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The optimisation of conditions for the extraction of banana juice with a pectolytic enzyme preparation

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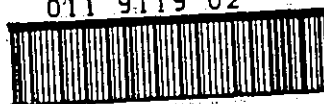


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THE OPTIMISATION OF CONDITIONS
FOR THE EXTRACTION OF BANANA JUICE
WITH A PECTOLYTIC ENZYME PREPARATION

by

FAUSTO PENAFIEL VILLARREAL

A Master's thesis
submitted in partial fulfilment of the requirements
for the award of
Master of Philosophy of the Loughborough University of Technology

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ABSTRACT

The aim of the work was the optimisation of conditions for the extraction of juice from surplus bananas using pectolytic enzymes. Juice extracted in this way could be used to make other products, e.g. beverages, wine, vinegar. Bananas were supplied by Geest Industries Ltd., and were of known history and variety (Cavendish). The samples were allowed to ripen under controlled conditions of temperature and relative humidity, and were enzyme-treated at the same degree of ripeness as determined by sugar content ($18.0 \pm 0.3\%$ w/w). A commercial blend of pectolytic enzymes, known as "Ultrazym 100 Special", was used. This preparation has pectinesterase, polygalactaronease and pectic lyase activity. The activity of this preparation in breaking down the pectic substances, to release the juice from the banana pulp, was investigated by assessing the following parameters: incubation temperature, incubation time, enzyme concentration and sodium metabisulphite concentration. The sodium metabisulphite was added in order to retard browning reactions in the extracted juice.

A maximum yield of juice of 74.6% w/w was obtained with an enzyme preparation concentration of 0.05% w/w, an incubation temperature of 50°C, an incubation time of 60 minutes and with a sodium metabisulphite concentration of 0.06% w/w. This yield

represents an 83.9% efficiency in terms of sugar extraction.

Variation in the different reaction parameters produced only slight changes in pH and titratable acidity of the extracted juice, however a significant positive correlation was found between the juice yield and juice titratable acidity. Total sugar in the extracted juice was found to vary over a small range (21.5 to 23.5% w/v), and no correlation was found between the total sugar concentration and the yield of extracted juice. Reducing sugar in the extracted juice was found in a far higher proportion than sucrose in the whole series of experiments.

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1. INTRODUCTION

1.1 General Introduction

The world production of bananas in 1966 was about 23,700,000 metric tonnes, of which 62% was produced in Central and South American countries.¹ Ecuador, for example, produces an average of 2,000,000 metric tonnes per annum of bananas for international trade, of which about 60% is exported.² The remaining 40% is regarded as "surplus" in that they are not exported due to handling and marketing problems.² Finding ways of utilising these surpluses is obviously of great commercial importance.

Attempts have been made to extract the sugar-rich juice of surplus bananas for use as a beverage or for fermentation to give vinegar and other products. The remaining extracted pulp can then be used for animal feed. However, the method involving juice extraction by pulping the peeled fruit with water and separation of the aqueous suspension by centrifugation of the slurry, gives a product with very low sugar content (about 3.5% w/v)³, which is lower than the minimum required (8% w/v) for producing minimum strength vinegar.⁴ A slightly modified

method uses the direct fermentation of the slurry. However a slow rate of fermentation results because of the formation of a thick layer of purée on top of the reaction mixture.⁴

In order to obtain the banana juice for vinegar production and other processes pectolytic enzymes have been used to break down the cell walls and hence release more of the sugar solution. However the long periods of incubation normally used in the reported procedures would tend to make industrial application uneconomical.

1.2 Classification of bananas^{1,5}

Bananas have been classified into two main groups within the family of Musaceae: Musa and Ensete. The former contains more than 32 distinct species and at least 100 sub-species. The banana Musa group contains all of the edible varieties which have been sub-divided into four groups: Eumusa, Rhodochlamys, Australimusa and Callimusa. The Eumusa group contains the major edible species of bananas in two sections: Musa acuminata and Musa balbisiana. Cavendish and Gross Michel varieties fall within the Musa acuminata section. These are the principal varieties in the world banana trade.

1.3 Constituents of banana fruit and changes during ripening

1.3.1 Coloured constituents

The green peel of unripe fruit contains 51.7 to 102.9 μg of Chlorophyll per g of fresh weight. This decreases to nil in the peel of ripe fruit. The total amount of the yellow carotenoid pigments, both xanthophylls and carotenes, was found to remain almost constant during ripening.⁶ The total carotenoid content of the peel of ripe Cavendish banana was found to be 5 to 6 $\mu\text{g/g}$ of fresh weight and of the pulp was found to be 0.6 to 1.0 $\mu\text{g/g}$ of fresh weight.⁷ In another investigation, the amount of xanthophylls was found to range from 5 to 7 $\mu\text{g/g}$ of fresh weight and the amount of carotenes from 1.5 to 3.5 $\mu\text{g/g}$ of fresh weight.⁶

Scales of peel colour have been devised as rough guides to ripeness of banana fruits. The colour index numbers 1 and 7 correspond to a green and to a yellow flecked brown peel colour, respectively, with intermediate index numbers corresponding to the intermediate peel colour.⁶

1.3.2 Acidic constituents

The presence of thirty non-volatile organic acids has been reported in Gross Michel pulp, among which malic and

citric were found to be the major constituents.⁸ Shikimic, quinic, glycolic, succinic, pyroglutamic, tartaric, lactic, glutamic, aspartic, glutaric and several keto-acids have been identified as minor constituents.^{5,8}

The pH of banana pulp at the green peel stage is generally about 5.3 ± 0.3 , but this falls during ripening to 4.5 ± 0.3 at the fully ripe yellow peel stage.⁶ The titratable acidity at the fully ripe stage varies over a wide range, for example, the titratable acidity (cm^3 of 1M NaOH to neutralize 100 grams of pulp) at the fully ripe stage has been reported as 3.66 for Gross Michel, 4.07 for Lady Finger, 4.20 for Lacatan, 7.20 for Plantain and 5.60 for Red Banana varieties.⁶

As ripening proceeds the titratable acidity of the pulp increases to a peak and then declines. For example, a maximum titratable acidity of 5.8 cm^3 was found for Cavendish bananas just prior to harvesting (yellow green peel colour stage.) This figure declined to 1.2 cm^3 when the harvested fruits were kept at $25 \pm 2^\circ\text{C}$ for 5 days, being then at an "eating ripe stage."⁹

1.3.3 Starch and sugar

Bananas at the green peel colour stage have a starch content of 20 to 25%, but this drops to 1 to 2% at the fully ripe stage. At the same time total sugars rise from 1 to 2% to about 15 to 20%.⁵ The amount of amylose in the starch of some Indian varieties, after about 100 days of growth, was found to be 20.8% (Basrai), 20.0% (Harichai), 20.2% (Rajeli), 21.2% (Lalkel) and 19.8% (Safed Velchi), the rest being amylopectin.¹⁰

The major sugars found in banana pulp are sucrose and the reducing sugars glucose and fructose. In addition, small amounts of maltose and a trisaccharide, 6^G- β -fructosylsucrose, have been reported.^{11,12} The pulp of Cavendish banana was found to contain amounts of 0.5%, 5.0%, 8.4% and 10.3% w/w reducing sugars at four stages of ripeness classified as green, yellow green, yellow and speckled brown, respectively. In the same samples sucrose amounts of 0.5%, 5.1%, 8.9% and 8.2% w/w were found. Total sugars, therefore amounted to 1.0%, 10.1%, 17.3% and 18.5% w/w at the above ripeness categories, respectively.¹¹ Although total sugars in the fully ripe stage are normally in the range 17 to 20%, in Indian Cavendish a total sugar content, at the fully ripe

stage, of 14.5% was reported.⁹

Gas liquid chromatographic separation of banana sugars has given an estimate of 2.6% w/w fructose, 3.0% w/w glucose and 9.0% w/w sucrose, but the variety of the bananas and ripeness were not specified in this report.¹³

A paper chromatographic analysis of Ghanaian bananas showed the presence of fructose, glucose and sucrose and respective mean values of 2.5, 4.1 and 9.0% w/w were reported, but again the variety and ripeness were not specified.¹⁴

1.3.4 Pectic substances, cellulose, hemicellulose and lignin

Cellulose, hemicellulose and lignin make up the cell walls and fibres of fruit tissue,¹⁵ whereas pectic substances are cementing materials, localized mainly in the middle lamella and the primary cell wall of higher plants.¹⁶ The major types of pectic substances are protopectin, pectin and pectic acid. Both protopectin and pectin are highly methylated, whereas pectic acids have relatively few methyl ester groups (see Figure 1 page 11). Pectin consists of short chain soluble material which is normally obtained by the break down of the long-chain insoluble protopectin.^{16,17}

As ripening advances pectin rises at the expense of the protopectin. For example, at the green peel colour stage the pectin content (as calcium pectate) of some varieties is 0.21% (Lady Finger), trace (Lacatan) and 0.04% (Red Banana) with a corresponding protopectin content (as calcium pectate) of 0.50, 0.59 and 0.87%, whereas at the fully ripe stage pectin rises to 0.68, 0.46 and 0.63%, respectively and protopectin declines to 0.35, 0.34 and 0.48% respectively.⁶

