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The Influence of a Pectinase and Pectinase/hemicellulases Enzyme Preparations On percentage Pineapple Juice Recovery, Particulates and Sensory Attributes

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Abstract: Two commercial enzyme preparations (from *Aspergillus niger*), a pectinase and a liquid pectinase/hemicellulases were used singly or in combination at a rate 0.03% (w/w) in a two step extraction of pineapple juice at 35, 37.5 and 40°C for 30 min. The percentage juice recovery, soluble sugars, total phenolics, titratable acidity, viscosity and turbidity of the recovered juice were measured to ascertain the influence of the enzyme preparations on extraction against the control. The ready to serve pineapple juice (RTS) was rated for acceptance on a 5 point hedonic scale.

Key words: Pineapple juice, pectinase, hemicellulases, total phenolics

INTRODUCTION

Pineapple has long been one of the most popular of the non-citrus tropical and subtropical fruits, largely because of its attractive flavour and refreshing sugar-acid balance (Bartolomé *et al*., 1995). Pineapple juice is largely consumed around the world, mostly as a canning industry by-product, in the form of single strength, reconstituted or concentrated and in the blend composition to obtain new flavours in beverages and other products (Arthey, 1995; and Carvalho *et al*., 2008). Canned pineapple and pineapple juice market has increased fourfold worldwide since 1984 from 1.3-5.6 million tons (fresh fruit equivalent). In 2004, two-thirds of the pineapple exported consisted of juice (single or concentrated) and the remaining one third was canned (rings, slices or cubes) (Vagneron *et al*., 2005). More recently, the consumer market has been equally receptive to limpid fruit juice either obtained by the traditional clarification processes using gelatine, bentonite (Carvalho *et al*., 2008) as well as by processes Using Ultrafiltration (UF) and Microfiltration (MF) membranes. Enzymes are used to ensure optimal juice yield and a quality product that ensure consumer appeal. The use of pectinases and hemicellulases preparations in pineapple pulp maceration not only facilitates easy pressing and increased juice recovery but also ensures the highest possible quality of the end products (Kilara, 1982; Kashyap *et al*., 2001) by facilitating the easy with which both UF and MF are carried out. This is as a result of their ability to degrade the cell walls thus significantly lowering the viscosity of the recovered juices hence minimizing membrane fouling (Carvalho *et al*., 2008). Hence in the present paper, we sought to study the influence of pectinases and hemicellulases on the quantity and quality of the recovered pineapple juice.

MATERIALS AND METHODS

Raw material: The pineapple fruits *(Ananas comosus* L.) were obtained from Da Run Fa supermarket-Wuxi city and used immediately or stored at 5°C for not more that seven days before being used. Fluka BioChemika17389 (EC 3.2.1.15), a pectinase preparation from *Aspergillus niger* and Folin-Ciocalteau reagent, were purchased from Sigma-Aldrich. Rapidase Pineapple, a liquid pectinase and hemicellulases preparation from *Aspergillus niger* (DSM-Netherlands). All others reagents were of analytical grade. The pineapple fruit was washed under running tap water before removing the crown and peeling using a stainless steel knife. The flesh intact with the core was sliced to approximately 2x2x1 cm before being mashed in a warring blender for 1-2 min. The pH of the pineapple pulp was recorded to the range of 3.70-4.20 using a pH meter model delta 320 (Mettler-Toledo Instruments-Shanghai).

The enzyme treatment of the pineapple pulp was conveniently carried out in three batches each with 36 samples distributed evenly within the temperature ranges used. All the samples were subjected to two steps of juice extraction. In batch I, 500 g of fresh pineapple pulp was pressed to extract the juice in the initial step (herein, step 1) after being incubated in 35, 37.5, or 40°C for 30 min. Subsequently, in step 2, the pulp residue was mixed with water in the ratio of 1:3 (w/w) before treatment with 0.03% (w/w) Pectinase from *Aspergillus niger* (Sigma-Aldrich) herein referred to as Fluka and a liquid Pectinase and hemicellulase from *Aspergillus niger* (DSM-Netherlands) herein referred to as Rapidase or their mixture at 35, 37.5, or 40°C for 30 min with constant stirring. The reaction was terminated by immersion of the samples in boiling water (99±1°C) for 5 min to inactivate enzymes before juice extraction.

