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Quality of soursop juice after pectinase enzyme treatment

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A pectinase enzyme was used at 0–0·1% concentrations to help in the extraction of soursop juice. The incubation times were from 1–3 h. The juices obtained were evaluated for yield, titratable acidity, pH, ascorbic acid, total soluble solids, viscosity, turbidity and sugar contents. Results indicated that the use of enzyme was beneficial as it increased the yield of juice by 41%. There were significant increases in acidity and Brix and significant decreases in viscosity and turbidity. There were no significant changes in the ascorbic acid and sugar contents. Sensory evaluation results indicate that the extracted juice was superior in quality to a commercial product.

INTRODUCTION

The use of enzymes in modifying the nature of food products has exciting potential. Besides the traditional uses of enzymes in cheese production, fruit juice clarification, alcohol production, meat tenderisation, etc., newer uses such as in corn syrup processing, vegetable protein hydrolysis, flavour modification and fruit juice processing are finding wide application (Braddock, 1981).

In fruit products enzymes have been exploited for the following purposes: to break down all polymeric carbohydrates such as pectin, hemicelluloses and starches, thus increasing the yield of juice by enabling better pressing of the pulp; to improve the yield of substances contained in fruit, e.g. acids, colouring or aroma substances; to clarify juices; to liquefy the entire fruit pulp for maximum yield (Gerhartz, 1990).

Rombouts & Pilknik (1971) reported that the use of pectinases during juice recovery resulted in viscosity reduction and less gel formation. The important advantage of viscosity reduction was that it allowed a high degree of juice concentration. Pectinase treatment of citrus juice liquids such as pulp wash is now common and concentrations above 70°Brix are achieved (Braddock Kesterson, 1975).

Current reports on the use of enzymes in tropical fruit juice processing are limited. The present work was carried out to determine the effects of using pectinase enzyme in the extraction and clarification of soursop juice. Soursop has great potential for juice processing and the parameters examined were the levels of enzymes to be used and time of incubation with regard to the quality of the juice obtained.

MATERIALS AND METHODS

Fruits

Soursop fruits (*Annona muricata* L.) were obtained from Bukit Tinggi, Bentong, Pahang at the mature green stage. The fruits were first allowed to ripen at room temperature before being processed. Fruits did not ripen at the same time; therefore, those that ripened earlier had to be processed first and frozen at −20°C to maintain their quality while waiting for the remaining fruits to ripen within the next 1—2 days.

Preparation of puree

Fruit pulps were separated from skin and seeds by hand and macerated using a Bhurr mill. Water was added in the ratio of 1 : 1 to facilitate the maceration process as well as to help extract more juice from the pulp. The maceration process was repeated four times in order to get a smooth-textured puree.

Enzyme treatment of puree

After maceration, the puree was pasteurised at 85—90°C for 10 min to inactivate the natural enzymes or microbes present, then cooled down to 37—40°C before the addition of test enzyme

The enzyme used was Pectinex Ultra SPL (ULT) (Novo Nodisd Ferment Ltd., Switzerland) at 0, 0·025%, 0·05%, 0·075% and 0·1% levels with three different incubation times of 1, 2 and 3 h. Triplicate samples (200 g puree each) were used for each treatment. The amount of enzyme used was based on weight of puree

(v/wt). The puree was incubated in a water bath at 35—40°C. After incubation, the enzyme was inactivated by heating the sample at 85—90°C for 10 min.

Yield of juice

Pulpy material was first separated from the juice by centrifugation at 3000 rpm for 15 min. The juice was then filtered through a Whatman No. 4 filter paper using a Buchner funnel attached to an aspirator model A-35 (Japan) giving a vacuum pressure of 25 mm Hg. The volume of juice obtained from each sample was measured using a 200-ml volumetric flask and the weight of juice was recorded.

Determination of total titratable acidity and pH

The total titratable acidity was determined by titrating 15 ml of diluted juice (20:80; juice:distilled water) with 0·1N NaOH. The acidity value was calculated as malic acid content. The pH value was determined using 15 ml of undiluted juice and a digital pH meter. Buffer solutions at pH 4·0 and 7·0 were used as reference.

