

Isolation of polysaccharides from pineapple fruit pulp and their enzymatic liquifaction

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Abstract: Pineapple fruit pulp polysaccharides as alcohol insoluble solids (AIS) were isolated and liquefied individually and/or in combination with commercial pectinase NS3000 and cellubrix enzymes. Individual enzymes at lower dosages (<100ppm) were unable to release monosaccharides whereas significant amount of monosaccharide were released at higher dosage (1000 ppm). However, pectinase NS3000 was found more effective in liquefying AIS. Combined effect showed that both enzymes have the same potential of releasing total monosaccharides (4.0% of the total monosaccharides); the major differences were in the rate of individual monosaccharides release. After 1h of hydrolysis, monosaccharide releases by combined enzyme treatment were almost twice than that of individual enzyme. In all the cases, free run-juice yield by enzymatic hydrolysis was found higher than that of control and the higher extraction rate was found at the higher enzyme dosages. The results revealed that the quality parameters like juice colour, cloudiness, soluble solids and the release of glycosidically bound monosaccharide were improved by enzymatic liquefaction.

Keywords: Alcohol insoluble solids, juice yield, enzymatic hydrolysis, cell wall polysaccharides, glycosidically bound monosaccharide

Introduction

From the realization of the demand of tropical fruit and fruit products in international markets, many countries began the processing of tropical fruits during the last two decades. Fruit juices, nectars and drinks are the most popular products made from tropical fruits. The consumption of fruit juices from citrus and tropical fruits together accounts for about 70% of the fruit juice present in the world market (Ramadan and Thomas, 2007; Brito *et al.*, 2008). Nowadays people are much health conscious and demand of improved quality fruit juices, which is close to natural, healthy and nutritional. Among the wide range of fruit juices available in the market, cloudy fruit juices contain more antioxidants, which protect against heart disease and cancer, and are thought to be one of this category (Oszmianski *et al.*, 2007). That is why cloudy fruit juices, particularly from tropical fruits like jackfruit, papaya, pineapple; banana, mango etc are becoming a fast-growing part of the fruit juice sector.

Pineapple, as a tropical fruit, is one of the most appreciated fruit due to its very attractive aroma

and very nice flavour. Though pineapple is mainly processed into canned products, pineapple juice also a dominant part in the fruit juice sector, which ranks fourth in volume of fruit juice consumed (Grassin and Fauquembergue, 1996). The processing of pineapple into cloudy juice is very difficult due to low hydrocolloid content of pineapple juice (Wiil, 1999). The analysis of pineapple juice cloud indicated enormous content of coarse cloud relative to fine, which is a probable reason of poor cloud stability of pineapple juice. Therefore, degradation of pineapple coarse cloud or selective extraction of fine cloud from fruit pulp might be the one approach to overcome this problem.

Now-a-days enzymes are essential tool in the juice processes, both in terms of juice yield, quality (like cloudiness) improvement and cost saving. It is expected that besides the improvement of juice volume and colour, enzymatic liquefaction of pulp increases soluble components, which offer a number of advantages in producing juice, such as aroma, phenolic components and cloudiness (Buchert *et al.*, 2005). Application of exogenous enzymes leads to

the degradation of the fruit cell walls or the selective extraction of some of their components, allowing the formation of new types of finished products and fruit derivatives. Glycosidase side activities in commercial pectinase preparations (Wrolstad *et al.*, 1994) facilitate aroma enhancement, which accumulated as non-volatile and flavour-less compounds in a great number of fruit and plant tissues (Sarry and Günata, 2004). Release of higher neutral sugars is one of the indirect measures of aroma improvement by enzymatic treatment.

As has been stated pineapple fruit juice concerned to this study thought to be very sensitive to process into cloudy due to lack of fine cloud compared to coarse cloud (Will *et al.*, 1999). Mechanical size reduction or addition of hydrocolloids is the common practice to improve cloudiness of pineapple juice and nectar (Carle, 1998). Though improvement of juice yield by enzyme treatment has already been well-established, studies concerning the quality improvement like cloudiness are still very limited and also contradictory. Many researchers have still been continuing research in support of quality improvement by enzymatic treatment (Ramadan and Thomas, 2007; Brito *et al.*, 2008). In this study, a combination of pectinase NS3000, a new pectinase preparation and Cellubrix, a cellulase preparation were used to liquefy the pineapple fruit pulp polysaccharides (AIS) aiming to assess the increase of soluble component in finished products, which will ultimately help to produce more cloudy pineapple juice with improved functionality. Besides AIS liquefaction, pineapple fruit pulp was also hydrolyzed to assess the quality parameters like juice colour, total soluble solids, cloudiness, and glycosidically bond sugars release.