Table 1: Effect of enzyme treatment on percent juice recovery from batches of pineapple pulp

		% Mean Yield at different temperature±SD*			
Batches	Enzyme Treatment	35°C	37.5°C	40°C	Average
I	Fluka	78.69±2.241458	78.69±1.580548	79.02±2.137031	78.8
	Rapidase	80.2±2.895174	79.18±1.409764	78.75±5.426162	79.38
	Mixture	77.69±0.800083	79.23±1.851738	80.4±2.551673	79.11
	Control	71.86±6.883998	71.72±5.188201	65.95±4.712041	69.84
II	Fluka	82.07±1.640772	80.36±1.274925	85.42±4.703913	82.62
	Rapidase	83.82±1.403424	82.56±3.636404	91.28±1.920009	85.89
	Mixture	86.28±1.610041	83.81±3.229721	93.33±3.02048	87.81
	Control	68.57±5.951322	70.02±3.825001	66.75±4.694958	68.45
III	Fluka	81.99±2.371694	84.56±2.36204	89.33±1.209352	85.29
	Rapidase	83.55±4.089747	91.3±2.805536	93.17±3.731876	89.34
	Mixture	89.46±4.174254	88.83±7.226129	94.93±1.146138	91.07
	Control	69.43±1.038123	71.36±3.298884	69.89±1.628199	70.23

SD*-Standard deviation

In step 1 of batch II, 500 g of fresh pineapple pulp was treated with Fluka, Rapidase or their mixture under similar reaction conditions to batch I. In step 2, the enzyme-treated pineapple pulp residue was mixed with water in the ratio of 1: 3 (w/w) and the juice was further extracted.

In batch III, the above enzymes were employed under similar reaction conditions in both steps of juice extraction from pineapple pulp and residue.

The control followed similar process trends as in batches I-III but without enzyme treatment.

The following analyses were carried out for the recovered juice. The percentage juice recovery and Total Phenolics (T.P) were estimated for each batch after dual extraction, while °Brix, Titratable Acidity (T.A), turbidity and viscosity were done for each step of extraction. The percent soluble sugar in the juice was determined using an Abbe type refractometer (WZS-I model, made in Shanghai) and expressed as °Brix. The turbidity of pineapple juice was recorded at 650 nm using a Leng Guang 722 spectrophotometer (Shanghai Analytical Instruments Factory) since at this wavelength; other browning components do not interfere with turbidity measurements (Sreenath and Santhanam, 1992). The turbidity was also visualized by the amount of sediment formed by adding 2 volumes of ethanol containing 0.5% HCL to 1 volume of juice at 30°C as a test of depectinization (Aehle, 2004). The titratable acidity of the juice expressed as percent citric acid (equation 3) was determined according to the method of James (1995). The viscosity of the juice from each step of extraction was measured at 20°C using an Ostwald viscometer and calculations done following equation (2). Total polyphenols of the pooled juice was determined according to the method of Fernandez de Simon *et al*., (1992) with a little modification. Briefly, 100 ml juice samples were concentrated to 25 ml with a rotatory vacuum evaporator (ZX98-1 Rotavapor, Shanghai Organic Chemistry Institute, Shanghai, China) at 50°C for 40 min. The concentrated juices were extracted twice each with 15 ml of diethyl ether and ethyl acetate. The fractions were pooled and evaporated to dryness before the residue was redissolved in 10 ml of 50% methanol (basic sample extract, BSE). Two milliliters of BSE were mixed with 8 ml of deionized water and 10 ml of acetate buffer (pH, 5.0) (extract 1, E1).One milliliter of E1 was mixed with 0.5 ml of FCR and made up to 10.0 ml with Na_2CO_3 after 30 sec. After standing for 30 min at 40°C, the absorbance at 765 nm of the final blue solution was measured using a Unico UV-2102 PCS spectrophotometer (Corp., Unico, China). Blank solution was prepared and measured identically, but without analyte. The values are reported as μg ml^{-1} Gallic Acid Equivalents (GAE) as estimated from the Gallic acid standard curve with the equation y = 0.0015x-0.0100 with R^2 being 0.9945 as shown in (Fig. 1).

