STUDIES ON THE PROCESSING OF NEW ZEALAND GRAPEFRUIT JUICE

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"The safest position is somewhere between arrogance based on unrecognised ignorance, and arrogance based on unwarranted certainty."

Professor John Yudkin
"Pure, White and Deadly", 1972

ABSTRACT

The likely origin of the New Zealand grapefruit (NZGF) is discussed and present and future trends in its production and utilisation presented. Early and late season samples of NZGF juice were analysed for the presence of the enzymes pectinesterase, polygalacturonase and ascorbic acid oxidase, no trace of the latter two being found. Samples of juice from NZGF harvested at regular intervals from July until December 1973 were analysed for yield, total soluble solids, titratable acidity, pH, pectinesterase activity, and ascorbic acid content.

The average yield of juice obtained (35.6% w/w) was significantly lower than that reported from overseas for true grapefruit. The level of total soluble solids remained fairly constant in the range 12.0 to 12.6%, while the pH of the juice increased throughout the season from 2.95 to 3.40. The titratable acidity was within the range 1.0 to 2.0 grams of citric acid per 100 ml of juice, while the Brix : acid ratio varied from 5.02 to 10.03. The level of pectinesterase in the juice (which increased as the season progressed) was comparable with that found in overseas citrus juices, while the level of ascorbic acid in the juice declined over the season from 32.4 to 23.2 mg/100 ml, in agreement with overseas trends. With the exception of yield, the compositional characteristics of NZGF juice reported here do not differ markedly from overseas grapefruit juices.

The important role which pectinesterase plays in the destabilisation of citrus juice cloud is outlined and possible methods for inactivating the enzyme are described. As the application of heat is the only method in commercial use, factors affecting and methods for studying the thermal inactivation of enzymes are discussed. As the major objection to most of these methods is the way in which the heating and cooling lags are evaluated, a new method which adequately describes these thermal lags has been developed for determining the thermal resistance of pectinesterase in NZGF juice.

A digital computer was programmed to determine (using a trial and error technique) the constants in two expressions which relate the equivalent effect of unsteady-state heating and cooling of NZGF juice to the inactivation of pectinesterase. One expression assumed that the rate of inactivation was exponentially related to temperature; in this case the constant was the z value. The other expression assumed that the rate was related to temperature according to the Arrhenius equation, in which case the constant was the activation energy. The two constants were evaluated for both low and high pH juice. It was found that the latter expression using the Arrhenius equation described the change in rate of inactivation with temperature more adequately than the former expression. From these expressions the times required at different temperatures to inactivate pectinesterase in NZGF juice of varying pH were calculated. The application of these results to the industrial processing of NZGF juice is discussed.

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SECTION I

THE ORIGIN OF NEW ZEALAND GRAPEFRUIT VARIETIES

It was not with an apple that Eve tempted Adam, according to one tradition, but with a citrus fruit - a primitive citron called etrog or Adam's apples. (Kefford, 1966). Citrus fruits with their attractive colours and distinctive flavours continue to tempt the appetites of men, and as the richest natural sources of vitamin C, they are important to human nutrition. More citrus fruits are consumed directly as human food than any other kind of fruit, and the world crop of citrus fruits is second only to the grape crop, much of which, however, is utilised in fermented liquors.

New Zealand is a signatory to the Codex Alimentarius Commission Standards on fruit juices which are likely to become accepted internationally within the next three years. Once this happens, any grapefruit products exported from New Zealand will have to meet the Codex standards. Therefore it is important to the citrus processor to know how New Zealand grapefruit compare with overseas species and varieties.

The botanical or horticultural names of the major citrus fruits, together with the taxonomic relationships between them, are summarised in Table I, after Swingle and Reece (1967) and Hodgson (1967).

Table I

Classification of Citrus Fruits

General Name	Botanical Name	Varietal Groups
Sweet orange	*Citrus sinensis*	Normal oranges Naval oranges Blood oranges Low-acid oranges
Bitter or sour orange	*Citrus aurantium*	Bitter (Seville) oranges Bittersweet oranges
Mandarin	*Citrus reticulata*	Common mandarins Tangerines Satsuma mandarins Mediterranean mandarins Small-fruited mandarins
Grapefruit	*Citrus paradisi*	Pale-fleshed grapefruits Red-fleshed grapefruits
Pummelo or Shaddock	*Citrus grandis*	Common pummelos Pigmented pummelos Low-acid pummelos
Lemon	*Citrus limon*	Acid lemons Low-acid lemons
Lime	*Citrus aurantifolia*	Small-fruited acid limes Large-fruited acid limes Low-acid limes

The origin and significance of the name "grapefruit" are obscure. According to Webber (1943), who made a comprehensive search of the literature, the earliest recognisable mention of grapefruit occurred in Barbados (West Indies) in 1750 under the name "forbidden fruit", from which the species designation *paradisi* was assigned in 1830. The first known use of the term grapefruit occurred in 1814 in Jamaica, in which it was referred to as a special and smaller kind of

shaddock whose flavour somewhat resembled that of the grape. It seems more likely, however, that the name was derived from the fact that the fruits commonly occur in small clusters rather than singly, as with most shaddocks (pummelos). Early in the present century, the name pomelo was proposed and for a time was used by American horticulturists. It was not accepted by the industry, however, and has now virtually disappeared.

