ± 0.12	-0.31b ± 0.04	-0.34b ± 0.12
b* values	13.24a ± 0.36	10.49a ± 0.53	10.28a ± 0.08	10.60a ± 0.52	9.86a ± 0.40	10.47a ± 0.09	9.86a ± 0.40
Durian rind slurries							
L* values	67.71bc ± 0.62	67.11bc ± 1.32	68.01c ± 1.49	66.72abc ± 0.36	65.74ab ± 1.20	66.86abc ± 0.31	65.74ab ± 1.20
a* values	-0.77a ± 0.29	1.37bc ± 0.39	1.13b ± 0.32	1.41bc ± 0.22	1.74c ± 0.47	1.52bc ± 0.16	1.74c ± 0.47
b* values	6.89a ± 1.91	7.40ab ± 0.04	6.88a ± 0.74	7.46ab ± 0.29	8.27b ± 1.11	7.68ab ± 0.20	8.27b ± 1.11

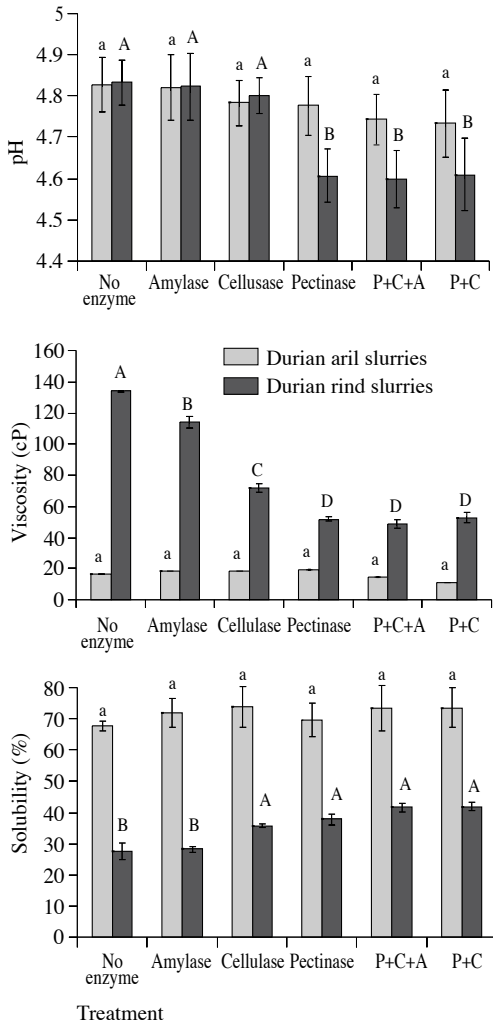
A = amylase, C = cellulase, P = pectinase, P+C+A = pectinase + cellulase + amylase, P+C = pectinase + cellulase

only b* values of DRH slurry treated with pectinase and cellulase (P+C) were significantly ($p < 0.05$) higher than that of DR slurry. The higher a* and b* values of DRH slurries indicate its tinge to be on the red/yellow sides compared with the control samples. The increase in a* value of DRH slurries was due probably to the release of pigments during incubation of DR with enzymes or other chemical substances that could influence colour.

Commercial pectinase enzyme is often added during winemaking to increase the phenolic content of wines that affect the wine colour (Kelebek et al. 2007), and the main pigment responsible for the wine colour has been identified as anthocyanins (Mazza 1995). Interestingly, pectinase and cellulase enzymes have been used to disrupt the cell wall of orange peel, sweet potato and carrot to release the carotenoids in the chloroplasts and cell fluids of the plants (Cinar 2005). The types of pigments present in DRH slurries, however, require further investigations.

During hydrolysis of polysaccharides, changes in pH may be used to indicate the progress of the hydrolysis process. The pH values of DA slurries with different types of enzymatic treatment did not show significant difference ($p > 0.05$) from each other (Figure 1). The pH of DAH slurries was maintained at around 4.8 which was the pH of sodium acetate buffer. No significant difference ($p > 0.05$) was noted in the pH values of DRH slurries treated with amylase or cellulase from the control sample. The pH values of DRH slurries treated with pectinase, combination of pectinase, cellulase and amylase (P+C+A) and combination of pectinase and cellulase (P+C), however, were significantly ($p < 0.05$) lower than other DRH slurries and control samples. The lower pH values attained after enzymatic treatment may be taken as an indicator of hydrolysis.