Materials and Methods

Materials

The fresh pineapples of Smooth Cayenne variety produced in Costa Rica (Freshdelmonte) were bought from super market in Austria. Cellulolytic enzyme preparation (Cellubrix) and pectinolytic enzyme preparation (Pectinase NS3000) kindly supplied by Novozyme, Switzerland were used for the enzymatic treatment of pineapple pulp.

Chemicals

All chemicals were supplied by Sigma Aldrich, Austria. Xylan, carboxymethyl cellulose and pectin were used as substrates in the assays of the enzyme activities. Xylose, glucose and galacturonic acid were used as monosaccharide standards. Guar gum,

carboxymethyl cellulose and pectin were used to improve the quality of cloudy pineapple juice.

Preparation of pineapple fruit mash

After rinsing the fruit in tap water, the shell and core were removed using a stainless steel knife and the flesh was cut into small pieces. Fruit pieces were blended into pulp by using kitchen blender. The pulp was pasteurized (85°C for 10 min) to inactivate the endogenous enzymes, cooled down and stored frozen. The pulp was brought to room temperature for the experiments.

Preparation of alcohol insoluble solids (AIS) from pineapple fruit pulp

The alcohol insoluble solids (AIS) was obtained after washing the fruit pulp with 95% alcohol three times and a final wash with acetone. The procedure was repeated twice and finally washed with acetone before final air-drying. Sequential extraction of AIS was carried out according to the method of Frügel *et al.* (2003). Little modification was done only in centrifugation and pectic fraction isolation. Pectic fraction was isolated by 50 mM NaOH, hemicellulose fraction by 4M NaOH and residual pellet was cellulose fraction. At each step, samples were diafiltrated with 10 kD membrane centrifuge tube at 2000 rpm. The retentate was washed with water two times and the supernatant volume was made 50 ml and then freeze-dried.

Activity assays of commercial enzyme preparations

The xylanase, cellulase and pectinase activities of the enzymatic preparations were measured by assaying the xylose, glucose and galacturonic acid liberated from xylan, CMC and polygalacturonic acid, respectively as described by Qin *et al.* (2005). The liberated sugars were assayed by reaction with di-nitrosalicylic acid (DNS) reagent (Miller, 1959). Endo-polygalacturonase (Bailey and Pessa, 1990), pectin lyase (Manachini *et al.*, 1988), β -glucosidase, β -xylosidase activities were measured using polygalacturonic acid, pectin, X-Glu (5-bromo-4.4 dichloro-3-indolyl- β -D-glucopyranoside) and X-Xylo (5-bromo-4.4 dichloro-3-indolyl- β -D-xylopyranoside), respectively. Pectin methyl-esterase was assayed by titration of the liberated carboxyl groups of pectin using an automatic titrator.

Enzymatic liquefaction of alcohol insoluble solids of pineapple fruit pulp

Five mg of alcohol insoluble solids were suspended in 1 ml of 20 mM phosphate buffer at pH

4.0, which is close to the pH of diluted pineapple pulp. The tubes were incubated in a laboratory incubator at 45°C and 130rpm. When the temperature was reached, the enzyme solution was added. The enzyme concentrations used were 25 ppm, 100 ppm and 1000 ppm for assessing the effect of the individual enzyme preparations. Samples were collected at intervals (0 h, 0.5 h, 1 h, 2 h and 4 h). One tube was also removed just before the addition of enzyme (control). The content of the each tube was heated at 99°C for 10 min and centrifuged for 30 min at 10,000 g and 4°C. The supernatant was recovered for subsequent analysis of released sugars. The combined effects of cellulase and pectinase were assessed by orthogonal loading of above individual dosages.

Enzymatic liquefaction of pineapple fruit pulp

Previously frozen stored pineapple AIS was brought to room temperature and 50 g pulp was weighed for each experiment. Pineapple pulps with added enzymes were incubated at 45°C for 60 min in a shaker at 160 rpm. The enzyme concentrations used for alcohol insoluble solids liquefaction was also used for pulp liquefaction. After loading, they were heated up to 85°C for 10 min to inactivate the enzymes and then cooled down on ice immediately. Mash samples treated with no enzyme and only with distilled water were processed similarly to serve as a control.