The major enzymes found in banana are those that hydrolyse the methyl esters to carboxyl groups; these are known as pectinesterases (PE). The presence of 3 molecular forms of PE in ripe banana pulp has been reported. They may be isolated by successive extractions with water, 0.15M NaCl and 0.15M NaCl at pH 7.5.^{5,18} A further three forms of PE have been detected in banana pulp by electrophoresis.¹⁹ Some investigators reported that there is an increase in the total PE activity, when the peel turns from green to yellow.¹⁸ The presence in banana pulp of enzymes that degrade pectic acids (polygalacturonases) has also been reported.¹⁹

It has been reported that the pulp of unripe bananas contains 0.49% lignin, 0.15% cellulose and 0.21% hemicellulose, giving a total of 0.85% fibre. During ripening lignin

decreases slightly, cellulose increases slightly and hemicellulose decreases to about half the content at the green peel colour stage. Accordingly, the pulp of ripe bananas contains 0.46% lignin, 0.19% cellulose and 0.12% hemicellulose, giving a total of 0.77% fibre. Part of the sugar and acid accumulation during ripening may be due to the hydrolysis of hemicellulose.⁶

1.3.5 Phenolic compounds and enzymatic browning

It has been reported that polyphenoloxidase (PPO) occurs in particularly high concentrations in mushrooms, potato tubers, peaches, apples, avocados, tea leaves, coffee beans, tobacco leaves and bananas.²⁰ Thomas and Nair in 1971, extracted PPO from pulp of Cavendish, Poovan and Red Banana varieties at "75%" maturity.²¹ The activity of the enzyme was tested with some mono- and diphenolic substrates. Among the monophenolic compounds, PPO showed maximum activity with *p*-cresol followed by tyramine. Similarly, PPO activity was assayed with some diphenolic substrates among which the enzyme was most active towards dopamine.²¹ Dopamine (3,4-dihydroxyphenylethylamine) has been found to be the primary substrate of banana PPO in browning reactions.⁵ Banana pulp and peel

appear to contain about 8 $\mu\text{g/g}$ fresh weight and 700 $\mu\text{g/g}$ fresh weight of dopamine at harvest respectively. As ripening advances these amounts rise by about 30-60%. A sharp decrease in dopamine was found at a ripeness level identified by yellow flecked brown peel colour.⁵

Total phenolic compounds in ripe banana fruits have been found to amount to about 0.53g/100g dry weight. The presence of the pelargonidin-3-monoside in banana pulp has been reported.²² The leucoanthocyanin containing fraction extracted from the bananas was shown to be the only pectinesterase inhibiting compound present.¹ Polymerization of tannins and concomitant loss of astringency has been observed in the ripening banana. It has been suggested that the tannins may be the main enzyme controlling compounds in the ripening banana.^{1,5}

1.3.6 Moisture

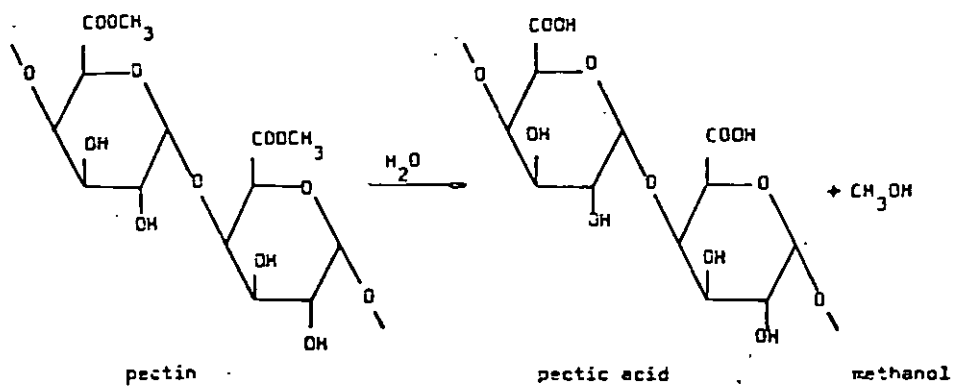
Moisture contents of 71% and 75% were found in Cavendish pulp at green unripe and flecked brown ripe stage, respectively.¹¹ The additional 4% moisture might be regarded as a product of carbohydrate break down in respiration, although water might also migrate from the peel to the pulp by osmosis caused by the rise in pulp sugar concentration.⁶ Moisture

content, in general, increases during ripening from about 69% (\pm 4%) to about 74% (\pm 3%).⁵

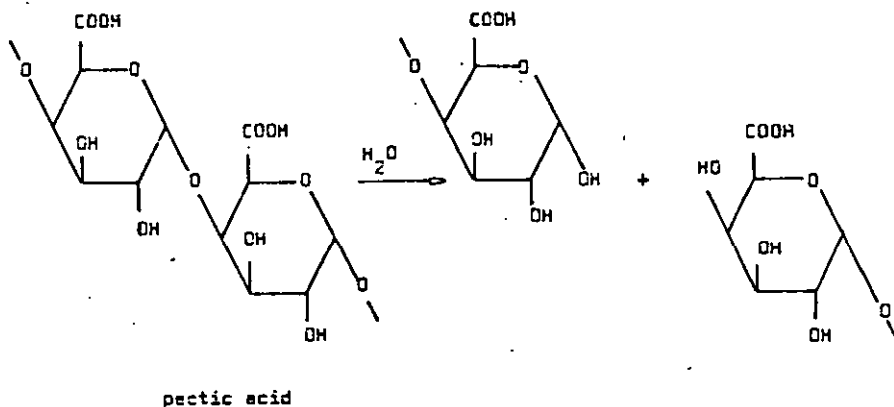
1.4 Application of pectolytic enzymes

1.4.1 Degradation of pectic substances by pectolytic enzymes

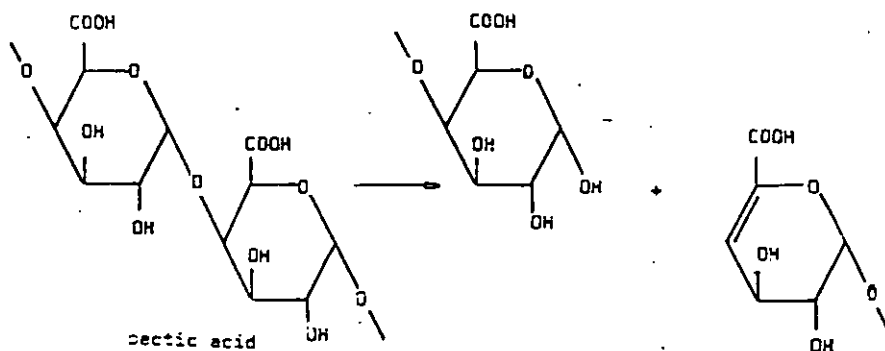
There are three major types of pectolytic enzymes: pectinesterases, hydrolases and lyases. Pectinesterase catalyzes the hydrolysis of methyl ester groups, transforming pectin into low methoxyl pectin and pectic acid (see Figure 1). Hydrolases catalyzes the hydrolysis of the α -1,4-glycosidic linkages of pectic substances. Pectin is hydrolyzed by the hydrolase known as polymethylgalacturonase, whereas pectic acid and low methoxyl pectin is hydrolyzed by the hydrolase known as polygalacturonase (see Figure 1). Some hydrolases split the glycosidic linkages at random (endo enzymes) others only at the reducing end of the chain (exo enzymes). Lyases catalyze the hydrolysis of the glycosidic linkages in pectic substances by a β -elimination mechanism. Pectin lyases split glycosidic bonds in pectin, whereas pectic lyases split glycosidic bonds in pectic acids and low methoxyl pectin (see Figure 1). Both endo and exo enzymes have been isolated.^{16, 17, 20, 23} It has been reported that commercial



Hydrolysis of pectin by pectinesterase to give pectic acid (and low methoxyl pectin)



Hydrolysis of pectic acid by polygalacturonases



Hydrolysis of pectic acid by pectic lyase

Figure 1 Reactions catalysed by pectolytic enzymes

pectolytic enzyme preparations contain, in addition to varying amounts of the above mentioned pectolytic enzymes, also cellulases, xylanases, arabanases, galactanases, glycosidases, proteases, esterases and oxido-reductases.¹⁶

1.4.2 Effect of pectolytic enzymes on banana pulp

Several investigators have examined the effect of pectolytic enzymes on the extraction of juice from banana pulp. Yields from Cavendish banana pulp of 82 and 78.5% w/w of juice have been obtained respectively with an enzyme powder and with a mouldy bran produced by growing a strain of Aspergillus aureus. The banana pulp was incubated with the enzyme preparation at 27-29°C for 16 hours and the juice was extracted by racking the enzyme treated pulp for a further 24 hours.²⁴

A liquid enzyme concentrate obtained from Aspergillus niger has been used to extract the juice from Cavendish banana pulp. The pulp was treated with 0.5-0.6% v/w enzyme and incubated at room temperature (25-30°C) for 16-18 hours. The juice was extracted by racking the enzyme-treated pulp for a further 24 hours. Under these conditions yields of

77-88% w/w of juice have been claimed. Similar treatments have been applied to other varieties of banana and yields of 70-79% (Poovan), 78% (Ladam), 60% (Sambrani), 82% (Rasabale), 77% (Chandrabale), 71% (Nendran), 85% (Pottiveran), 75% (Patti Palayan), 89% (Robusta) and 74% (Kadubale) were reported.^{17,25} By increasing the enzyme concentration to 0.75% v/w followed by a further addition of 0.25% v/w of enzyme to clarify the extracted juice, the reaction time could be reduced to 4 hours, although the racking time for juice separation remained unchanged (24 hours).²⁶ By increasing the enzyme concentration to 1.5% v/w, the addition being made all in one step, the reaction time could be reduced to 3 hours to obtain, it is claimed, a 91% w/w non-clarified juice.²⁶ A variation in the method, which involves heating the pulp to 70°C, cooling to 40°C and addition of the liquid enzyme at 1.0% v/w level, was found to give, it is claimed, 87% w/w juice with 4 hours time of reaction.²⁶

The addition of potassium metabisulphite to the heated pulp was found to increase the yield of non-clarified juice, but the concentration of the preservative used was not specified.²⁶ This promising result suggests that further

investigation should be carried out into the effect of added sulphur dioxide on the extraction process.