Determination of ascorbic acid, soluble solids content and viscosity

Ascorbic acid was determined using the 2,6-dichlorophenol indophenol dye titration method (Ranggana, 1977). Total solids content was determined using a hand refractometer model Otago with a scale of 0—32°Brix. The viscosity measurement was made using a Brookfield viscometer. Sample juice was filled to the 200-ml level mark in a 200-ml beaker and a reading was taken using spindle No. 2 rotated at 30 rpm. A standard with 98 cps was used as reference.

Determination of turbidity and reducing sugars

Turbidity was measured using a turbidometer model TRM-L, Drott (Austria) by pouring the juice into a cuvette measuring 5·5 cm × 20 cm, and taking a direct turbidity reading in national turbidity units (NTU). Hexamethylene tetramine solution was used as standard. Sugars were determined by high performance liquid chromatography (Hunt *et al.*, 1977). The standard solutions used were glucose, sucrose, fructose and maltose.

Sensory analysis

The clear juice obtained was subjected to sensory analysis to assess its flavour characteristics. A quantitative descriptive analysis technique (Sidel *et al.*, 1983) was used and comparisons were made with a freshly prepared juice and a commercial juice, both of which were non-enzyme treated. Prior to evaluation, both the clear juice and the fresh juice had to be prepared so that they had the same chemical properties as the commercial juice (Brix = 14°; acidity = 0·21% and pH = 3·23). Evaluations were made by 20 panellists. Data were analysed by analysis of variance.

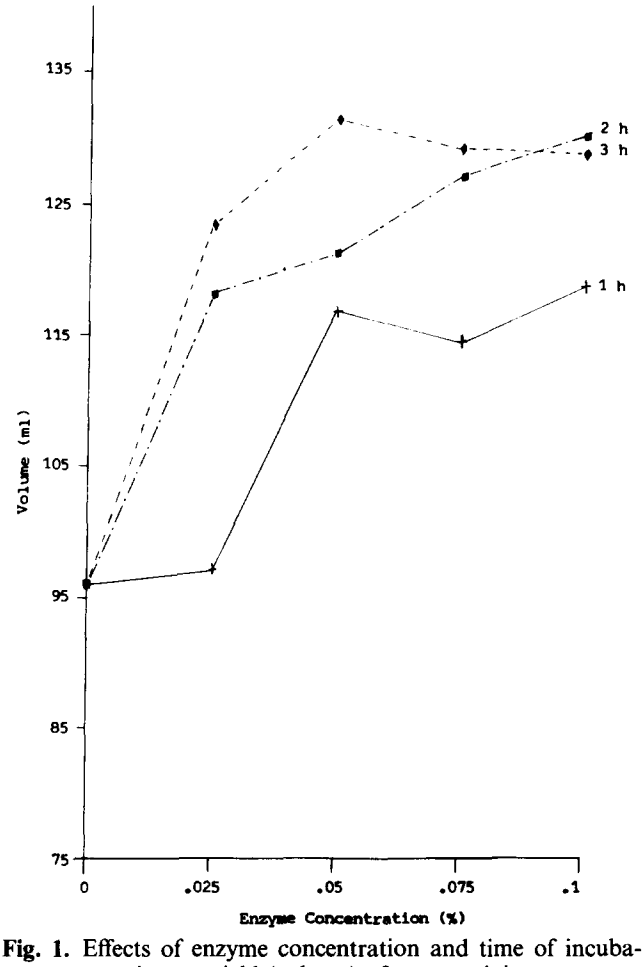

Fig. 1. Effects of enzyme concentration and time of incubation on yield (volume) of soursop juice.

RESULTS AND DISCUSSION

Yield of juice

The effects of using different enzyme concentrations and time of incubation on the yield of juice are shown in Fig. 1. Generally, the larger the amount of enzyme used and the longer the time of incubation, the greater the yield of juice obtained. Untreated pulp gave a low yield. From 200 g of puree, the juice obtained was only about 95·4 ml, while the treated sample produced a maximum of 134·4 ml. The difference is about 41%.