In fruit juice industry, the use of commercial pectinase increases the extraction yield, reducing sugars and



Error bars indicate standard deviation of three measurements
 P+C+A = pectinase + cellulase + amylase,
 P+C = pectinase + cellulase
 ABCA significant difference between DR and DRH slurries is represented with different uppercase letters; $p < 0.05$
 abcA significant difference between DA and DAH slurries is represented with different lowercase letters; $p < 0.05$

Figure 1. Effect of enzyme treatment on pH values, viscosity and solubility of durian aril and durian rind slurries

galacturonic acid content (Baciu and Jördening 2004). It is possible that a certain level of pectin and cellulose degradation had occurred in the slurries of DRH (pectinase), DRH (P+C+A) and DRH (P+C)

that caused a decrease in pH. However, since no significant drop in pH occurred in DRH slurries treated with cellulase, it is likely that most of the acid produced was due to the pectinase hydrolysis. The polysaccharides from the rinds of durian are composed of pectin as the main component and starch as a contaminant (Hokputsa et al. 2004). With enzymatic treatment, pectin and other polysaccharides could be partially or completely disintegrated and thus followed by the reduction in pH, viscosity and an increase in solubility.

There was no significant difference ($p < 0.05$) in the viscosity values between the DAH and DA slurries (Figure 1). As DA is composed mostly of soluble components such as sugars, organic acids, vitamins and other compounds (Voon et al. 2006), the enzyme treatments did not affect its viscosity. The viscosity of DRH on the other hand was dependent on the types of enzymes used for hydrolysis. Without enzyme treatment, the viscosity of DR slurries was significantly ($p < 0.05$) higher than DR slurries hydrolysed with enzymes. The order of viscosity of the slurries was: Control sample (DR) > DRH (amylase) > DRH (cellulase) > DRH (pectinase), DRH (P+C+A) and DRH (P+C). No significant difference ($p > 0.05$) in viscosity of DRH (pectinase), DRH (P+C+A) and DRH (P+C) slurries was noted. It is possible to suggest that the viscosity attribute of DR slurries was mostly due to the presence of pectin and cellulose (Hokputsa et al. 2004). During the enzymatic treatment, pectinase breaks down the pectin molecules that led to a reduction of water holding capacity and consequently, free water is released to the system and reduces the viscosity (Lee et al. 2006).

Most plant cell walls consist of microstructures composed of cellulose embedded in a polysaccharide and the protein matrix surrounded by an outer layer composed mainly of pectic material (Andersson et al. 1994). Siroth et al. (2000) reported that cellulase and pectinase destroy

the structural formation of the matrix responsible for trapping starch granules in cassava pulp. Therefore, treatment of pulp with cellulase and pectinase not only improved starch liberation but also increased the pulp's susceptibility to amylase for starch hydrolysis.

In terms of the effects of viscosity reduction of DRH, the order of enzymes' effectiveness may be ranked as; amylase < cellulase < pectinase. This is acceptable since pectinase has been reported to contain 6–7 times stronger activity than cellulase for pectin degradation (Sreenath and Santhanam 1992). Even though the combination of pectinase and cellulase may synergistically decrease viscosity due to the degradation of high molecular weight polysaccharides (Sreenath et al. 1995), such an effect was not found in DRH (P+C+A) or DRH (P+C) slurries.

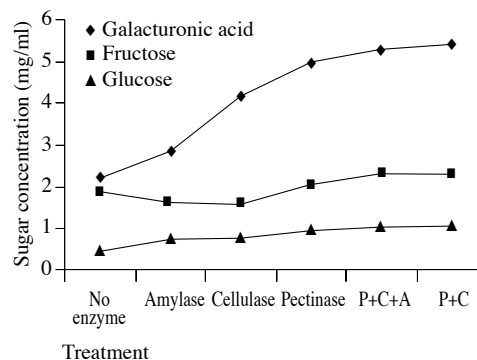
Solubility is relevant to the composition characteristics of powder product as it affects sensory attribute such as the taste perception, and is affected by water soluble compounds such as sugar, carbohydrate, protein, vitamins and minerals. On the other hand, the insoluble compounds mainly include fibre and fat. In all cases, DA and DAH slurries were higher in solubility compared to that of DR and DRH slurries (Figure 1). The solubility values of DA and DAH slurries were not significantly ($p > 0.05$) different from each other, and this trend is similar to the influence of enzyme treatment on viscosity of DA slurries. All types of enzyme treatment used had no effect on viscosity or solubility of DA slurries.