Table 1 gives the yields and chemical characteristics of juice extracted from pulp by various processes.^{17,24,25,26} However, the data are insufficient to be able to draw firm conclusions about the effect that varying reaction conditions and enzyme concentration has on the pectolytic enzyme activity and on the chemical characteristics of the extracted juice, and further research needs to be performed in this area. The effect of added sulphur dioxide on the pectolytic enzyme activity and on the juice chemical characteristics also needs investigation. Table 1 shows, however, that there were significant differences in the yield and chemical characteristics of the juice extracted from different varieties of bananas and hence it is very important to indicate the varieties used in reporting work in this area.

1.4.3 Factors affecting pectolytic enzyme activity

It has been reported that pectolytic enzymes are generally active over a range of 5 to 55°C, although it has been pointed out that different preparations might vary in their stability to heat.^{27,28}

Table 1

Yield and some chemical characteristics of banana juice extracted with pectolytic enzymes

Enzyme	Enzyme concentration	Incubation temperature, °C	Incubation time hr.	Banana variety	Juice yield % w/w	pH	Titrateable acidity % w/v as malic acid	Total sugar % w/v	Reference no.
Enzyme powder	-	27-29	16	Cavendish	82.0	4.76	0.41	-	
Mouldy bran	-	27-29	16	Cavendish	78.5	4.46	0.41	-	24
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Cavendish	77.0-88.0	4.3-5.3	0.37-0.69	17.0-18.0	17&25
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Poovan	70.0-79.0	-	0.58	13.0-14.0	
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Ladam	78.0	-	0.73	22.0-24.0	
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Sambrani	60.0	-	0.73	21.0	
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Rasabale	82.0	-	0.69	18.5-19.0	
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Chandrabale	77.0	-	0.47	13.0	
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Nendran	71.0	-	0.76	22.0-28.0	
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Pottiveran	85.0	-	0.91	24.0	
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Patti Palayan	75.0	-	0.62	20.0	
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Robusta	89.0	-	0.45	18.0	
Liquid enzyme	0.5-0.6% v/w	25-30	15-18	Kadubale	74.0	-	0.50	21.0	
Liquid enzyme	0.75% on pulp + 0.25% v/w on juice	25-30	4	Cavendish	85.0	-	0.44-0.46 ⁽²⁾	-	
Liquid enzyme	1.5% v/w	25-30	3	Cavendish	91.0 ⁽¹⁾		0.54 ⁽²⁾	-	26
Liquid enzyme	1.0% v/w	40	4	Cavendish	87.0		-	-	

(1) Non-clarified juice.

(2) As anhydrous citric acid.

Plant and fungal pectinesterases have been found to show a maximum activity when the pH of the substrate falls within the limits of 4.3 to 8.5 and 3.6 to 5.0 respectively, whereas optimum pH ranges for plant and fungal polygalacturonases were found to be 2.5 to 6.0 and 3.5 to 6.5 respectively.²⁷

It has been reported that tannins, formaldehyde and oxidized apple juice may inactivate polygalacturonases.²³ Phenolic compounds such as catechins, leucoanthocyanins, chlorogenic acid and p-coumaric acid have been shown to inhibit pectinesterase activity.²⁹ Enzyme treatment of about 20 hours was required to extract the juice from apple pulp and this long treatment was attributed to the probable inhibition of the pectolytic enzymes by the low molecular weight apple polyphenols. The time of incubation was reduced to 1-2 hours, when polyvinylpyrrolidone, which binds polyphenols, was added to the pulp.³⁰

Moderate concentrations of sulphur dioxide, used to preserve grape pulp showed no inhibitory effect on pectolytic enzymes.³¹ The commercial enzyme "Pectinol-O" was found to be unaffected in grape juice extraction by up to 1000 ppm of sulphur dioxide.³² The effect of sulphur dioxide on pectolytic enzymes used in banana juice extraction has not been investigated systematically.

1.5 The present work

The investigation reported in this dissertation was undertaken in order to determine the optimum reaction conditions and enzyme concentration for banana juice extraction using pectolytic enzymes, and in particular to try to reduce the reaction times reported in earlier investigations in order to avoid spoilage and in order to make the process more commercially viable.

In previous work it was suggested that metabisulphite should be used when banana juice is extracted. There appears to be a lack of data on the possible inhibitory effect of metabisulphite on pectolytic enzyme activity. The present investigation includes a systematic study on these possible inhibitory effects.

Very little systematic data has been reported for the chemical changes that banana juice may undergo during extraction under different conditions. In the present investigation, extracted banana juice was analysed in order to assess systematically the chemical changes which take place during enzymatic extraction under different conditions.

2. MATERIALS AND METHODS

2.1 Materials

Banana fruits of the Musa Cavendish variety were supplied by Geest Industries Limited and were stored at 13°C and 95% relative humidity.

The fungal pectolytic enzyme preparation "Ultrazym 100 Special" was used, supplied by Novo Enzyme Products Limited.

2.2 Sampling of banana fingers and determination of sugar concentration

Banana fingers at the fully yellow peel ripeness stage were sampled at each end and in the centre according to Figure 2. All the banana sampled were of length (L) between 14 and 16.5 cm.

Two methods of determining the sugar concentration were used.

Sugar concentration - Method 1³³: The banana pulp sample was chopped into fine pieces and transferred to a 250 cm³ conical flask with 3 x 50 cm³ hot 95% methanol. The flask was held in a boiling water bath for 1 hour and

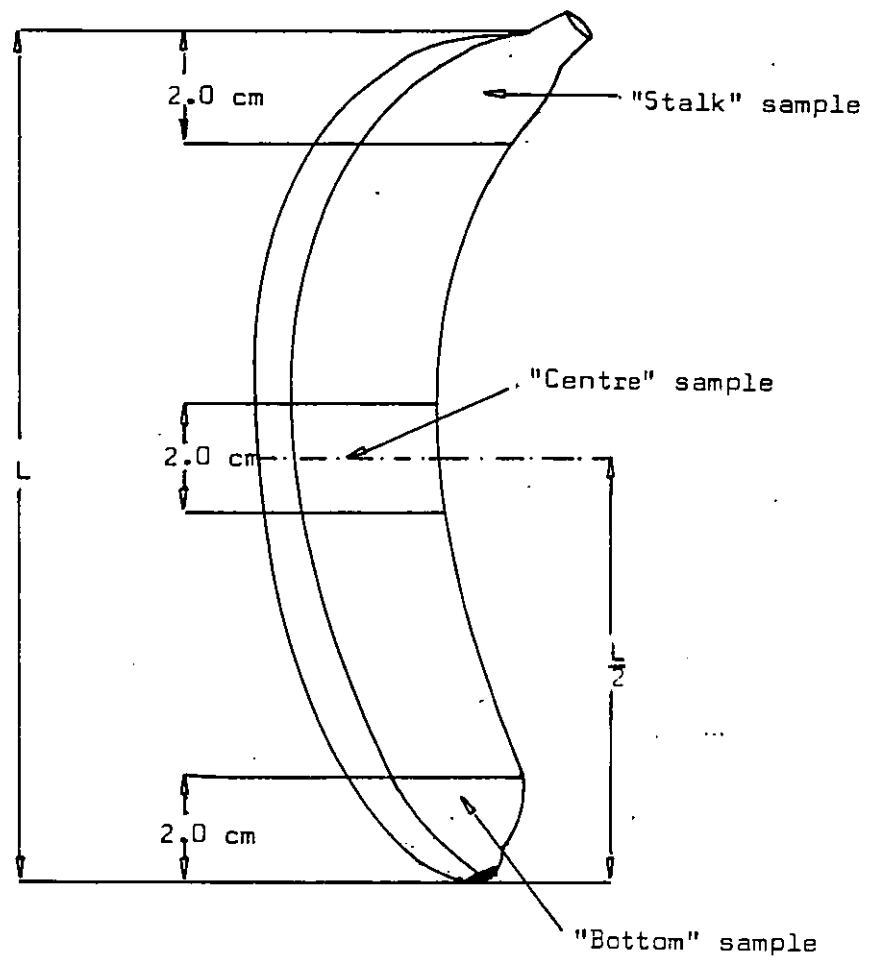


Figure 2 Sampling of pulp for sugar determination along the length of the banana finger.