HPLC analysis of anhydrous galacturonic acid and neutral sugars

The anhydrous galacturonic acid (AGA) and other sugar components were separated using a Dionex DX-500 Bio-LC system, with a Carbopac PA1 and PA20 columns (250 mm × 4 mm) in combination with a Carbopac guard column (25 mm × 4 mm) (Dionex Corp., Sunnyvale, CA). All analyses were carried out at a temperature of 30°C and a flow rate of 1ml/min for PA1 column and 0.5 ml/min for PA20 column. The neutral monosaccharides were eluted isocratically using 15 mM NaOH for 35 min in PA1 column and 2 mM for 20 min in PA20 column. The AGA was eluted using a gradient reaching 170 mM sodium acetate and 100 mM NaOH. The column was washed with 100 mM NaOH for 15 min and re-equilibrated to starting conditions for 20 min before the next injection.

Determination of juice yield

When the temperature of the enzyme treated pulp came to the room condition, the mash was poured into a funnel lined with cheese cloth. The volume

of free-run juice draining into a measuring cylinder per minute was recorded over a total of 6 min. All determinations were carried out in triplicate and the averages reported.

Analysis of juice quality parameters

The pH was determined on all juice samples using pH meter (ProMinent, Germany). Total soluble solids (^oBrix) was determined using a digital Pocket Refractometer, Pal-3. Colour determination of pineapple juice was done by using a Hunter colour meter (Lange, Germany). Three Hunter parameters, namely “L” (Lightness), “a” (redness and green) and “b” (yellowness and blueness) were measured. Cloudiness was determined by diluting two folds with water and measuring absorbance at 660nm. The higher absorbance indicated the greater cloudiness of the juice.

Results and Discussion

Isolation and fractionation of alcohol insoluble solids (AIS)

Alcohol insoluble solids (AIS) of the pineapple fruit mash was prepared and fractionated into pectin, hemicellulose and cellulose (Table 1). The amount of AIS obtained from 100g pulp was 2.88 g of which pectin, hemicellulose and cellulose contributed 19.2 %, 10.9 % and 20.10 % respectively. The recovery of fraction is only 50 %. This might be explained by the loss during extraction and purification steps.

Activity profile of enzyme preparations

The activity profile of the two preparations indicated that at pH 3.6 (pH of natural pineapple juice) and at room temperature, the major activity as well as the side activities of the enzyme preparations are very low (Table 2). Cellubrix showed practically no activity other than cellulase at this condition. Pectinase NS3000 (NS), a new product of Novozyme, showed comparatively better side activities than their previous product, pectinase Ultra SP (SP). Among the enzyme preparations, pectinase NS3000 showed good stability at pH 3.6. Findings related to SP are very close agreement to those reported by Buchert *et al.* (2005). When the incubation temperature increased to 45°C (used for pineapple mash treatment), the activity of all enzyme preparations increased significantly (data not shown).

Table 1. Isolation and sequential extraction of pulp alcohol insoluble solids (AIS)

Samples	Amount taken (mg)	Extracted (mg)*	(%) recovery	% basis on recovery
Pulp	1440			
AIS (extracted)	200			
Pectin	-	21.8 (0.1)	10.9	21.73
Hemicellulose	-	38.4 (0.05)	19.2	38.24
Cellulose	-	40.2 (0.01)	20.10	40.03
Total		100.4 (0.2)	50.2	

*Values are means \pm SD of three samples**Table 2.** Activity profile of enzyme preparations at pH 3.6 and room temperature (25°C)

Enzyme preparation	CE nkat/mL	PME nkat/mL	PG nkat/mL	PL nkat/mL	XYL nkat/mL	β -GLU nkat/mL	β -XYL nkat/mL
Cellubrix	123	0	0	0	16	0	0
Pectinase Ultra SP	0	5	8	0	15	0	0
Pectinase NS3000	53	22	44	0	15	0	0

CE= Cellulase, PME= Pectin methylesterase, PG= Polygalacturinase, PL= Pectin lyase, XYL= Xylanase, β -GLU= β -glucosidase, β -XYL= β -xylosidase, nkat= nanokatal**Table 3.** Total sugar release (%) with individual and combined enzymes treatment

Individual effect ^A	25ppm	100ppm	1000ppm		
Cellubrix	0.05 (0.1) *	0.1 (0.01)	1.91 (0.2)		
Pectinase NS	0.29 (0.02)	0.65 (0.1)	2.65 (0.05)		
Combined effect	Release of monosaccharide at				
	0 h	0.5 h	1.0 h	2.0 h	4.0 h
C1P4 ^B	0	2.92 (0.1)	4.73 (0.5)	8.58 (0.1)	11.36 (0.01)
C4P1 ^C	0	2.06 (0.0)	4.16 (0.05)	7.61 (0.2)	9.55 (0.05)

^AFor individual effect enzymatic hydrolysis was conducted for 1h period^BEnzyme dosages were 25ppm cellubrix and 1000ppm pectinase NS3000^CEnzyme dosages were 1000ppm cellubrix and 25 ppm pectinase NS3000*Values are means \pm SD of three samples