Statistical analysis: The SPSS (Chicago, IL) statistical analysis system was used for analysis of the data. The statistical significance difference was assessed by one-way analysis of variance.

Significant differences ($p \leq 0.05$) among treatments were detected using Duncan's multiple range test.

RESULTS AND DISCUSSION

Juice recovery: The percentage juice recovery was estimated as given in equation (1) and since each batch consisted of 36 samples, hereby means and standard deviations are reported (Table 1).

$$\text{Pulp weight} - \text{Total soldwaste} \times \frac{100}{\text{pulpweight}} \quad \ldots\ldots 1$$

The highest percentage juice recovery (80.4±2.551673) in batch I was achieved using a mixture of the two enzymes and at extraction temperature of 40°C and the natural pH of the pineapple pulp against a percentage of 65.95±4.712041 from the control. Percentage juice

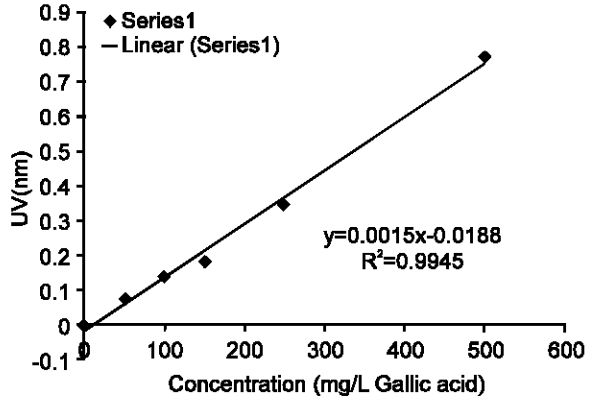

Fig. 1: Folin-Ciocalteau Gallic acid standard curve. Equation used in calculation was y = 0.0015x-0.0100

recovery after addition of Fluka, Rapidase or their mixture in batch I within the temperature range used during extraction was not significant (78.8-79.11% on average). However, in batch II and III there was a 9-13% increase in juice recovery respectively as compared to batch I and these is captured in Table 1 which shows the percentage mean yield at different temperature± standard deviations. Overall, the highest percentage juice recovery was recorded as 94.93±1.146138 in batch III when a mixture of the two enzymes was used at the extraction temperature of 40°C against a control value of 69.89±1.628199. This significantly translates to about 25% increase in juice recovery.

Several researches have shown enzymatic treatment results in higher yields of fruit and vegetable products (Sreenath *et al*., 1994; Czukor and Nyarady, 1999; Demir *et al*., 2000 and Will *et al*., 2000). This is due to the partial or complete degradation of the cell wall and middle-lamina pectins, other polysaccharides and cell-substances (Dörreich, 1996) thus increasing press capacity which results in increased juice yield, high carotene and dry matter content of the product (Demir *et al*., 2000).

°Brix values: Despite the fact that prior to extraction all sliced pineapple pieces were thoroughly mixed in an effort to achieve homogeneity, Brix values still fluctuated between 11.7 °Brix and 14.1 °Brix for the pooled juices (data not shown) with juices obtained by use of enzymes showing higher values. Will *et al*. (2000) noted that it is quite difficult even on a laboratory scale to put together homogeneous batches from various raw materials.

Another reason for the fluctuation could be due to the use of different enzyme products for pulp treatment, differential degree of pressing and losses as a result of human error.

However, these results (showing an increase in °Brix for enzyme treated pulp) are well comparable to those reported by Bhardwaj *et al*. (2005) and Demir *et al*. (2000) who noted an increase in dry matter content of plum must and carrot puree respectively where the latter was measured at 20°C after using an immobilized pectinase.