the solution was decanted. The solids were blended with 80% methanol in a kitchen blender at $\frac{1}{2}$ speed for 4 minutes. The blended material was held on boiling water bath for 0.5 hour and filtered. The methanol was removed by heating on a boiling water bath and the remaining aqueous extract was clarified with saturated neutral lead acetate solution and made up to 200 cm³ with distilled water. The reducing sugar content and sucrose content were obtained by the standard Lane-Eynon procedure.³³

Sugar concentration - Method 2: The banana pulp sample was blended with 50 cm³ hot 95% ethanol in a kitchen blender at $\frac{1}{2}$ speed for 4 minutes. After consecutive filtrations and washings with hot 95% ethanol, the solids were blended with 50 cm³ hot 80% ethanol at $\frac{1}{2}$ speed for 4 minutes. After consecutive filtrations and washings, the filtrates were collected and the alcoholic extract was clarified with saturated neutral lead acetate solution. The sugar solution was filtered and the precipitated material was washed with 3 x 25 cm³ hot 80% ethanol.¹⁴ The ethanol was removed under vacuum on a rotary evaporator at 40°C and the aqueous extract remaining was made up to 200 cm³.³⁴ The reducing sugar content and sucrose content were obtained by the standard Lane-Eynon procedure.³³

2.3 Extraction of banana juice

The centre portion of each peeled finger (see Figure 2) was removed and analysed for sugar concentration as described above using Method 2. Provided the sugar concentration was in the range $18.0 \pm 0.3\%$ w/w then the remainder of the banana finger was used for juice extraction.

Standard juice extraction method: The banana finger, minus the centre portion, was weighed and sliced, and to the slices sodium metabisulphite (S% w/w) was added as a fine powder. The mixture was blended in a kitchen blender for 4 minutes at $\frac{1}{2}$ speed and the enzyme preparation was added (E% w/w) to a weighed amount of the puree. After mixing thoroughly, the enzyme treated puree was incubated in a thermostatically controlled water bath (for M minutes at T °C). The enzyme-treated puree was then centrifuged in pre-weighed centrifuge tubes at 4000 r.p.m. for 25 minutes. The juice was decanted immediately after centrifuging and was stored at 11°C until analysed. The centrifuge tubes plus residues were weighed.

Four series of experiments were carried out. In the first series the sodium metabisulphite concentration (S% w/w) was fixed at 0.06% w/w, the enzyme concentration (E % w/w) was fixed at 0.05% w/w, the incubation time (M minutes) was fixed at 120 minutes and the following incubation temperatures (T °C) were used: 20°C, 30°C, 40°C, 50°C and 60°C.

In the second series of experiments the sodium metabisulphite concentration was fixed at 0.06% w/w, the enzyme concentration was fixed at 0.05% w/w, the temperature was fixed at 50°C (the optimum temperature of incubation found in the first series of experiments) and the following incubation times were used: 30, 45, 60 and 120 minutes.

In the third series of experiments, the sodium metabisulphite concentration was fixed at 0.06% w/w, the temperature was fixed at 50°C, the time was fixed at 60 minutes (the optimum time of incubation found in the second series of experiments) and the following enzyme concentrations were used: 0, 0.01, 0.03, 0.05, 0.07 and 0.10% w/w.

In the fourth series of experiments the temperature was fixed at 50°C, the time was fixed at 60 minutes, the enzyme concentration was fixed at 0.05% w/w (the optimum enzyme

concentration found in the third series of experiments) and the following sodium metabisulphite concentrations were used: 0, 0.02, 0.04 and 0.06% w/w.

2.4 Analysis of banana juice

The juice extracted in each experiment was analysed for pH, titratable acidity, reducing sugars and sucrose. The pH of the juice was measured by the procedure described in the international standard ISO 1842 for liquid products.³⁵ Titratable acidity of juice was determined by the visual method of titration described in BS 4288.³⁶ Reducing sugars and sucrose were determined by the standard Lane-Eynon method.³³

3. RESULTS AND DISCUSSION

3.1 Selection of bananas according to sugar concentration

In order to obtain meaningful results for the juice extraction optimisation experiments and for the chemical analysis of the extracted juice samples, it was considered of primary importance that the bananas used were at exactly the same degree of ripeness as defined by sugar concentration. It was decided therefore that each banana finger should be sampled for sugar concentration before extraction and should be used only if the sugar concentration was within defined limits.

A preliminary investigation was necessary to determine if the sugar concentration varied along the length of banana fingers, so that the position for sugar concentration sampling could be decided, and also in order to define the sugar concentration limits in the subsequent extraction experiments. It was considered that if the sugar concentration varied along the length of the finger then the variation would be most apparent between the ends (which tended to remain green longer during ripening) and the centre and hence the banana fingers were sampled as shown in Figure 2, page 19. Two methods of determining the sugar concentration were used

that differed in the sugar extraction steps. The first method was the standard general AOAC method (when starch is not to be determined),³³ the second method was the modified procedure suggested by Dako et al., and Southgate in order to minimise inversion of sucrose.^{14,34}

The results for 10 banana fingers (2 from each of 5 hands) using Method 1 are given in Table 2 and for a second group of 10 bananas (2 from each of 5 hands) using Method 2 are given in Table 3. All the banana fingers used were at the fully yellow peel ripeness stage.

From Table 2 it can be seen that there is no significant difference (99% probability level) between the total sugar concentration at the three sampling positions. This is confirmed by the data for total sugar concentration in Table 3. It was therefore decided that banana fingers to be used in the juice extraction experiments would only be sampled at the centre position in order to obtain an estimate of the total sugar concentration throughout the finger. It was further decided that banana fingers to be used in the juice extraction experiments should have a sugar concentration of $18.0 \pm 0.3\%$ w/w as determined by Method 2. Method 2 was preferred to Method 1 since the sugars did not appear to be

Table 2 Percentage sugar concentration in the "stalk", "centre" and "bottom" samples by Method 1

Finger No.	"Stalk" samples			"Centre" samples			"Bottom" samples		
	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars
	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w
1	9.6	6.1	15.7	8.8	6.3	15.1	9.5	5.5	15.0
2	9.2	5.9	15.1	8.0	6.3	14.3	9.2	6.5	15.7
3	9.2	5.7	14.9	8.0	6.3	14.3	8.3	6.2	14.5
4	9.1	7.1	16.2	8.9	8.4	17.3	8.8	6.8	15.6
5	8.6	7.2	15.8	9.4	7.8	17.2	7.8	7.5	15.3
6	8.8	7.2	16.0	8.0	8.4	16.4	7.6	7.7	15.3
7	9.2	5.9	15.1	8.8	5.5	14.3	7.2	7.3	14.5
8	10.0	5.1	15.1	9.6	6.1	15.7	8.0	6.2	14.2
9	9.2	5.1	14.3	9.6	5.5	15.1	8.8	5.9	14.7
10	9.6	6.6	16.2	8.8	5.8	14.6	8.8	6.3	15.1
Means	9.2	6.2	15.4	8.8	6.6	15.4	8.4	6.6	15.0

Student t-test on total sugars data indicates that there is no significant difference (99% probability) between the total sugar concentrations at the "stalk", "centre" and "bottom" sections.

Table 3 Percentage sugar concentration in the "stalk", "centre" and "bottom" samples by Method 2

Finger No.	"Stalk" samples			"Centre" samples			"Bottom" samples		
	Reducing sugars	Sucrose	Total Sugars	Reducing sugars	Sucrose	Total sugars	Reducing sugars	Sucrose	Total sugars
	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w
1	4.0	13.7	17.7	4.3	13.4	17.7	4.2	13.5	17.7
2	5.7	12.5	18.2	6.2	12.1	18.3	5.4	12.9	18.3
3	6.2	12.1	18.3	6.9	11.3	18.2	5.9	12.3	18.2
4	7.5	10.6	18.1	7.6	10.7	18.3	7.4	10.6	18.0
5	7.1	10.8	17.9	7.5	10.6	18.1	7.2	10.9	18.1
6	5.0	12.0	17.0	5.2	12.4	17.6	4.9	12.3	17.2
7	5.4	12.5	17.9	5.5	12.4	17.9	5.4	12.6	18.0
8	6.5	11.4	17.9	6.4	11.4	17.8	6.4	11.3	17.7
9	7.5	10.3	17.8	7.1	10.8	17.9	7.4	10.3	17.7
10	7.0	11.0	18.0	6.8	11.5	18.3	6.9	11.1	18.0
Means	6.2	11.7	17.9	6.3	11.7	18.0	6.1	11.8	17.9

Student t-test on total sugars data indicates that there is no significant difference (99% probability) between the total sugar concentrations at the "stalk", "centre" and "bottom" sections.

fully extracted by the Method 1 procedure (total sugars averaged 15.3% w/w by Method 1 and 17.9% w/w by Method 2).

In addition it can be seen that the milder conditions employed in Method 2 led to less inversion than Method 1.