According to Hodgson (1967), the grapefruit almost certainly originated in the West Indies, for it is not described in the old literature and was not known in Europe or in the Orient until after its discovery in the Western Hemisphere. That it was derived from the pummelo is certain, but whether by somatic mutation or natural hybridisation is not known. However, it is the opinion of Hodgson (ibid.), based on observations of numerous natural hybrids of the pummelo in northeastern India, Sikkim, and eastern Nepal, that the grapefruit originated as a natural hybrid.

Although the grapefruit was said to be common in Jamaica and probably throughout the West Indies, it remained for Florida to introduce the grapefruit to the American consumer and to develop a commercial industry. This fact explains why, with the sole exception of Redblush (Ruby), all the grapefruit varieties of commercial importance have originated in Florida and apparently trace back to the original introduction.

The so-called New Zealand grapefruit is not a true

grapefruit but is a natural hybrid of obscure origin with tangelo characteristics. That it originated in the Orient is suggested by Bowman's statement (1956) that it was brought to Australia (presumably the fruit) from Shanghai by a Captain Simpson early in the 19th Century. The earliest description of the Poorman orange (as the variety was then called by the Australians), given in a New South Wales nursery catalogue of 1820, indicates that the original introduction might have been a shaddock (pummelo).

According to Bowman (ibid.) the Poorman was taken to New Zealand by Sir George Grey, who established his home on Kawau Island in the Hauraki Gulf about 1855. About 1861, Grey provided propagation materials to John Morrison of Warkworth, and for many years the most commonly grown strain of this fruit was known as Morrison's seedless. The name 'New Zealand Grapefruit' (hereafter referred to as NZGF) was later given to this and other thin-skinned high quality strains of Poorman oranges. A popular strain being planted at present is known as 'Golden Special' and is probably a variation of Morrison's strain. 'Lippiatts' is another strain which has been grown and which arose as a seedling of the Poorman orange.

The earlier plantings of NZGF were of trees propagated on either sweet orange or rough lemon rootstocks which grew to a very large size, and although these trees were heavy bearers, harvesting costs were high. The trend with recent plantings has been to propagate trees on trifoliata rootstocks, and this is resulting in smaller trees

which are easier to handle and which can also be planted closer together to maintain yields per acre. In fact, double planting at about 230 trees per acre is now not uncommon, but the average density of trees over all plantings is at present only about 145 trees per acre (Fletcher, 1971).

The NZGF is medium-large, oblate to broadly obovate to nearly globose. Despite having numerous seeds, it is monoembryonic. It has a pale orange-yellow colour at maturity, with a medium-thick rind. The flesh colour is yellowish orange, coarse-textured and juicy, the flavour being pleasantly subacid with a trace of bitterness. Compared with true grapefruits, it matures very early, but holds on the tree exceptionally well without loss in colour. The tree is vigorous, large and prolific, with dark green leaves, and the petioles are suggestive of mandarin or bitter orange rather than grapefruit (Hodgson, ibid.).

The only other kind of grapefruit which is grown in New Zealand is the Wheeny grapefruit. This is a late variety, ripening from November to March. It originated as a chance seedling at Wheeny Creek near Kurrajong, New South Wales, Australia, and was first introduced into New Zealand about 1935. The fruit is pale yellow, large, thin skinned, and very juicy, somewhat similar in appearance to American and Jamaican grapefruit varieties. It is not a true grapefruit, however, but a natural hybrid, probably with a Seville orange parent. Interest in Wheeny grapefruit has waned in recent years, and production of this variety is not important in New Zealand at present.

As mentioned earlier in this section, New Zealand is a signatory to the Codex Alimentarius Commission Standards on fruit juices. The draft Codex Standards on citrus juices (Codex Alimentarius Commission, 1971) require that grapefruit juice be extracted from the species Citrus paradisi Macfadyen, the true West Indian grapefruit. The New Zealand Food and Drug Regulations (1973) regulation 197, state that grapefruit juice shall be the expressed juice of mature grapefruit of the species Citrus paradisi, or of hybrids of that species, or of hybrids of the species Citrus grandis.