For the purpose of comparison the juices obtained were weighed and recorded as in Fig. 2. The maximum amount of juice was obtained using a 0·05% enzyme concentration with 3 h incubation. Higher levels of enzymes used with the same incubation time did not increase the yield. The time of incubation is also a main factor to be considered. It is apparent that when the enzyme level was increased to 0·075%, a reasonably high yield could be obtained with only a 2-h incubation period. The economics of the whole process then has to be based on-time of incubation versus the extra cost of enzyme used.

Titratable acidity and pH

The total titratable acidity for enzyme-extracted juice increased significantly from 0·41% to 0·49% for the 1, 2

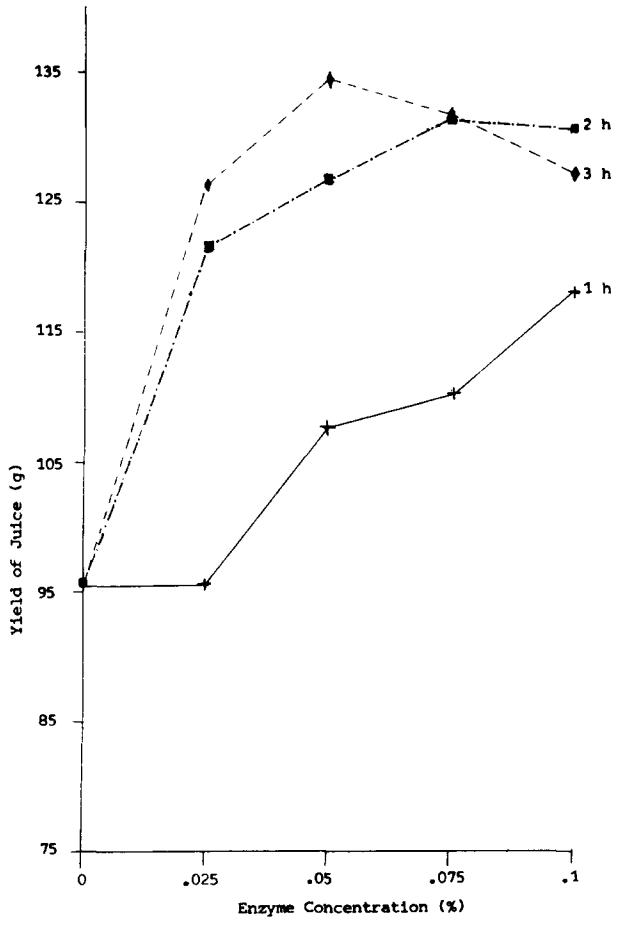

Fig. 2. Effects of enzyme concentration and time of incubation on yield (weight) of soursop juice.

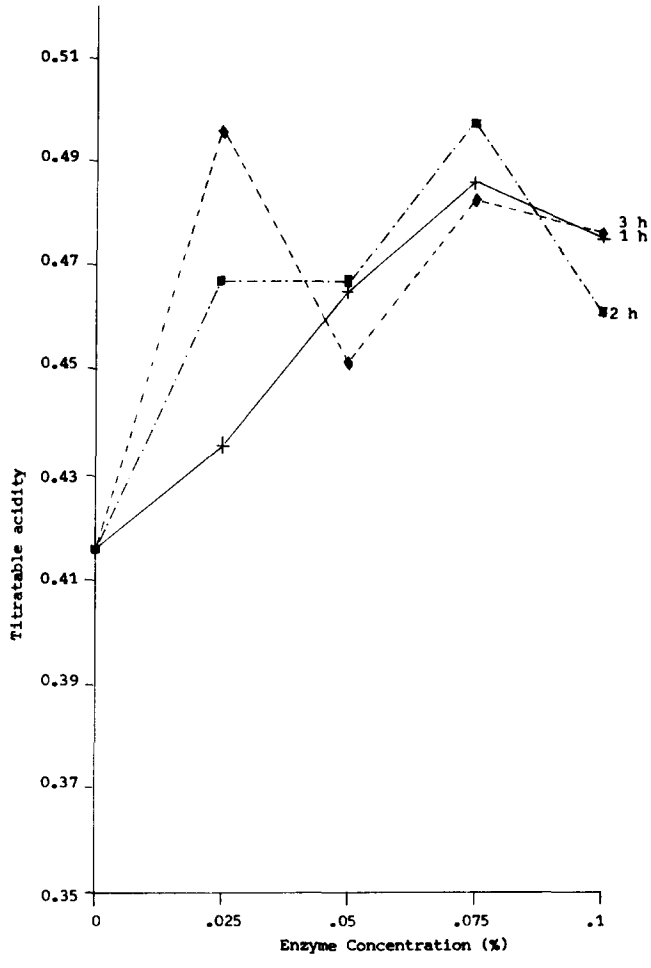

Fig. 3. Effects of enzyme concentration and time of incubation on titratable acidity of juice.