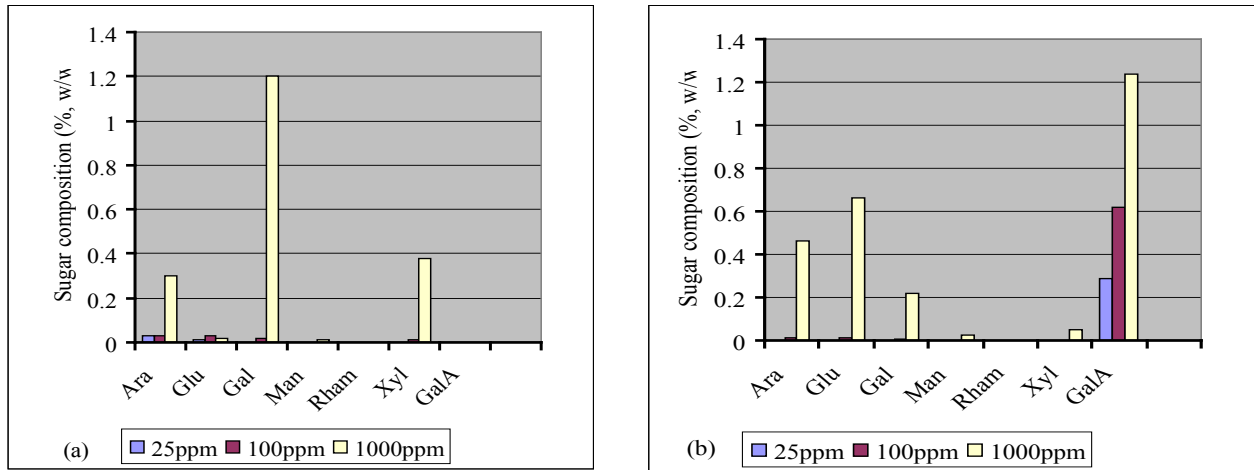


Figure 1. Release of monosaccharide constituents of the water insoluble fraction of pineapple pulp AIS treated with (a) Cellubrix (b) Pectinase NS3000. (All weights are expressed as per cent of original AIS)

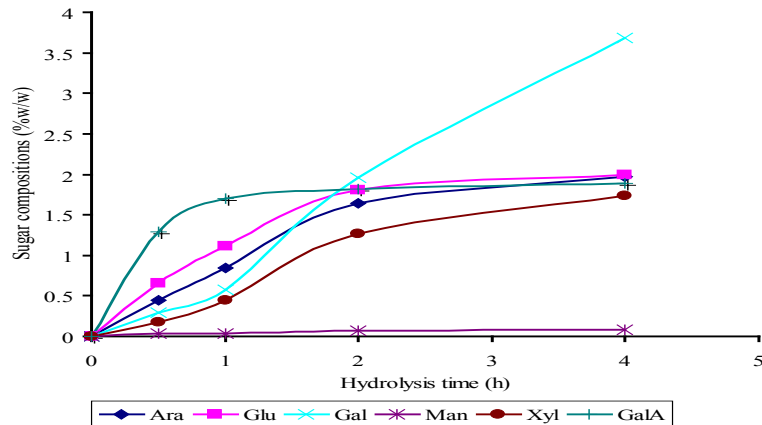


Figure 2. Kinetics of monosaccharide release by a combined effect of cellulase and pectinase (25 ppm and 1000 ppm) of water insoluble fraction of pineapple pulp alcohol insoluble solids (AIS). (All weights are expressed as per cent of original AIS)

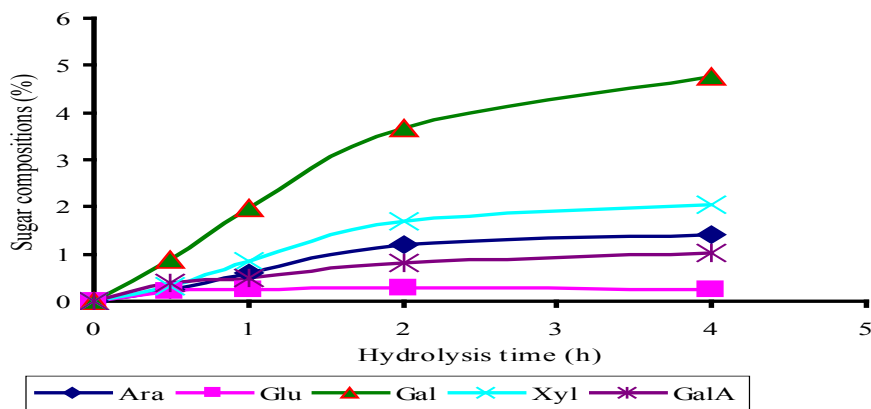


Figure 3. Kinetics of monosaccharide release by a combined effect of cellulase and pectinase (1000 ppm and 25 ppm) of water insoluble fraction of pineapple pulp AIS. (All weights are expressed as per cent of original AIS)