No significant difference ($p > 0.05$) in solubility of DR slurry with DRH (amylase) slurry was noted, indicating that amylase was not a suitable enzyme for this treatment. However, the solubility of the DR slurries increased after treatment with cellulase, pectinase, combination of pectinase, cellulase and amylase (P+C+A) and combination of pectinase and cellulase (P+C). The increased solubility of DRH

was due mainly to the liberation of soluble components such as galacturonic acid and simple sugars into the slurries.

Polysaccharides of plant origin such as cellulose, hemicellulose and pectin can be hydrolysed using pectinase, cellulase and β -glucosidase enzymes to produce glucose, fructose, galactose, arabinose, xylose, rhamnose and galacturonic acid (Nishio and Nagai 1979; Marshall et al. 1985). The α -amylase has been known to randomly hydrolyse α -1,4-glucosidic bonds of starch into dextrans, and this digestion may degrade amylose into maltose and glucose. As there was no significant difference in pH values of DA and DAH slurries (Figure 1), HPLC analysis was conducted only on DR and DRH slurries.

The concentration of glucose and fructose increased slightly with enzyme treatment (Figure 2). This is acceptable since starch is not the main component in the DR (Hokputsa et al. 2004). As could have been expected, galacturonic acid increased with enzyme treatment, and was the highest in DRH treated with pectinase and cellulase. This confirms previous suggestion that DR consists mainly of pectic polysaccharides which can be degraded into galacturonic acid (Hokputsa et al. 2004).



P+C+A = pectinase + cellulase + amylase
P+C = pectinase + cellulase

Figure 2. Effect of enzyme treatment on concentration of galacturonic acid, fructose and glucose of DR slurries. Results are mean values of duplicate analyses

In many cases, pectinase has been shown to be more effective in hydrolyzing polysaccharides of DR. This agrees with research in other agricultural waste such as that of grape peel (Grohmann and Baldwin 1992). A better degradation could be achieved when pectinase was combined with cellulase than when only pectinase was added to peel. This suggests that cellulase is needed when using pectinase for hydrolysis purpose (Grohmann et al. 1994). The use of pectinase and cellulase enzymes disrupts the cell wall of DR. Perhaps, due to the synergistic factor that the galacturonic acid release was higher in DR slurries treated with P+C+A and P+C as compared to that treated with cellulase or pectinase alone.

Conclusion

This preliminary result indicated the possibilities of modifying physical properties of durian rind slurries by using enzyme treatments. The enzyme treatment, however, had no effect on physical properties of durian aril slurries. Of all enzymes used, pectinase was the most effective in hydrolyzing polysaccharides in durian rinds than was cellulase or amylase. Optimization of durian rind hydrolysis can be expected to increase the utilization of durian waste for the production of alcohols, organic acids or other glucose-derived fermentation products.

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Abstrak

Durian (*Durio zibethinus*) sejenis buahan tropika Asia Tenggara. Penggunaan enzim amilase, selulase dan pektinase untuk mengubah suai sifat fizikal sluri pulpa durian (durian aril, DA) dan kulit pangs durian (durian rind, DR) telah dikaji. Rawatan dengan pektinase, kombinasi pektinase dan selulase, dan kombinasi ketiga-tiga enzim telah menghasilkan sluri DR dengan pH rendah, keterlarutan tinggi serta kelikatan yang rendah berbanding dengan sluri yang dieram tanpa penambahan enzim. Walau bagaimanapun, pengolahan enzim tidak menunjukkan kesan yang sama pada sluri DA. Analisis gula dan asid galacturonik hasilan enzim terawat sluri DA dan DR telah membuktikan bahawa terdapat kesan sinergistik **pektinase dan selulase bagi memecahkan dinding sel DR**. Kesimpulannya, pektinase mempunyai kesan yang paling ketara dalam memecahkan polisakarida pada DR berbanding dengan **selulase dan amilase**. Kajian awal ini menunjukkan kemungkinan mengubah suai ciri-ciri fizikal sluri DR dengan menggunakan pengolahan enzim.