and 3 h of incubation at the 0·025 enzyme concentration but not at 0·05, 0·075 and 0·1% concentrations (Fig. 3). The acidity values at the latter three concentration levels were almost the same for the three incubation times.

This indicates that, when 0·025% enzyme was used, a longer time was needed to increase the acidity of juice, but if the enzyme concentrations were increased to 0·05%, 0·075% and 0·1%, only 1 h incubation was sufficient to increase the acidity of the juice. The dominant acid in soursop is malic acid. According to Paull & Chen (1983), the malic acid content of mature soursop fruit increased 7 times after the fruit was harvested compared to only 4 times for citric acid. Atmayer *et al.* (1942) reported that ripe soursop fruit contained 0·7—1·04% malic acid. The value is high compared to fruits in this experiment. From the processing standpoint, acidity is important in determining the quality of fruit juices. It contributes to the development of flavour by maintaining a proper sugar—acid ratio; it provides a thirst-quenching effect by encouraging saliva formation in the mouth (Taylor, 1990); and at the same time it helps to act as a mild preservative.

The original pH value of the puree was 3·73 which was within the optimum range 2·5—5·5 for the pectinase reaction (Fellers, 1990). However, after the enzyme reaction, the pH value changed as shown in Fig. 4. For each level of enzyme used, the decrease, as a result of incubation time, was not significant for the first hour of incubation. Only after 2 h and 3 h of treatment did the pH values decrease significantly from the pH of the original juice. Nevertheless, the values for 2 and 3 h incubation are almost the same.

According to Woodroof & Phillips (1981) a decrease in pH from 4·5 to 3·0 could increase the shelf life about 3 times. In this experiment, enzyme treatment only decreased the pH of juice from 3·7 to 3·58; thus the keeping quality was expected to remain the same.

The effects of enzyme concentration and time of incubation on the ascorbic acid, soluble solids content and viscosity of juice are shown in Table 1. The ascorbic acid content in soursop is normally around 26·9 mg/100 g which is about equal to the content in starfruit (25·8 mg/g) (Siong *et al.*, 1988). In this case, the ascorbic acid content in the starting material was considered low (6·3 mg/100 g), probably because most of the nutrient had been lost during puree preparation and storage. The enzyme treatment did not seem to increase the ascorbic acid content significantly.

The use of enzyme at various levels significantly increased the soluble solids content from 6·8°Brix to 7·3°Brix within the first hour of incubation. Increasing the incubation time to 2 and 3 h did not cause any significant increase in the total soluble solids content. When the Brix values were compared with the acid val-