Enzymatic liquefaction of isolated cell wall polysaccharides

Alcohol insoluble solids (AIS) were considered as suitable preparations to describe cell wall polysaccharides (Femenia *et al.*, 2007). Therefore, AIS were incubated with commercial enzymes individually or combined to assess their ability to hydrolyze the cell wall polysaccharides. When Cellubrix was added alone to the AIS monosaccharide release was hardly observed at low dosages of enzymes (Figure 1a). Only at higher dosages (1000 ppm) higher amount of galactose and considerable amounts of arabinose and xylose were released, which indicated that hemicellulose was more susceptible to cellubrix. The release of lesser extent of glucose indicated that cellubrix alone was not able to hydrolyze cellulosic materials.

As could be expected, no galacturonic acid was released due to action of cellubrix. On the other hand, with the increase of pectinase NS3000 (PN) dosages the galacturonic acid release was increased (Figure 1b). Due to wide range of side activities of PN several neutral sugars were released. Interestingly, PN was able to release significant amount of glucose.

A kinetic study with combined effect of cellubrix and pectinase NS3000 has been shown in Figure 2 and Figure 3. When a combination of lower cellulase and higher pectinase activities were used the polysaccharide constituents released were mainly arabinose, glucose, galactose, xylose and galacturonic acid and to a lesser extent mannose. Except galactose all other sugars released reached maximal concentration after 2h of reaction. However, the release of the pectic sugars was not observed at the same rate. The galacturonic acid was released faster, then followed by glucose, and later followed by arabinose.

When a combination of higher cellubrix and lower PN enzymes was applied, the release of galactose was found same with no mannose released (Figure 3). The yield of glucose was increased as compared to single effect. Concerning to galactose, the rate of change was lower by the combined effect with higher cellubrix than in higher PN.

The total sugars released by the action of single and combined effects are presented in Table 3. After 1h hydrolysis, the total sugar released was about twice by combined effect than by single effect when compared to a higher dosage.

The success of the liquefaction methods greatly depends on the ability of the enzymes to liquefy cell wall materials (Will *et al.*, 2002). It was observed that both cellubrix and pectinase NS3000 (PN) were able to release monosaccharides from AIS of pineapple

fruit pulp indicating that fruit cell walls were liquefied. But a synergistic effect of cellulase and pectinase was found by a more effective liquefaction of the cell wall polysaccharides. The predominant non-cellulosic polysaccharides in pineapple cell walls are glucuronoarabinoxylan and xyloglucan together with galactoglucomannan (Smith and Harris, 1995). The formation of foam and viscosity of pineapple juice was mainly due to galactoglucomannan, a gum like hemicellulosic compounds (Chen and Yamamoto, 1978). The release of significant amounts of galactose, glucose and mannose were evident for the presence of this gum. Pectic galacturonan content of pineapple was reported by many researchers (Jarvis, 1984). The yield of galacturonic acid by PN indicated the presence of pectic polysaccharides. Both combinations release considerable amounts of xylose and glucose that make evident the presence of xyloglucan, another important hemicellulose compound present in pineapple fruit (Femenia *et al.*, 2007).

Enzymatic liquefaction of pineapple fruit mash

Effect on juice extraction rate

The pattern of free-run juice with a single effect or in combination of the combined enzymes is shown in Figure 4. In all cases juice extraction rate was much higher than the control, but pectinase NS3000 (PN) showed better extraction effect individually than cellubrix. As shown in Figure 4a increase of cellubrix dosage did not result in any profound difference in juice flow. Juice flow increased with increasing dosages of PN enzyme but the sufficient yields has already been reached at the enzyme dosages of 100 ppm (Figure 4b).