3.2 Optimisation of conditions for the extraction of banana juice

Optimisation of conditions for the extraction of banana juice was assessed in the experiments reported here in terms of maximisation of yield of extracted juice, or, where two or more reaction conditions gave the same maximum yield, then also in terms of minimum incubation time or minimum enzyme concentration. Optimisation from an economic standpoint involves other factors and will probably differ from optimisation based on maximum yield, however the data given in this dissertation can be used in assessing the optimum economic conditions. For example, if the length of incubation time is critical from an economic standpoint, then the results given in this dissertation can be used to balance shorter incubation time, and hence lower yields, against running costs.

A pectolytic enzyme preparation was used to break down the pulp to permit juice extraction. The activity of the enzyme preparation is influenced by incubation temperature, incubation time, enzyme concentration and possibly by the presence of metabisulphite, which is added as a preservative for the banana juice.²⁶ When the incubation temperature was investigated, in the first series of experiments, the incubation time, enzyme concentration and metabisulphite concentration were kept constant at levels which appeared (from preliminary experiments) to be near to the optimum. This gave an optimum incubation temperature, which was then used in subsequent experiments. Similarly in the second series of experiments, an optimum incubation time was determined, which was used in subsequent experiments. Similarly in a third series of experiments an optimum enzyme concentration was determined, which was used in subsequent experiments. In the fourth series of experiments, an optimum level of metabisulphite was determined, although the optimum here was defined in terms of maximum juice yield obtainable with a sulphur dioxide concentration of 300 ppm and below. This limit of 300 ppm of sulphur dioxide was set

because levels much above this figure can lead to off-flavours if the juice is used for beverages, and also because many countries limit the sulphur dioxide in fruit juice to about this level.³⁷

3.2.1 Variation in incubation temperature

In table 4 the juice yields obtained at different incubation temperatures are given and the data is plotted in Figure 3. It can be seen that the amount of juice obtained increased with temperature up to 50°C, at which a mean yield of 74.5% w/w juice was obtained. At 60°C the yield was lower than at 50°C, although it was still higher than the yields obtained at 20 and 30°C. This suggests that there is partial inactivation of the enzyme preparation presumably due to heat denaturation at 60°C. These results are in agreement with previous reports that pectolytic enzymes are generally active over a range of 5 to 55°C.^{27,28}

Table 4 Effect of incubation temperature on juice yield and chemical characteristics of the juice⁽¹⁾

Incubation temperature °C	Total sugar in raw bananas ⁽²⁾ % w/w	Juice yield ⁽³⁾ % w/w	pH	Titrateable acidity % w/v as malic acid	Reducing sugar % w/v	Sucrose % w/v	Total sugar % w/v
20	17.7	66.5	4.7	0.33	22.1	0.3	22.4
20	17.7	65.8	4.7	0.33	21.0	0.3	21.3
20	18.0	67.0	4.7	0.33	21.3	0.2	21.5
20	17.8	67.3	4.7	0.33	20.9	0.3	21.2
20	17.7	67.1	4.7	0.33	21.0	0.1	21.1
Means	17.8	66.7 ^a	4.7	0.33	21.3	0.2	21.5
Standard deviations		0.54					0.47
30	18.1	70.1	4.6	0.33	22.6	0.5	23.1
30	17.9	68.5	4.7	0.32	21.6	1.1	22.7
30	17.7	68.0	4.7	0.31	22.0	0.4	22.4
30	17.7	69.6	4.6	0.33	21.7	0.5	22.2
30	18.0	70.7	4.6	0.34	23.0	0.3	23.3
Means	17.9	69.4 ^b	4.6	0.33	22.2	0.6	22.7
Standard deviations		1.00					0.41
40	18.0	72.5	4.6	0.33	22.1	0.2	22.3
40	17.7	72.4	4.6	0.33	22.0	0.2	22.2
40	17.7	70.3	4.6	0.32	22.2	0.2	22.4
40	17.9	70.3	4.6	0.32	22.4	0.2	22.6
40	18.0	72.2	4.6	0.34	21.8	0.8	22.6
Means	17.9	71.5 ^c	4.6	0.33	22.1	0.3	22.4
Standard deviations		1.02					0.16
50	18.0	74.7	4.6	0.36	21.8	0.9	27.7
50	17.8	74.2	4.6	0.36	27.1	0.1	22.2
50	17.7	73.7	4.6	0.36	21.5	0.5	22.0
50	18.1	75.5	4.6	0.36	22.0	0.5	22.5
50	17.9	74.5	4.6	0.36	21.8	0.5	22.3
Means	17.9	74.5 ^d	4.6	0.36	21.6	0.5	22.3
Standard deviations		0.59					0.24
60	18.3	71.2	4.6	0.34	23.1	0.2	23.3
60	18.2	70.0	4.6	0.35	23.1	0.1	23.2
60	18.0	69.6	4.6	0.36	23.1	0.1	23.2
60	18.1	70.8	4.6	0.36	23.4	0.2	23.6
60	17.9	70.6	4.6	0.34	22.2	0.2	22.4
Means	18.1	70.4 ^e	4.6	0.35	23.0	0.2	23.1
Standard deviations		0.57					0.40

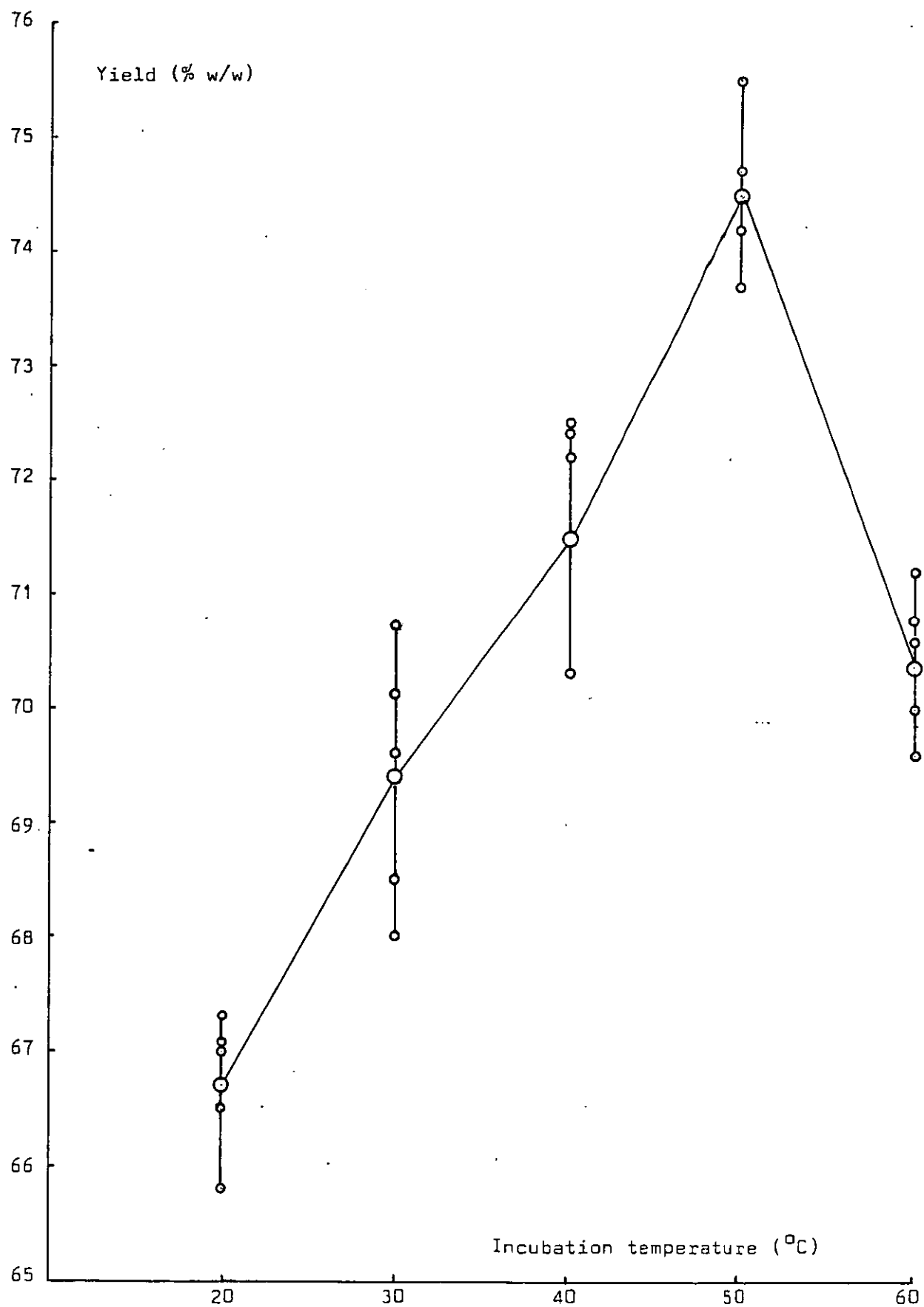
(1.) Incubation time = 2 hours. Enzyme concentration = 0.05% w/w. Sodium metabisulphite concentration = 0.06% w/w.

(2) Control stage of ripeness. Specification: 18.0 ± 0.3% w/w total sugars.

(3) Mean values for juice yield are not significantly different (95% level Student t-test) if they have the same superscript.

Figure 3 Effect of incubation temperature on juice yield.