However, the above discussion has indicated that New Zealand grapefruit is unique, belonging to neither of the above-mentioned species. Therefore, at this point in time, all NZGF juice sold in New Zealand fails to meet the Food and Drug Regulations. Furthermore, NZGF juice could never meet the Codex Alimentarius Commission Standards from a species point of view. The proper course would seem to be to have a special designation in both the Codex Standards and the New Zealand Food and Drug Regulations for NZGF juice. Because of the recent increases in NZGF juice processing, there would appear to be some urgency in this matter.

SECTION II

PRODUCTION OF CITRUS FRUITS

The world production of citrus fruits exceeds 40 million tonnes, an increase of over 30% in the last 6 years, as shown in Table II.

Table II

World Production of Citrus Fruits

Year	Million Tonnes
1966	30.81
1967	33.66
1968	33.11
1969	36.74
1970	37.53
1971	38.88
1972	40.21

Source: The State of Food and Agriculture, F.A.O., Rome (1973).

The proportion of the crop consumed in the form of processed citrus products continues to increase and there has been an attendant expansion in international trade in citrus juices and concentrates.

The main producers of citrus fruits in 1972 are shown in Table III.

Table III

Main Producers of Citrus Fruits in 1972

Country	Million Tonnes
North America	11.28
Latin America	9.03
Western Europe	6.30
Near East	2.44
Africa	2.33

World production of grapefruit in 1972 was less than three million tonnes, the main areas of production being shown in Table IV.

Table IV

Main Areas of Grapefruit Production in 1972

Country	Million Tonnes
Florida	1.40
Texas	0.324
Israel	0.316
California	0.170
Argentina	0.144
TOTAL	2.353

New Zealand production of citrus fruits in 1972 was 619,000 bushels or approximately 5630 tonnes (Ministry of Agriculture and Fisheries, 1973), an extremely small quantity

when compared with world production. Of this quantity, 37% or 2080 tonnes was NZGF.

As New Zealand is situated climatically at the very southern limit for citrus culture, it is only in the relatively frost-free, sheltered pockets on the eastern littoral of the North Island from Gisborne north, that conditions are sufficiently congenial for commercial production of citrus fruits.

The citrus industry is concerned primarily with producing fruit for the home market. With strong competition from other countries more favourably suited for citrus and closer to world markets, there has been little prospect for developing export outlets.

Nevertheless, the citrus industry has expanded steadily since World War II, and this expansion has increased in tempo in recent years. The total area in citrus doubled in 5 years from the time of the Department of Agriculture's (now the Ministry of Agriculture and Fisheries) orchard survey in 1963 until the last survey in 1968. Unfortunately, the results of the 1973 orchard survey will not be available until later this year (Fletcher, 1973), but in the four years from 1968 to 1972, the total citrus acreage had doubled again, as Table V shows.

Table V

Growth in New Zealand Citrus Production

Year	Total Acres in Citrus	Total Acres in NZGF	NZGF as % Total Citrus	Total Acres in Wheeny
1963	890			
1968	1781	309	19	34
1970	2769	572	21	32
1972	3528	939	27	32

Table VI shows the relative importance of the four main citrus growing regions in New Zealand in 1972.

Table VI

Relative Importance of Four Main Citrus Growing Regions in New Zealand in 1972

Region	Total Area in Citrus		Production of NZGF		
	Acres	% N.Z. Total	Bushels	% Total NZGF	% Total Citrus Crop
Kerikeri	838	23.7%	25,000	10.9%	4.6%
Auckland	281	8.0%	56,000	24.4%	9.1%
Bay of Plenty	1841	52.3%	140,000	61.0%	22.6%
Gisborne	568	16.0%	9,000	3.7%	1.5%
TOTALS	3528	100.0%	230,000	100.0%	37.8%

The first interesting point which emerges from Table VI is the undisputed claim of Bay of Plenty as the

main region for growing NZGF. Producing over twice as much as its nearest rival Auckland, it is the obvious area for the establishment of a large scale citrus processing industry. This fact is substantiated when it is considered that a sizable portion of the crop grown in the Auckland region finds its way onto the fresh market to satisfy the demand of the Auckland metropolis.

The second point is the greater yield of NZGF per acre compared with other citrus fruits, namely 38% of the total production from 27% of the total area under citrus. In fact, these figures are not as spectacular as the 1970 ones when 21% of the total area under citrus was NZGF, producing 37% of the total crop. The apparent drop in production per acre can be explained by the large acreage of very young NZGF trees which have been planted between 1970 and 1972. When these trees reach their full productive capacity within the next 10 to 12 years, NZGF will account for the major portion of the total citrus crop on a bushels per acre basis.