When combination of the two enzyme preparations was used, higher juice flow was observed in the combination with higher PN (Figure 4c). It is apparent that there is almost no difference in juice extraction rate by using PN individually or in combination with cellubrix. Due to the presence of higher hemicellulose and cellulose content in pineapple cell wall materials, either single enzyme with numerous side activities or combination of different enzyme blends with higher hemicellulase and cellulase activities are necessary for higher juice yield. The cell wall of pineapple fruit has a middle lamella and a primary wall. The middle lamella acts as an intercellular sticking substance and is mainly composed of pectin. Pectin of the middle lamella is the main substrate of pectolytic enzyme preparation (Anastasakis *et al.*, 1987; John *et al.*, 2003). The progressive degradation of cellulose fibrils led to loss of wall strength and its breakdown

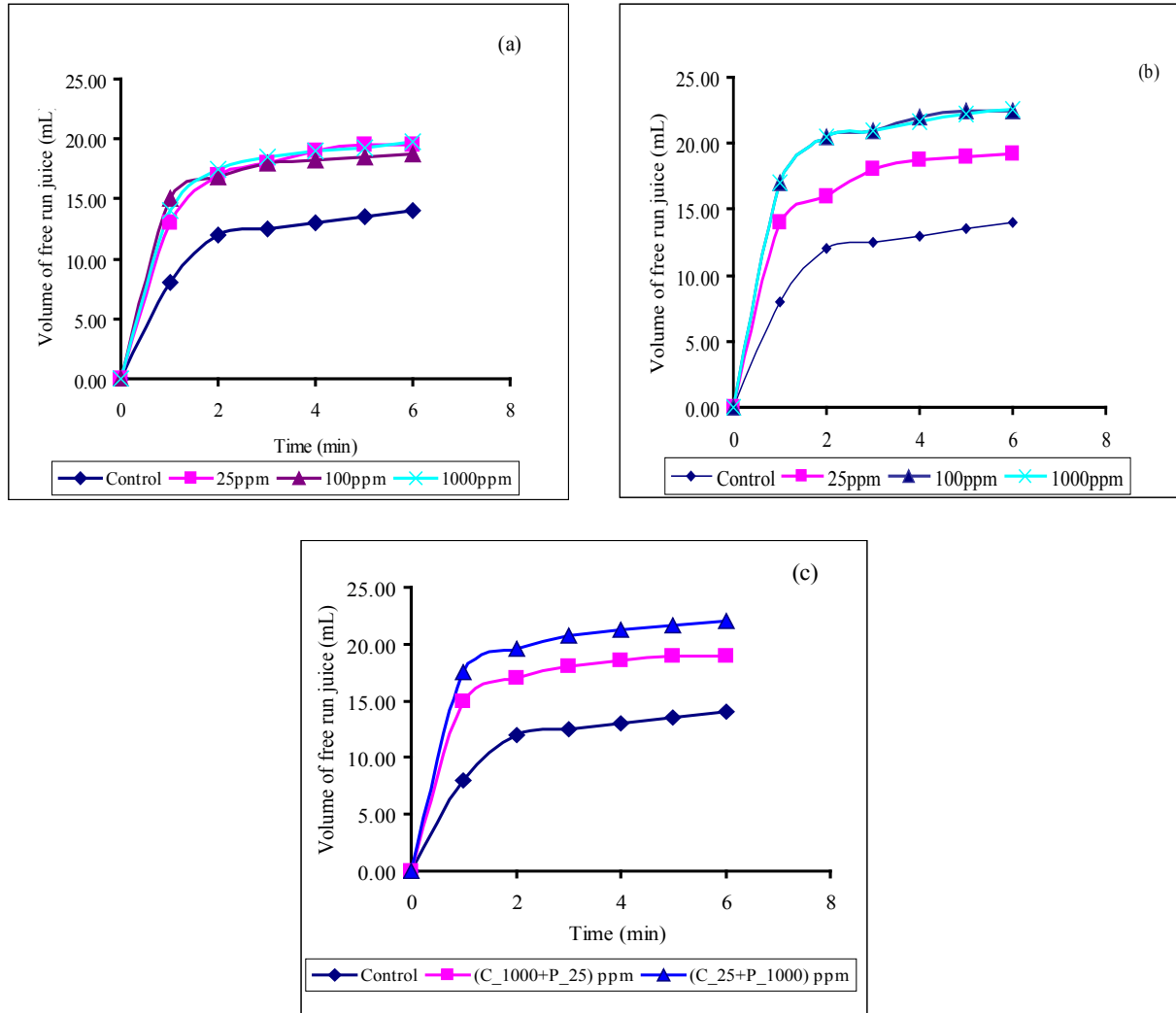


Figure 4. Effect of cellulolytic and pectinolytic enzyme preparations on flow rate of pineapple juice (a) pectinolytic enzyme preparation with 25, 100 and 1000 ppm dosages (b) cellulolytic enzyme preparations with 25, 100 and 1000 ppm dosages and (c) combined effect of pectinolytic and cellulolytic enzyme preparations (control = no enzyme, C_1000+P_25 = 1000 ppm cellulix and 25 pectinase NS and C_25+P_1000= 25 ppm cellulix and 1000 ppm pectinase NS)

into fragments should make juice recovery easier (Grassin and Fauquembergue, 1996), which should result in higher yield. The higher juice yield by PN3000 along or in combination were evident of this phenomenon.