Individual values (o) and means of 5 replicates (O)



3.2.2 Variation in incubation time

In Table 5 the juice yields obtained at different incubation times are given and the data is plotted in Figure 4. It can be seen that with a 30 minute incubation time a mean yield of juice of 71.7% w/w was obtained, which represents 96.1% of the maximum obtained at 60 minutes of 74.6% w/w. Increasing the incubation time to 120 minutes did not significantly affect the juice yield. It may well be that from an economic standpoint an incubation time of 30 minutes, with a juice yield slightly less than the maximum, might be the most commercially viable conditions.

The incubation times reported here are considerably less than those used by previous workers, e.g. 3-4 hours²⁶ or even as high as 15 to 18 hours.^{17,24,25}

3.2.3 Variation in enzyme concentration

In Table 6 the juice yields obtained at different enzyme concentrations are given and the data is plotted in Figure 5. It can be seen that 25.9% w/w juice was obtained from banana pulp with no enzymatic treatment. An enzyme concentration of 0.01% w/w increased the yield of juice by about 2.4 times.

Table 5 Effect of incubation time on juice yield and chemical characteristics of the juice⁽¹⁾

Enzyming time minutes	Total sugar in raw bananas ⁽²⁾ % w/w	Juice yield ⁽³⁾ % w/w	pH	Titrateable acidity % w/v as malic acid	Reducing sugar % w/v	Sucrose % w/v	Total sugar % w/v
30	18.1	72.0	4.6	0.32	22.3	0.1	22.4
30	17.7	71.6	4.6	0.32	21.9	0.2	22.1
30	18.3	71.1	4.6	0.33	23.5	0.2	23.7
30	17.8	72.2	4.7	0.33	22.1	0.1	22.2
30	18.1	71.8	4.7	0.33	22.2	0.3	22.5
Means	18.0	71.7 ^a	4.6	0.33	22.4	0.2	22.6
Standard deviations		0.38					0.58
45	18.0	73.2	4.6	0.36	22.2	0.4	22.6
45	18.1	72.8	4.6	0.34	22.5	0.3	22.8
45	17.7	72.8	4.6	0.34	21.8	0.2	22.0
45	17.9	73.0	4.6	0.35	22.7	0.2	22.9
45	18.0	72.0	4.6	0.34	22.5	0.3	22.8
Means	17.9	72.8 ^b	4.6	0.35	22.3	0.3	22.6
Standard deviations		0.41					0.32
60	18.1	75.0	4.6	0.36	23.0	0.0	23.0
60	18.1	75.5	4.6	0.36	23.0	0.0	23.0
60	17.8	73.8	4.6	0.35	21.6	0.6	22.2
60	18.2	74.7	4.6	0.36	23.6	0.0	23.6
60	18.0	74.1	4.6	0.36	23.1	0.0	23.1
Means	18.0	74.6 ^c	4.6	0.36	22.9	0.1	23.0
Standard deviations		0.61					0.45
120	18.0	74.7	4.6	0.36	21.8	0.9	22.7
120	17.8	74.2	4.6	0.36	22.1	0.1	22.2
120	17.7	73.7	4.6	0.36	21.5	0.5	22.0
120	18.1	75.5	4.6	0.36	22.0	0.5	22.5
120	17.9	74.5	4.6	0.36	21.8	0.5	22.3
Means	17.9	74.5 ^c	4.6	0.36	21.8	0.5	22.3
Standard deviations		0.59					0.24

(1) Incubation temperature = 50°C. Enzyme concentration = 0.05% w/w. Sodium metabisulphite concentration = 0.06% w/w.

(2) Control stage of ripeness. Specification: 18.0 ± 0.3% w/w total sugars.

(3) Mean values for juice yields are not significantly different (95% level Student t-test) if they have the same superscript.

Figure 4 Effect of incubation time on juice yield.

Individual values (o) and means of 5 replicates (O)

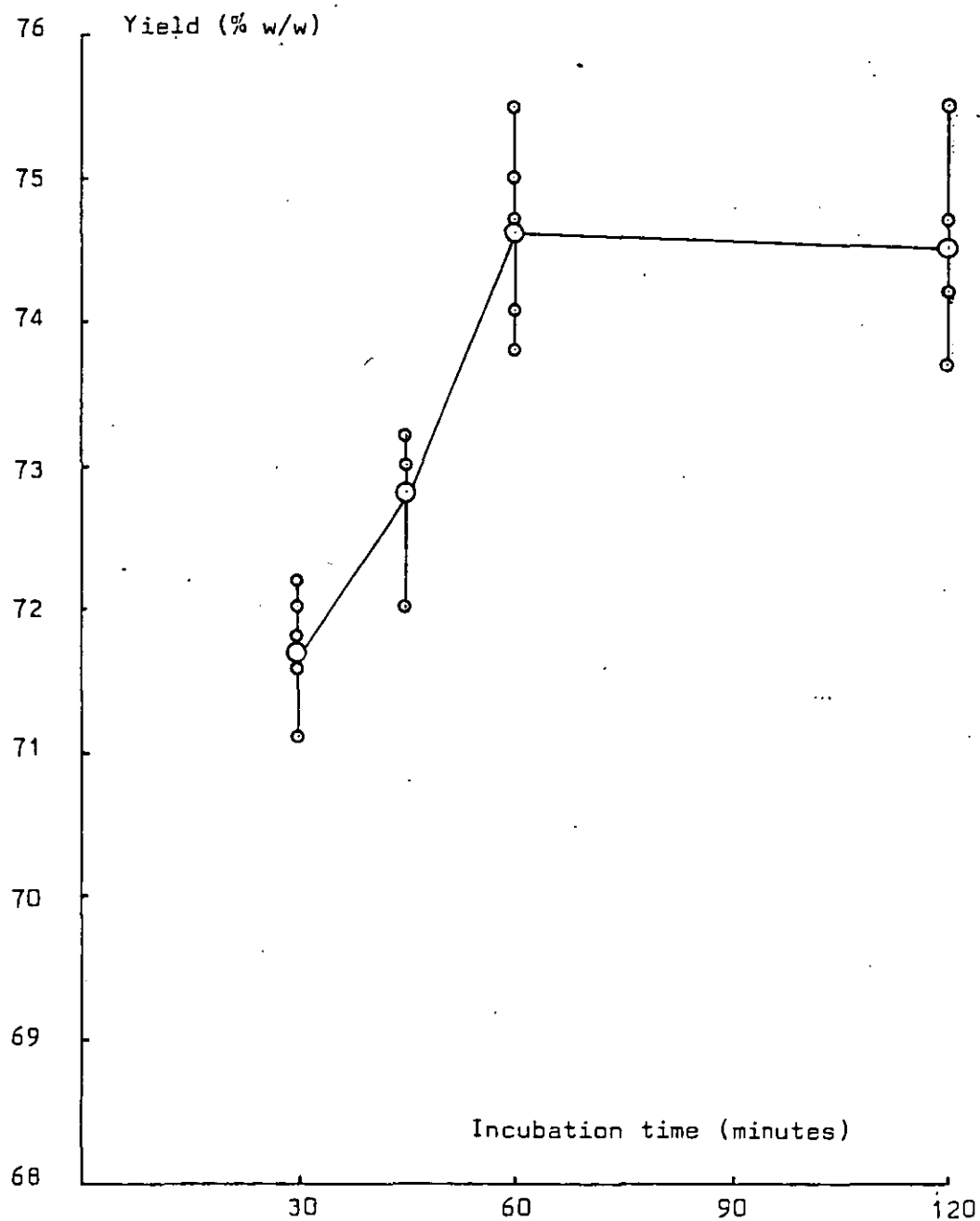
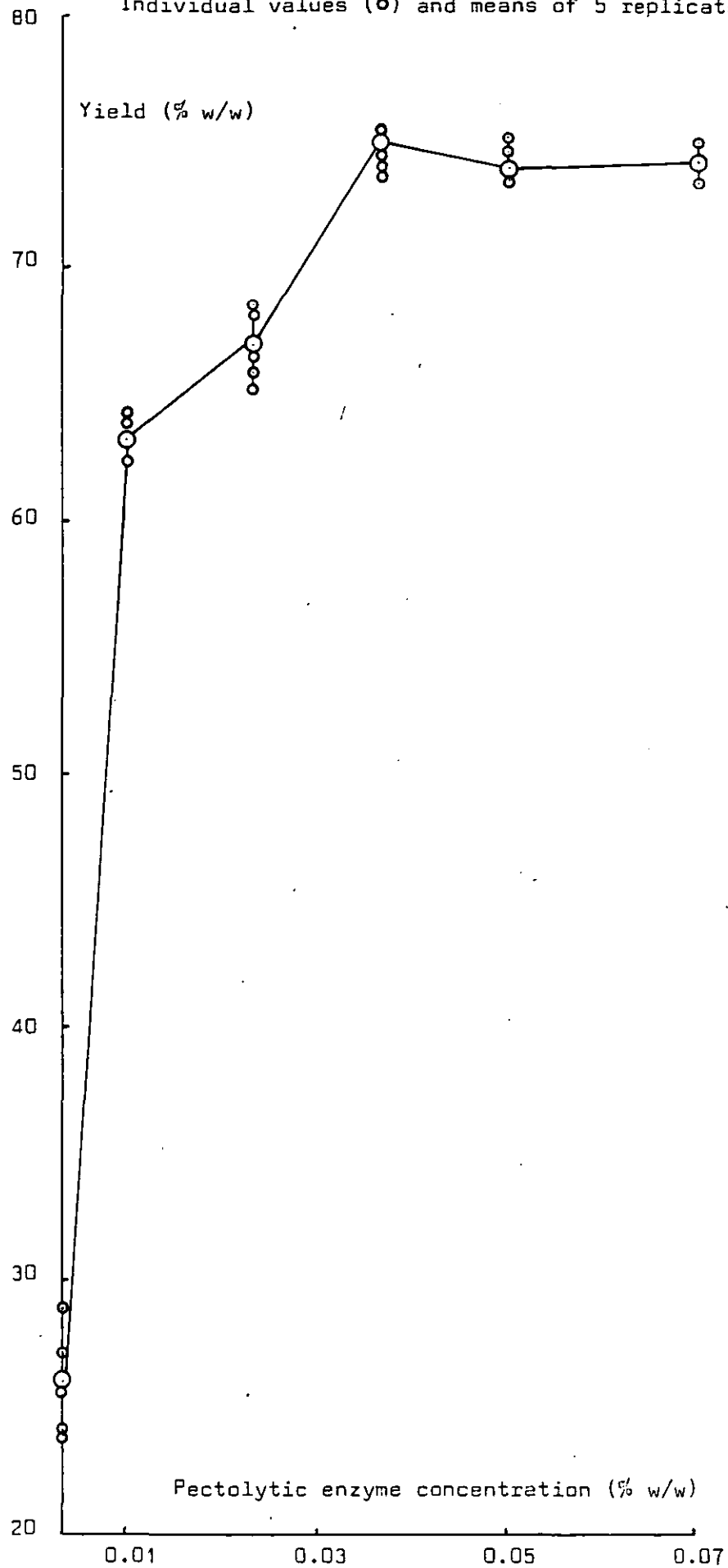