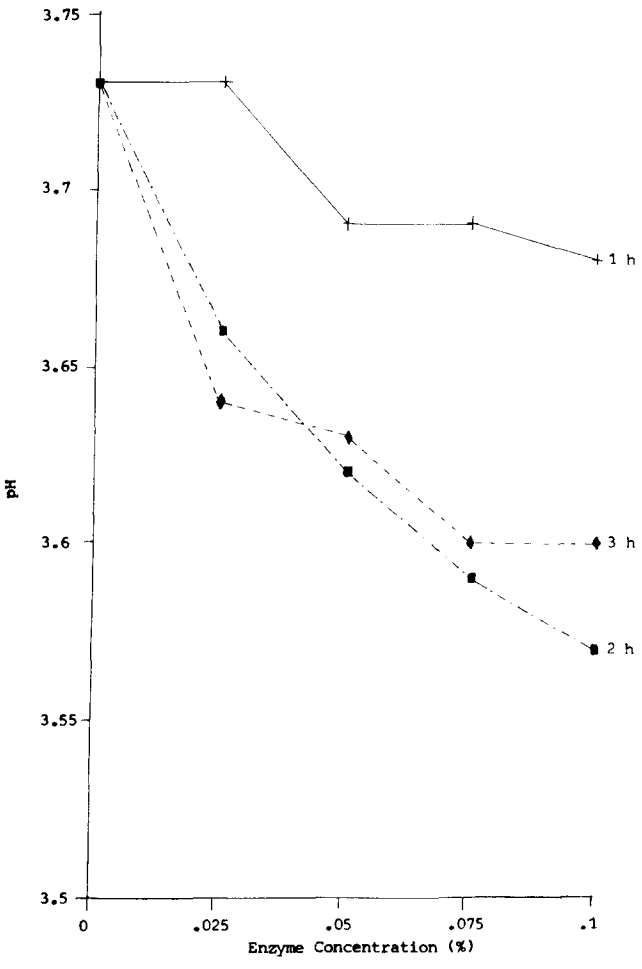

Fig. 4. Effects of enzyme concentration and time of incubation on pH of soursop juice.

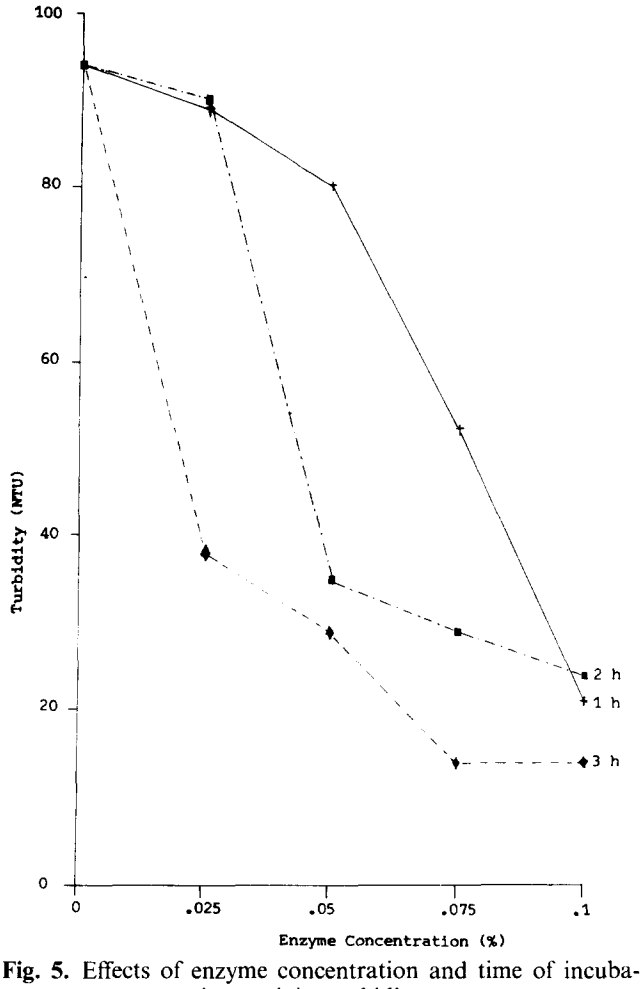

Fig. 5. Effects of enzyme concentration and time of incubation on juice turbidity.

ues it was found that, after enzyme treatment, the Brix/acid ratio decreased from 16·6 to 14·9. This is because the increase in Brix value was not in proportion to the increase in acid value. Nevertheless, for juice processing, a Brix/acid ratio of 14·9 is acceptable.