Effect on quality parameters of extracted juice

The effect of the cellulase and pectinase enzyme preparations on quality parameters of extracted juice is shown in Figure 5(a-d). Since the major color of cloudy pineapple juice is yellow, the amount of this pigment in pineapple flesh is an excellent measure of quality (Mehrlich and Felton, 1980). Figure 5a shows that pineapple juice treated with cellulase only

became lighter which corresponded to an increase in *L* value compared to control whereas the use of higher pectinase dosage alone or combined with cellulase made the derived juice darker.

It is noted that the degradation in *L* value might be influenced by a decrease in *b* value. But it can be seen in Figure 5b the change in *b* values due to higher pectinase dosages alone or combined with cellulase were improved. Interestingly, low dosages of pectinase NS made the juice lighter and bluish, which indicated the pigment destruction (Mehrlich and Felton, 1980; Rattanathanalerk *et al.*, 2005). As the *b* is the major indicator to describe the colour of pineapple juice the increase of *b* value strongly

supports the positive effect of enzyme in improving quality.

The absorbance readings at 660nm were used to measure the cloudiness (Owusu-Yaw *et al.*, 1988; Versteeg *et al.*, 1980) and the higher the absorbance reading the cloudier the juice. The effect of pulp enzyme treatment on cloudiness of pineapple juice has been shown in Figure 5c. The similar effect as of colour was observed for cloudiness. The higher pectinase or with combination of cellulase dosages resulted in more cloudy juice compare to control. Lower pectinase NS dosages made the juice clarified and clarification is acute at a dosage of 100 ppm PN. On the other hand, varying amounts of cellulase did not have noticeable effect in improving cloudiness. Total soluble solids levels of enzyme-treated juice were higher than that of control except C4P1. Higher pectinase NS dosages alone or in combination with cellulase were the most effective in increasing TSS. The raise could be partially accounted for by increase in soluble sugars, which may result from the conversion of insoluble pectin by pectinolytic enzymes and the action of cellulase on cellulose to produce soluble sugars (Schobinger *et al.*, 1981). The increase of 'b' value of enzyme treated pineapple juice might be due to release of more colour compounds during enzymatic hydrolysis. The carotenoid compounds remained intact and were released with cell wall materials to the juice during enzymatic

liquefaction. Regarding the cloudiness improvement reason might be the degradation of the insoluble cell wall polysaccharides into soluble molecules. As a result the soluble components were increased, which resulted in higher cloudiness as well as the TSS.

Effect on the release of monosaccharide

An increase in juice yield is mostly associated with an increase in sugars soluble in juice. As shown in Table 4, the treatment of fruit mash with only pectinase and cellulase enzymes has resulted in poor monosaccharides yield. Pectinase was found effective in releasing monosaccharides when they used either at higher dose or in combination with cellulase. When pectinase was applied with higher cellulase xylose release was the highest but no galacturonic acid was released.

Pectinase NS3000 alone could also be able to release arabinose, mannose, xylose and galacturonic acid. However, when it combined with cellulase, the amount of monosaccharides released was increased. Galacturonic acid is the predominant sugar released by the treatment with pectinase NS3000, followed by arabinose and then by mannose. Flavour active compounds were present intact with fruit or juices by glycoside bonds. It is reported that Glycosidase activities in enzyme preparations break down the bonds and release the monosaccharides into the juice as well flavour compounds (Acar, 1999; Borowaska,

Table 4. Carbohydrate composition of enzymatic hydrolyzed pineapple juice*

Enzyme	Glu (g/L)	Fru (g/L)	Gal ppm	Man ppm	Ara ppm	Xyl ppm	GalA ppm	Total Sugar (g/L)
Control	22.26	16.27	0	0	0	0	0	38.53
C ₂₅	21.85	14.29	0	27	0	6	0	36.17
C ₁₀₀	24.35	17.68	0	17	0	0	0	42.05
C ₁₀₀₀	23.43	16.29	0	21	15	6	152	39.91
P ₂₅	23.95	16.72	0	21	13	8	0	40.71
P ₁₀₀	23.90	16.78	0	24	10	13	95	40.82
P ₁₀₀₀	23.20	16.80	0	21	45	12	135	40.21
C1P4	22.12	16.30	0	28	52	22	216	38.74
C4P1	22.10	16.60	0	21	17	130	0	38.87

*(values are average of three experiments)