Total phenolics: Like in the case of °Brix values, fluctuations were also noted for total phenolic values (Table 2). However, we noted a significance rise in total phenolics for samples treated with enzymes as compared to the control with a mean difference of 137.5µg ml^{-1} (GAE). Figure 1 (Folin-Ciocalteau Gallic Acid Standard Curve) was used to estimate the total phenolics. The disadvantages of an increased release of phenolics include a poorer sensory quality of the juices, since increased phenolics have a tendency to enhance browning (Siebert, 1999) which calls for other measures to arrest thus an increase in corresponding cost (Will *et al*. 2000). We ascribe the increase in total phenolics to the degradation by pectinase activities on the cell wall and middle-lamina pectin of the fruit.

Demir *et al*. (2000) noted that besides increasing press capacity and the yield of juice up to 20%, pectinase has also a positive effect to achieve high carotene and dry matter content of the product.

Viscosity, Titratable Acidity (T.A) and turbidity: Juice extracted from enzyme treated pulp had a much lower viscosity as compared to the control. Equation (2) was used in calculating the relative viscosity of the juice and values reported as mPa.s (Table 2).

$$\text{Relative viscosity was given as}: \eta = \eta^{\circ}\frac{pxt}{p^{\circ}xt^{\circ}} \quad \ldots\ldots\ldots 2$$

Where η is the relative viscosity of sample, ηo is the viscosity of water (1.002mPa.s) at 20°C; pxt and $p^{\circ}xt^{\circ}$ are the sample and water densities respectively each multiplied by their flow time in an Ostwald viscometer.

Vaillant *et al*., 2001; Carneiro *et al*., 2002 and Kashyap *et al*., 2001; noted that the use of enzymes leads to the drop fruit juice viscosity, besides improving pressability of the pulp, disintegrating the jelly structure and making it easier to obtain the fruit juices thus higher yields. The treatment consisted of hydrolyzing soluble polysaccharides, which are responsible for the high viscosity of the juice, as well as liquefying the non soluble polysaccharides such as non soluble pectins, cellulose, hemicellulose and lignin from cell walls (Vaillant *et al*., 2001 and Grassin and Fauquembergue, 1996). Grassin and Fauquembergue (1996) noted that pineapple juice contains a small amount of pectin but a high hemicelluloses content type galactomannans, arabinogalactans and galactoglucomannans while insoluble parts are rich in arabinoxylans. These together with the presence of a natural gum (a neutral polysaccharide containing 70% sugars which are predominantly galactomannans (2.25 mannose : l

Table 2: Effect of enzyme treatment of pineapple pulp/residue on °Brix a, Titratable Acidity (T.A) b, viscosity c, turbidity d and total phenolics (T.P)[e]