The viscosity of the juice after enzyme treatment had generally decreased. This was also noted in many of the studies reported earlier (Baker & Bruemmer, 1972; Braddock, 1981) and is due to the hydrolytic action of enzymes on the cellulosic and pectic materials present in the juice. Loss in viscosity is undesirable in ready-to-drink juices or concentrates because the product will lose its body and destabilisation of the colloidal sys-

Table 1. Effects of enzyme on the ascorbic acid, total soluble solids and viscosity of soursop juice

Incubation time (h)	Enzyme conc. (%)	Ascorbic acid (mg/100 g)	Total soluble solids (°Brix)	Viscosity (cps)
	0·00	$0·969^{a}$	$6·83^{d}$	$12·2^{a}$
	0·025	$1·16^{a}$	$7·17^{abc}$	$7·43^{bc}$
1	0·05	$1·20^{a}$	$7·20^{abc}$	$7·70^{b}$
	0·075	$1·14^{a}$	$7·23^{bc}$	$6·77^{bc}$
	0·1	$1·14^{a}$	$7·30^{a}$	$5·27^{ef}$
	0·00	$0·969^{a}$	$6·83^{d}$	$12·2^{a}$
	0·025	$1·93^{a}$	$7·27^{abc}$	$6·33^{d}$
2	0·05	$0·775^{a}$	$7·20^{abc}$	$5·10^{ef}$
	0·075	$0·833^{a}$	$7·23^{abc}$	$6·23^{cd}$
	0·1	$0·794^{a}$	$7·18^{abc}$	$5·33^{ef}$
	0·00	$0·969^{a}$	$6·83^{d}$	$12·2^{a}$
	0·025	$1·16^{a}$	$7·27^{ab}$	$4·67^{f}$
3	0·05	$1·14^{a}$	$7·30^{abc}$	$4·68^{f}$
	0·075	$1·01^{a}$	$7·10^{c}$	$5·03^{ef}$
	0·1	$1·13^{a}$	$7·23^{abc}$	$5·23^{ef}$

Data are means of three readings.
Numbers with the same superscripts within the same column are not significantly different at 0·05% level.

Table 2. Reducing sugar contents of soursop juice after enzyme extraction

Incubation time (h)	Enzyme conc. (%)	Fructose (%)	Glucose (%)	Sucrose (%)	Maltose (%)	Total (%)
	0·00	$2·98^{bc}$	$2·49^{c}$	$0·02^{a}$	$0·21^{f}$	5·70
	0·025	$2·94^{abc}$	$2·63^{b}$	$0·07^{a}$	$0·20^{f}$	5·84
1	0·05	$3·09^{a}$	$2·61^{b}$	$0·03^{a}$	$0·32^{e}$	6·05
	0·075	$3·07^{a}$	$2·67^{ab}$	$0·04^{a}$	$0·32^{e}$	6·10
	0·1	$3·02^{ab}$	$2·75^{a}$	$0·04^{a}$	$0·37^{e}$	6·18
	0·00	$2·98^{bc}$	$2·49^{c}$	$0·02^{a}$	$0·21^{f}$	5·70
	0·025	$3·02^{ab}$	$2·65^{b}$	$0·06^{a}$	$0·34^{e}$	6·07
2	0·05	$2·87^{bc}$	$2·62^{b}$	$0·04^{a}$	$0·35^{ce}$	5·88
	0·075	$2·88^{bc}$	$2·63^{b}$	$0·04^{a}$	$0·37^{e}$	5·92
	0·1	$2·89^{bc}$	$2·67^{ab}$	$0·06^{a}$	$0·37^{bce}$	5·59
	0·00	$2·98^{bc}$	$2·49^{c}$	$0·02^{a}$	$0·21^{f}$	5·70
	0·025	$2·84^{c}$	$2·67^{ab}$	$0·06^{a}$	$0·38^{ab}$	5·95
3	0·05	$2·81^{c}$	$2·64^{b}$	$0·05^{a}$	$0·38^{a}$	5·88
	0·075	$2·83^{c}$	$2·64^{b}$	$0·04^{a}$	$0·39^{a}$	5·90
	0·1	$2·86^{bc}$	$2·64^{b}$	$0·03^{a}$	$0·40^{a}$	5·93

Data are means of three readings.
Numbers with the same superscripts within the same column are not significantly different at 0·05% level.