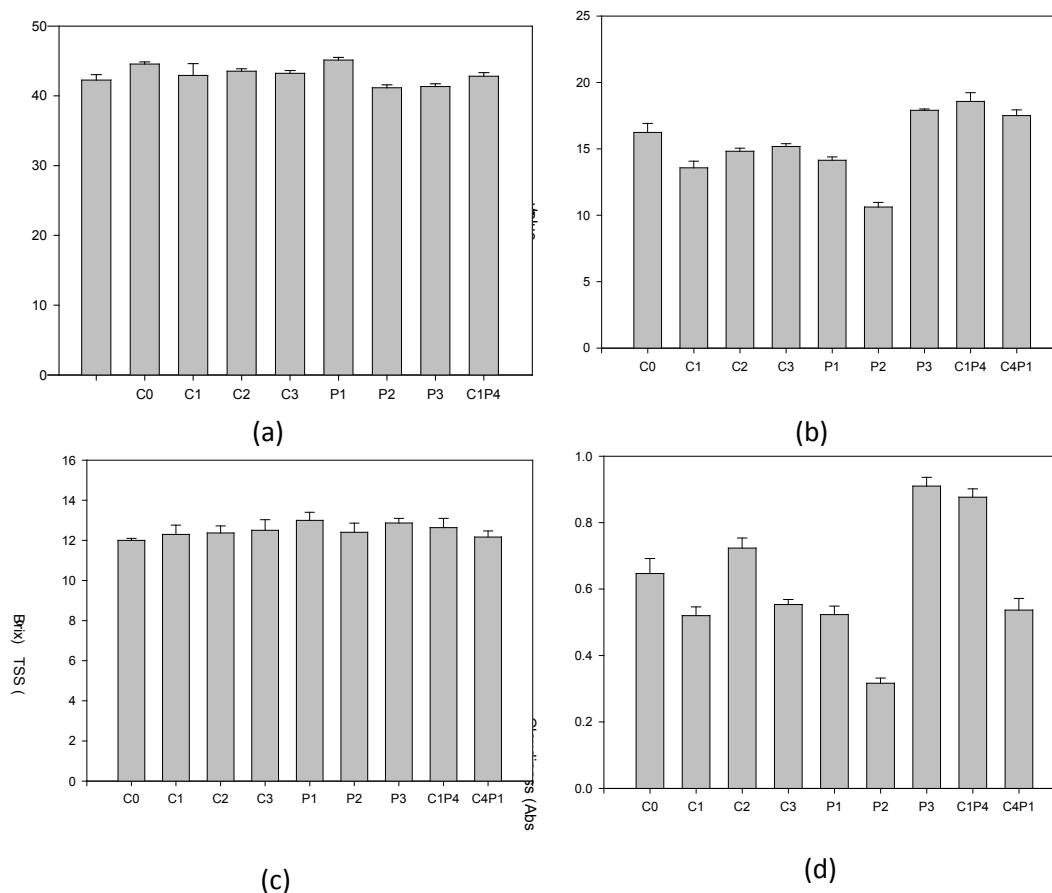


Figure 5. Effect of cellulolytic and pectinolytic enzyme preparations on quality parameters of pineapple juice on (a) brightness (L -value), (b) yellowness (b -value), (c) total soluble solids and, (d) cloudiness (absorbance at 660 nm). Samples: C0 represent juice with enzyme treatment; C1, C2, C3 represent 25, 100 and 1000 ppm cellulix treated; P1, P2, P3 represent 25, 100 and 1000 ppm pectinase treated; C1P4 represents 25ppm cellulix and 1000 and C4P1 represents 1000 ppm cellulix and 25 ppm pectinase NS)

2000; Ramadan *et al.*, 2007). In present study cellulix enzyme preparations alone were only able to release monosaccharides at higher dosages whereas PN was able to release monosaccharides even at lower dosage. On the other hand, a synergistic effect could be able to hydrolyze glycosidic bonds, which becomes evident by higher yield of monosaccharides. During production of bilberry and blackcurrant juices by enzymatic pressing, pectolytic enzyme alone was able to release monosaccharides (Buchert *et al.*, 2005). Using higher dose of enzyme might be the reason behind this ability.

Conclusion

The results of the present study showed that pectinase NS3000 alone or in combination with cellulix successfully hydrolyzed pineapple cell wall polysaccharides. Even so, enzymatic liquefaction by pectinase NS3000 or in combination with cellulix in pineapple fruit pulp improved the juice colour, total soluble solids and cloudiness that would significantly made easier to process the pineapple into cloudy juice with improved functionality. Nevertheless, the commercial enzymatic preparations stable at low pH

and with numerous side activities at rather higher dosages are found advantageous for liquefaction of pineapple fruit pulp.

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