			35°C		37.5°C		40°C	
Batch	Treatment	Parameters	Step 1	Step 2	Step 1	Step 2	Step 1	Step 2
I	Control	°Brix	14.0	4.8	14.6	4.2	14.5	4.6
		T.A (%)	1.128	0.433	1.139	0.318	1.130	0.280
		Viscosity	3.9	2.6	4.1	3.0	4.1	3.4
		Turbidity	1.518	1.538	1.501	1.522	1.504	1.524
		T.P*	437		400		450	
	Fluka	°Brix	14.5	4.6	15.2	5.4	15.0	5.1
		T.A (%)	1.152	0.398	1.140	0.342	1.143	0.335
		Viscosity	4.1	2.3	3.8	2.0	3.7	2.4
		Turbidity	0.828	1.128	0.816	1.424	0.845	1.548
		T.P*	594		600		591	
	Rapidase	°Brix	14.5	5.6	15.4	6.0	15.0	4.7
		T.A (%)	1.468	0.486	1.507	0.501	1.102	0.320
		Viscosity	3.8	2.0	3.6	1.9	3.0	2.4
		Turbidity	0.834	1.145	0.986	1.345	1.002	1.098
		T.P*	537		560		595	
	Mixture	°Brix	14.9	4.6	15.8	4.2	14.6	4.8
		T.A (%)	1.548	0.523	1.345	0.464	1.152	0.468
		Viscosity	3.1	2.0	3.8	2.1	4.1	2.4
		Turbidity	0.846	1.234	0.845	1.402	0.946	1.365
		T.P*	596		580		575	
II	Control	°Brix	14.8	4.0	14.9	4.5	14.1	4.8
		T.A (%)	1.130	0.318	1.128	0.433	1.139	0.315
		Viscosity	4.1	3.0	3.9	3.1	4.2	2.9
		Turbidity	1.423	1.556	1.545	1.654	1.496	1.502
		T.P*	425		440		435	
	Fluka	°Brix	15.0	5.4	15.2	4.8	14.9	5.3
		T.A (%)	1.150	0.402	1.152	0.335	1.149	0.398
		Viscosity	2.9	2.1	3.0	2.5	2.8	2.4
		Turbidity	0.845	1.326	0.946	1.502	0.875	1.524
		T.P*	597		601		597	
	Rapidase	°Brix	15.0	4.8	14.9	4.1	14.6	5.0
		T.A (%)	1.334	0.364	1.402	0.432	1.156	0.338
		Viscosity	2.6	2.2	2.6	2.4	2.9	2.3
		Turbidity	0.845	1.153	0.966	1.075	0.927	1.112
		T.P*	600		595		610	
	Mixture	°Brix	15.1	5.0	14.9	4.6	14.5	5.1
		T.A (%)	1.546	0.467	1.438	0.344	1.152	0.467
		Viscosity	2.8	2.0	2.9	2.1	3.0	2.6
		Turbidity	0.846	1.323	0.845	1.024	0.892	1.009
		T.P*	600		612		615	
III	Control	°Brix	14.5	4.0	14.1	4.4	14.3	4.2
		T.A (%)	1.342	0.456	1.167	0.334	1.153	0.289
		Viscosity	4.1	3.0	3.7	2.6	4.0	2.8
		Turbidity	1.235	1. 645	1.436	1.626	1.507	1. 562
		T.P*	435		437		497	
	Fluka	°Brix	15.0	5.1	14.8	4.9	14.5	5.2
		T.A (%)	1.153	0.364	1.150	0.398	1.402	0.335
		Viscosity	3.0	2.4	2.7	2.3	2.9	2.5
		Turbidity	0.867	1.143	0.976	1.075	0.872	1.098
		T.P*	605		600		630	
	Rapidase	°Brix	15.0	5.2	15.1	5.0	14.9	4.8
		T.A (%)	1.152	0.280	1.156	0.345	1.238	0.404
		Viscosity	2.4	2.0	2.7	2.2	2.9	2.3
		Turbidity	0.895	1.486	0.852	1.503	0.879	1.152
		T.P*	590		584		623	
	Mixture	°Brix	15.0	5.2	15.2	4.6	14.7	4.6
		T.A (%)	1.554	0.543	1.522	0.436	1.465	0.476
		Viscosity	3.1	2.6	2.6	2.3	2.9	2.7
		Turbidity	0.867	1.456	0.896	1.290	0.982	1.109
		T.P*	602		598		635	

*μg ml^{-1} (GAE),

a. The percentage of sugar in the juice was expressed as °Brix.

b. As percent citric acid (g/100 ml juice).

c. Relative viscosity reported as mPa.s and measured at 20°C.

d. Absorbance was measured at 650 nm.

e. Total Phenolics reported as μg ml^{-1} Gallic Acid Equivalents (GAE).