Figure 5 Effect of pectolytic enzyme concentration on juice yield.

Individual values (o) and means of 5 replicates (O).



Increasing the enzyme concentration above 0.01% w/w gave small increases in juice yield up to a concentration of 0.05% w/w. Increasing the enzyme concentration above 0.05% w/w produced no significant differences in the juice yield.

The optimum enzyme concentration i.e. the minimum concentration giving the maximum yield, is therefore 0.05% w/w, at which a juice yield of 74.6% w/w was obtained. This enzyme concentration appears to be considerably less than those used by previous workers (see Table 1, page 15), however comparison is difficult since most previous investigations have been carried out using liquid pectolytic enzyme concentrates rather than powdered preparations.

3.2.4 Variation in metabisulphite concentration

In Table 7 the juice yields obtained at different metabisulphite concentrations are given and the data is plotted in Figure 6. It can be seen that when the banana pulp was not treated with sodium metabisulphite, a yield of 67.6% w/w of juice was obtained. However in the absence of sodium metabisulphite considerable browning occurred. The yield of juice was significantly higher with sodium metabisulphite concentrations of 0.04% w/w and 0.06% w/w,

Table 7 Effect of sodium metabisulphite concentration on juice yield and chemical characteristics of the juice⁽¹⁾

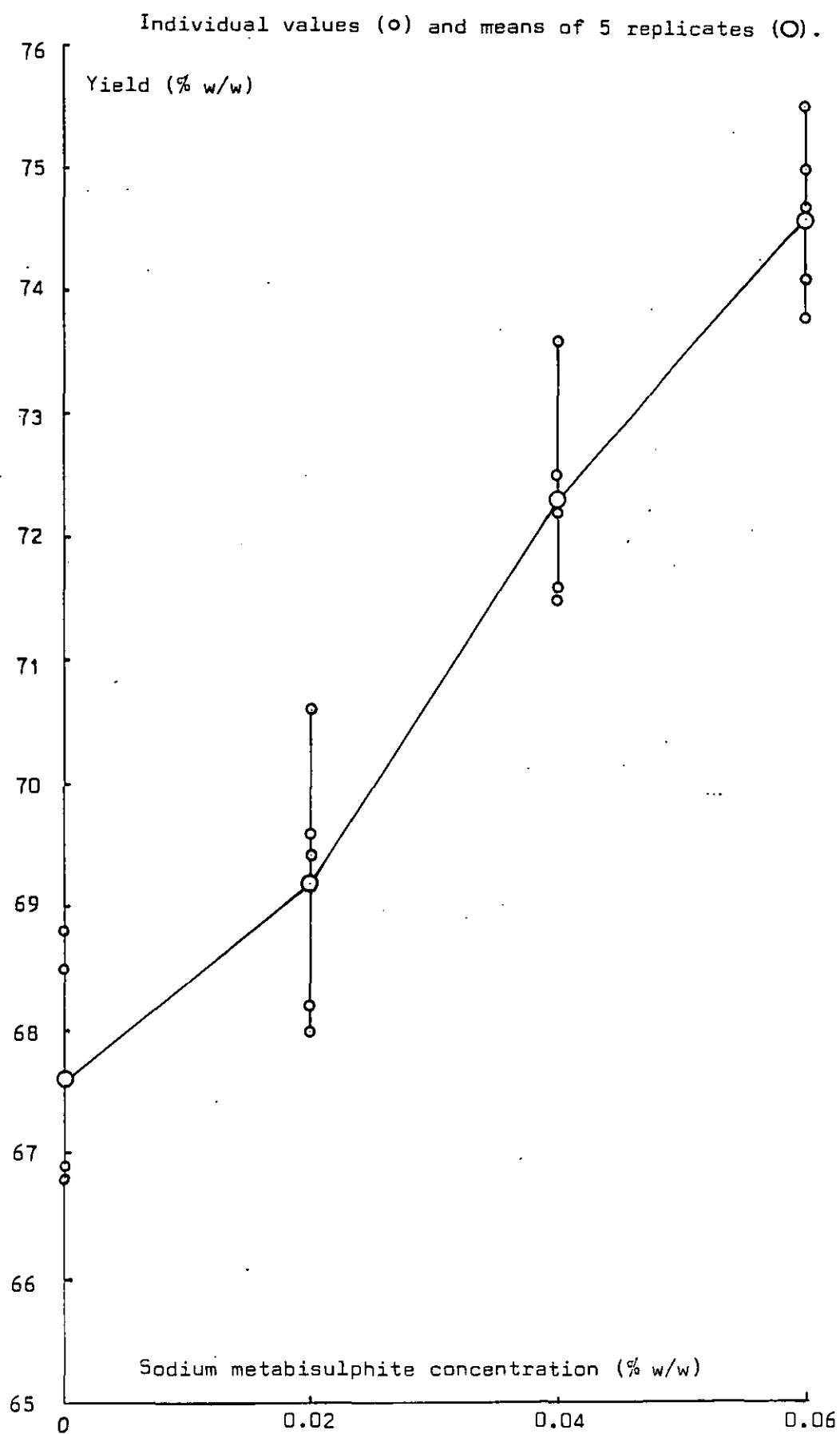
Bisulphite concentration	Total sugar in raw bananas ⁽²⁾	Juice yield ⁽³⁾	pH	Titrateable acidity	Reducing sugar	Sucrose	Total sugar
% w/w	% w/w	% w/w		% w/v as malic acid	% w/v	% w/v	% w/v
0.00	18.1	66.9	4.7	0.31	23.0	0.0	23.0
0.00	18.0	66.8	4.7	0.31	22.7	0.3	23.0
0.00	17.7	68.8	4.7	0.30	22.4	0.0	22.4
0.00	17.9	66.9	4.7	0.31	23.0	0.0	23.0
0.00	18.0	68.5	4.7	0.32	23.1	0.0	23.1
Means	17.9	67.6 ^a	4.7	0.31	22.8	0.1	22.9
Standard deviations		0.88					0.28
0.02	18.0	68.0	4.7	0.30	22.1	0.7	22.8
0.02	18.0	69.4	4.7	0.30	21.4	0.9	22.3
0.02	17.8	68.2	4.7	0.30	22.1	0.1	22.2
0.02	17.7	70.6	4.7	0.32	20.9	0.2	21.1
0.02	18.0	69.6	4.7	0.31	21.2	0.4	22.6
Means	17.9	69.2 ^a	4.7	0.31	21.7	0.5	22.2
Standard deviations		0.96					0.56
0.04	17.9	71.6	4.6	0.32	22.6	0.1	22.7
0.04	18.0	72.2	4.6	0.32	22.2	0.5	22.7
0.04	18.0	72.5	4.6	0.32	22.8	0.0	22.8
0.04	18.0	73.6	4.6	0.33	22.7	0.0	22.7
0.04	18.1	71.5	4.6	0.32	22.8	0.0	22.8
Means	18.0	72.3 ^b	4.6	0.32	22.6	0.1	22.7
Standard deviations		0.76					0.05
0.06	18.1	75.0	4.6	0.36	23.0	0.0	23.0
0.06	18.1	75.5	4.6	0.36	23.0	0.0	23.0
0.06	17.8	73.8	4.6	0.35	21.6	0.6	22.2
0.06	18.2	74.7	4.6	0.36	23.6	0.0	23.6
0.06	18.0	74.1	4.6	0.36	23.1	0.0	23.1
Means	18.0	74.6 ^c	4.6	0.36	22.9	0.1	23.0
Standard deviations		0.61					0.50

(1) Enzyme concentration = 0.05% w/w. Incubation time = 60 minutes. Incubation temperature = 50°C.