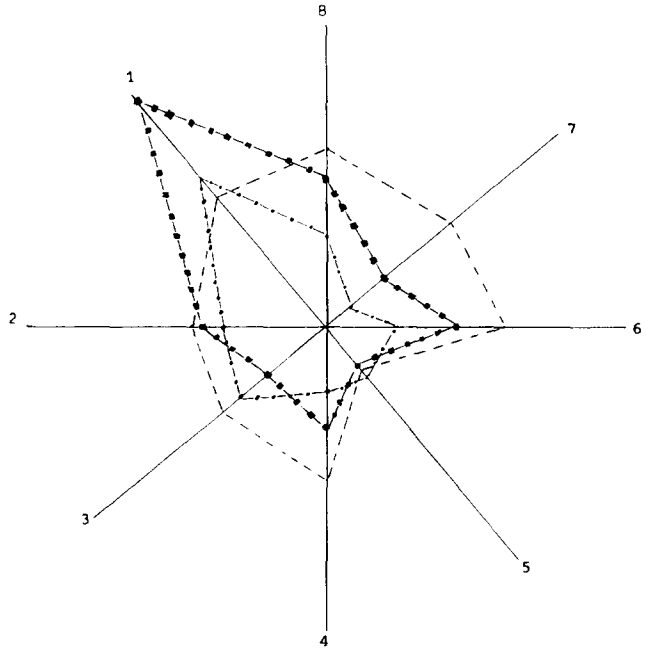

Fig. 6. Cobweb presentation of flavour characteristics in soursop juices. Flavour characteristics: (1) natural soursop; (2) burnt; (3) medicinal; (4) vomitting; (5) flat; (6) fermented; (7) rotten fruit; (8) biting. —•— Enzyme treated juice; —▪— fresh juice; - - - - commercial juice.

tems will result. However, in juice concentration it is important to reduce viscosity in order to increase the efficiency of the concentration process (Rombouts & Piknik, 1971). In this experiment, loss in viscosity helped to facilitate the filtration process. The decrease in viscosity was 62% (compared to that of untreated juice). There were no significant differences between the effects of different levels of enzymes and times of incubation. Screenath *et al.* (1987) reported that the use of enzyme Pectinex Ultra reduced the viscosity of mango juice by 74%. Turbidity tests on juice from untreated samples showed that, even after centrifugation, the sample showed high turbidity, indicating the presence of suspended colloidal particles within the system. Upon enzyme treatment, the colloidal system was offset; solid particles settled out resulting in clear juice. Turbidity readings decreased with the increase in levels of enzymes and times of incubation used (Fig. 5).

Results of sugar analysis on soursop juice before and after enzyme treatment are shown in Table 2. The types of sugars found in soursop were fructose, glucose, sucrose and maltose. The amounts of fructose and glucose were 100-fold higher than the amounts of sucrose and maltose present. However, the total sugar content reached 5—7%, which is expected of mildly acidic fruits similar to the Vietnamese guava (Yusof & Mohamed, 1987). Generally, the use of different levels of enzymes did not significantly increase the amounts of sugars, even though the Brix values increased as reported in the earlier section. In food processing it is often assumed that the total soluble solids (Brix) reading denotes the sugar content. In this experiment it is clear that the Brix reading could also be contributed to by acid content.

The panellists' average scores on the various flavour characteristics of the clear juice, compared to the control (freshly prepared juice) and the commercial juice, are presented in a cobweb configuration in Fig. 6. Among the three samples it was clear that the fresh juice had a significantly high natural soursop flavour. The enzyme-treated juice, however, had more flavour than the commercial juice; the latter was rated as having a significantly high fermented and rotten fruit flavour. The fact that the enzyme-treated juice was also rated low for the rest of the flavour characteristics (burnt, medicinal, vomiting and biting) also indicated that it was potentially acceptable to consumers.

CONCLUSIONS

The use of enzyme Pectinex Ultra SPL was found to be beneficial since it was able to increase the yield of juice extracted by 41%. The mechanism of action is that the enzyme degrades the polysaccharide materials present in the pulp, causing them to be broken into smaller fractions and thus facilitating filtration. For optimum yield, the level of enzyme was 0·075% with an incubation time of 2 h. At the same time, the use of enzyme resulted in significant increase of acidity and Brix; in fact a lower concentration (0·05%) and a shorter incubation time (1 h) sufficed for this. It also caused a significant reduction in viscosity and turbidity. The reduction in viscosity facilitated the filtration process by reducing clogging of the filter. Sensory results indicate that the enzyme-extracted juice had better flavour characteristics than a commercial juice.

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