Table 3: Average score on a 5 point Hedonic scale on organoleptic properties of the extracted juice

Batch	Treatment	Sweetness	Tartness	Colour	Odour*	Overall Acceptability
I	Control	4.7	3.2	4.7	2.8	4.2
	Fluka	4.0	4.1	3.6	2.4	3.6
	Rapidase	4.4	4.5	4.0	2.7	4.0
	Mixture	3.8	4.6	3.4	2.4	3.9
II	Control	4.6	3.4	4.5	2.9	4.6
	Fluka	4.2	4.3	4.0	2.5	3.4
	Rapidase	4.6	4.4	4.4	2.8	4.1
	Mixture	3.4	4.5	3.6	2.3	3.8
III	Control	4.2	4.2	4.6	2.8	4.1
	Fluka	3.7	4.4	3.8	2.2	3.4
	Rapidase	4.4	4.0	4.6	2.9	3.8
	Mixture	3.2	4.7	3.8	2.2	3.7

*On a 3-point Hedonic scale while the rest on a 5-point Hedonic scale.

galactose) make the juice extremely vicious leading to a quick drop in the ultra-filtration flux rate thus the juice production process. This explains why Rapidase which contains high galactomannanases and xylanases activities but low pectinases activities gave good results in regard to viscosity as was visualized with the frequency of membrane change during microfiltration of the juice (data not shown). Figure 2 shows the mean apparent viscosity values of juice across the treatments in batches.

Enzyme treated pulps did show higher titratable acidity as compared to untreated ones. Titratable Acidity (T.A) as % citric acid was calculated as given in equation (3) in accordance with the method of James (1995).

$$T.A(\%) = \frac{Tx192x10}{3x1000} \ldots\ldots\ldots 3$$

Where:

T is the mean titre (in ml) of 0.1M sodium hydroxide solution required to neutralize the acidity in 10.00 ml of the pineapple juice while 192 is the molecular weight (relative molecular mass) of citric acid.

Perez-Magarino and Gonzalez-SanJose (2000) and Bhardwaj *et al.* (2005) reported an increase in titratable acidity using commercial pectolytic enzymes on grape and plum musts respectively as compared to untreated samples. However, higher titratable acidity brings with it problems associated with decreased juice pH as a result of increasing acidity, basically a poor organoleptic property. Sairi (2005) showed pineapple juice having lower titratable acidity was better preferred compared to fresh juice following deacidification using electrodialysis with monopolar ion exchange membranes.

Juice clarity was also achieved by the use of enzymes as compared to the untreated samples. This was evident from turbidity measurements at 650 nm or testing for depectinization through visualizing the amount of sediment formed by adding 2 volumes of ethanol containing 0.5% HCL to 1 volume of juice at 30°C (Aehle, 2004). Perez-Magarino and Gonzalez-SanJose (2000) reported lower total pectin content in enzyme treated must than non-treated samples.

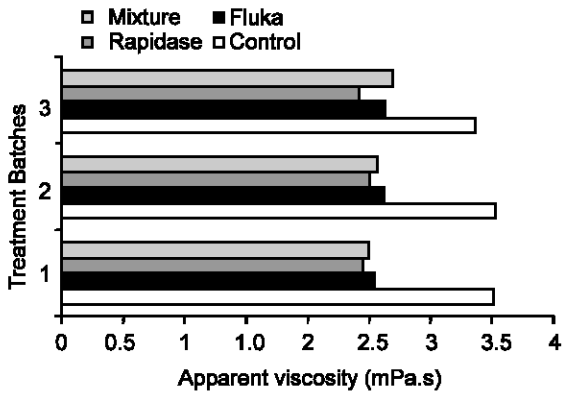

Fig. 2: Mean apparent viscosity values of juice across the treatments in batches

Sensory evaluation on Ready-to-serve (RTS): The sensory evaluation of the pooled pineapple Ready-to-serve (RTS) juice extracted at 37.5°C in each batch was conducted on a 5 point Hedonic scale to determine the acceptability of the processed juice. Generally, the control was considered more acceptable at an average score of 4.3 on the hedonic scale compared to a score of 4.0 the highest, among enzyme extracted juice. The rating for the juices in overall acceptability followed the order control > Rapidase > mixture > fluka. However, statistical analysis by one-way analysis of variance at ($p \leq 0.05$) did not show any significance difference. On the organoleptic properties assessed, the control scored high for sweetness, colour and odour but lowest in tartness which is associated with high level of acidity in the juice. Juice extracted using Rapidase scored better than that extracted with either Fluka or the Mixture of the two enzymes. Table 3 shows the average score on the tested organoleptic properties.

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