(2) Control stage of ripeness. Specification: 18.0 ± 0.3% w/w total sugars.

(3) Mean values for juice yields are not significantly different (95% level Student t-test) if they have the same superscript.

Figure 6 Effect of sodium metabisulphite on juice yield.



corresponding to 200 ppm and 300 ppm of sulphur dioxide respectively, and no browning was apparent. The maximum yield of 74.6% w/w was obtained with a sodium metabisulphite concentration of 0.06% w/w, although it is possible that if sensory and legal constraints permitted higher concentrations of sulphur dioxide, then higher yields might be obtained. Similar results for the effect of sulphur dioxide on the activity of pectolytic enzymes with banana pulp as substrate have been reported, although no data were given for the amount of sulphur dioxide added.²⁶

A possible explanation of the increased yields obtained with the added sulphur dioxide is that the activity of the pectolytic enzymes is increased because the sulphur dioxide reacts with phenolic compounds which might otherwise inhibit the enzymes.^{29,30}

3.3 Chemical analysis of extracted juice

3.3.1 pH

In Tables 4, 5, 6 and 7 the pH values are given for the juice extracted with the various different incubation temperatures, incubation times, enzyme concentrations and sodium metabisulphate concentrations, respectively. It can

be seen that the pH is not significantly affected by these factors, since in the whole series of experiments the pH range of the extracted juice was 4.6 to 4.7. These values are within the previously reported pH range of juice extracted from Cavendish banana pulp, i.e. pH 4.3 to 5.3.^{17,24,25}

3.3.2 Titrateable acidity

In Tables 4, 5, 6 and 7 the titrateable acidities are given for the juice extracted with the various different incubation temperatures, incubation times, enzyme concentrations and sodium metabisulphite concentrations, respectively. The values vary over only a small range, 0.29 to 0.36 g malic acid per 100 cm³ of juice, however there is a significant positive correlation between the titrateable acidity and the yield of juice (the correlation coefficient r is significant at the 99% level of probability). This positive correlation can be attributed to the greater breakdown of pectic substances to yield pectic acid.^{17,20,23}

In investigations reported previously the titrateable acidity of extracted banana juice ranged from 0.37 to 0.91 g malic acid per 100 cm³ of juice; the ranges quoted for juice

extracted specifically from Cavendish bananas were 0.37 to 0.69 g malic acid per 100 cm³ of juice^{17,24,25} and 0.44 to 0.54 g of anhydrous citric acid per 100 cm³ of juice.²⁶ A possible explanation for the lower values of titratable acidity obtained in the investigation reported here is that the bananas used were riper than in previous investigations. It is difficult to check on this, since the ripeness of the bananas used in previous investigations was not clearly defined.

3.3.3 Sugar concentrations

In Tables 4, 5, 6 and 7 the reducing sugar, sucrose and total sugar concentrations are given for the juice extracted with the various different incubation temperatures, incubation times, enzyme concentrations and sodium metabisulphite concentrations. It can be seen that the concentrations of total sugars in the juice vary over a very small range of means, 21.5 to 23.5% w/v, and that there is no significant variation apparent in relation to changes in the various factors. A test for correlation between juice yield and total sugar concentration of juice showed no significant correlation.

Reducing sugar was present at far higher concentrations than sucrose in all cases, which suggests that the pulping process leads to a mixing of the sucrose and the banana pulp invertase enzyme leading to almost complete inversion of the sucrose.

In previous investigations the total sugar content of extracted banana juice ranged from 13 to 28% w/v.^{17,25} The only values quoted for juice extracted from Cavendish bananas are 17.0 and 18.0% w/v, however the degree of ripeness of the bananas was quoted in these reports only in general terms, as "ripened fruits", and no quantitative data for the pulp sugar content was given.^{17,25} It is possible that the bananas had not reached the fully yellow peel ripeness stage and hence lower total sugar concentrations would be expected in the extracted juice, compared with those obtained in the present investigation.

3.4 Efficiency of sugar extraction

The optimisation process described above was defined generally in terms of maximum extracted juice yield. However an alternative way of considering optimisation is in terms of the efficiency of extraction of the sugar content of the pulp. This is particularly important if the product is to be

used for production of beverages or for fermentation purposes. For example, it has been reported that banana juice was obtained for fermentation, by a traditional method, with 3.5% w/v sugar content, and it proved necessary to add sugar cane to get a juice with a minimum of 8% sugar in order to produce a minimum strength vinegar.^{3,4}

Examination of Tables 4, 5, 6 and 7 shows that optimisation on the basis of extracted juice yield will be almost exactly the same as optimisation in terms of sugar extraction, since the total sugar content of the extracted juice varies over only a small range (21.5 to 23.5% w/v). However it is of interest to consider the efficiency of sugar extraction particularly under the conditions that give the optimum juice yield.

To deduce the efficiency of the sugar extraction it is necessary to know the concentration of sugar in the banana pulp. In the present work Cavendish bananas with means of 6.3% w/w of reducing sugar and 11.7% w/w sucrose (see Table 3, page 27) were used. This is equivalent to 18.6% w/w of reducing sugar (11.7% w/w of sucrose gives 12.3% w/w of reducing sugar) in the banana pulp. Under the optimum conditions with respect to extracted juice yield, 74.6% w/w of juice was obtained containing 22.9% w/v of reducing sugar

and 0.1% w/v of sucrose, which is equivalent to 23.0% w/v of reducing sugar. This is equal to 20.9% w/w of reducing sugar (extracted juice has a density of 1.10 g/cm^3). Hence $(20.9 \times 74.6/100) \text{ g}$, which is equal to 15.6 g, of reducing sugar are obtained from 100 g of banana pulp. Therefore $(15.6/18.6 \times 100) \%$, which is equal to 83.9%, of the sugar content of the banana pulp is extracted under optimal conditions. A similar calculation for the juice extracted from the banana pulp without addition of enzyme (25.9% w/w juice extracted, see Table 6, page 36 gives a sugar extraction efficiency of 29.6%. It is assumed in these calculations that the reducing sugars present in the extracted juice from pectin breakdown and other sources is negligible, a reasonable assumption since the total amount of pectin present in the pulp is less than 1% w/w and probably only a very small proportion of that will be hydrolysed to reducing oligosaccharides and monosaccharides.

It is not possible to compare this figure of 83.9% efficiency of sugar extraction with previously reported results, since insufficient data are given in the reports for the sugar extraction efficiencies to be calculated. In particular, the sugar concentrations in the banana pulps used for juice extraction are not given.

4.

CONCLUSIONS

The yield of juice extracted from banana pulp by the pectolytic enzyme preparation, increased with incubation temperature up to 50°C. At 60°C the yield was lower than at 50°C. These findings agree with previous reports that pectolytic enzymes are generally active over a range of 5 to 55°C.²⁷

In order to obtain the maximum yield of juice, an incubation time of 60 minutes or more was found to be necessary. However if economic factors are taken into account, then 30 minutes might prove to be the most useful incubation time since the yield obtained of juice of 71.7% w/w was not far below the maximum yield (96.1% of the maximum yield). The incubation times used in this investigation were far shorter than those reported by previous workers (see Table 1 page 15).

A maximum yield of juice was obtained by using a 0.05% w/w enzyme preparation and this yield was not increased with higher concentrations of enzyme. This optimum level of enzyme concentration was lower than concentrations of enzymes used by previous workers (see Table 1, page 15).

Levels of 0.02, 0.04 and 0.06% w/w of added sodium metabisulphite were shown to have no inhibitory effect on the

enzyme preparation, but rather the opposite, the yield of juice reached a maximum of 74.6% w/w at 0.06% sodium metabisulphite. The positive effect of metabisulphite on banana juice extraction by pectolytic enzymes agrees with findings of previous workers, although no quantitative data for the amount of sodium metabisulphite used were previously reported.²⁶ Browning of the extracted juice was suppressed for at least one week when using 0.06% w/w of added sodium metabisulphite.

The pH of the juice was not significantly affected by the extraction procedure, whereas titratable acidity increased slightly, but significantly, with juice yield.

The concentration of total sugars in the juice varied over only a small range in the whole series of experiments (21.5 to 23.5% w/v). No correlation was found between the yield of juice and the concentration of sugars in the juice. The sugars present in the juice were almost entirely reducing sugars with only very small amounts of sucrose being present.

Under the optimal studied conditions, 0.05% w/w enzyme and 0.06% w/w sodium metabisulphite at an incubation temperature of 50°C for 60 minutes, a juice yield of 74.6% w/w was obtained which is equivalent to 83.9% efficiency in extracting the sugar content of the pulp.

This investigation indicates a need for further work to be done in the following areas:

1. Assessment of a more precise optimum incubation temperature by carrying out experiments at temperatures between 45 and 55°C.
2. Assessment of the effect on yield and on the properties of the juice of adding more than 0.06% w/w sodium metabisulphite.
3. More detailed assessment of the relationship between enzyme concentration, incubation time and juice yield.
4. Assessment of the effect of different centrifugation speeds and times on the juice yield.
5. Assessment of procedures for further processing the extracted juice in order to produce products of commercial value.

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