SECTION III

FUTURE TRENDS IN NZGF PRODUCTION AND UTILISATION

Cartwright (1973) estimated supply and demand projections for New Zealand citrus from 1973 to 1977. As he pointed out at some length, the projections were logical developments of existing historical data and specific assumptions, not forecasts or predictions. Inadequacies in basic data limited the scope of his study. Table VII shows the projected supply of NZGF.

Table VII

Projections of Supply of NZGF

Year	Projected Supply (bushels)	Projected Supply as % of 1972 Supply	Excess of Projection over 1972 Supply
1973	271,000	117.8	40,900
1974	319,000	139.0	89,800
1975	395,600	171.9	165,500
1976	481,000	209.3	251,600
1977	590,000	256.8	360,800

Source: Cartwright (1973)

Even although the yield estimates used above resulted in the projections being conservative, rapid growth rates in supply are projected. An increase of more than 70% over the 1972 supply level is projected for 1975, while the

1977 supply is projected to be in excess of two and one half times the 1972 supply level.

Cartwright (ibid.) projected that the excess of supply over demand for NZGF in 1977 would be 139%, and in the absence of any action to stimulate demand, average retail prices in 1975 were conservatively projected to fall by 30% of the 1972 price for NZGF.

The projections implied early and drastic depressions in prices unless urgent steps were taken to stimulate consumer demand. In the absence of such action, growers of NZGF were projected to be placed under a severe cost-price squeeze, and this would be attended by pressure on processors and distributors to reduce their margins.

Current per capita consumption of all citrus fruits in New Zealand has been calculated at about 9 kg per person and most of this was eaten as fresh fruit (Fletcher, 1969). Home garden production would appear to add about a further 2.3 kg per head (Frampton, 1971). Estimated consumption figures for fresh citrus fruit in other countries with developed economies are shown in Table VIII.

Table VIII

Estimated Annual Consumption of Fresh Citrus Fruits

	1963	1965	1968
	kilograms per head		
United Kingdom	8.2	8.6	9.1
Canada	9.5	11.3	10.9
United States	10.0	13.2	11.8
West Germany	14.5	18.2	16.3
France	15.9	17.7	16.8

Source: Fruit - A Review (Commonwealth Secretariat, 1970).

It is apparent that consumption of fresh fruit in New Zealand is not particularly high when compared with other countries with high living standards. A centrally planned and directed programme of promotion could produce spectacular increases in consumption. Although citrus growers are giving this matter consideration through their national body, the Citrus Council, little has yet emerged.

In the case of NZGF, the obvious processed product to produce in an effort to increase consumer consumption is a high quality juice which could be available to consumers all the year round, compared to the fresh fruit which are only available for six months of the year. According to Tressler and Joslyn (1971), about 60% of the total per capita citrus consumption in the United States of America was in the form of juice, either as single strength or frozen concentrate. Figures such as those in Table IX show that

consumption of citrus fruits as juice is increasing.

Table IX

Estimated Consumption of Citrus Juice in Kilograms per Head

(Single Strength Equivalent)

	1959-60	1966-67
West Germany	1.0	2.0
Netherlands	1.1	3.4
United Kingdom	1.8	2.2
United States	11.2	12.1

Source: Processed Fruit and Vegetables (F.A.O., 1970).

No official data is available as to the New Zealand consumption of processed citrus fruit products. Demand for processed grapefruit has grown largely since the mid-1960s. It received considerable stimulus by the emergence in 1965 of single strength grapefruit juice marketed by town milk vendors. Until that time little interest had been shown by processors in processing fresh grapefruit. In 1970, milk treatment companies distributed nearly 1.8 million litres of grapefruit drink in New Zealand. This is equivalent to 0.6 million litres of single strength juice. Taking an average juice yield of 35% (see Chapter 2, Section III), this consumption represented 91,500 bushels of NZGF, or 46% of the total 1970 NZGF production.

The distributors consider there is a great potential for increased sales of the juice. At present

only 15 of the 40 or more milk treatment stations in New Zealand distribute the grapefruit drinks, and the South Island is virtually untapped.

As well as the milk treatment stations, several New Zealand processors are producing a canned grapefruit drink, but the quality is low. The main criticism of the grapefruit drink is its variable water content (Dover, 1973). Under the New Zealand Food and Drug Regulations (1973), regulation 222, a fruit drink need only contain 5 per cent by volume of fruit juice, although the actual level is generally much higher than this. Money (1971) indicated that milk treatment stations worked on a 1 : 3 dilution with water. i.e. 25% of juice. The drink has also been criticised for its excessive bitterness and unstable cloud.

The present study was undertaken to investigate aspects of the processing of NZGF juice and provide basic technological data that would enable citrus processors to produce a consistently high quality single strength juice. By so doing they would hopefully increase the per capita consumption of NZGF juice in New Zealand to cope with the predicted large production increases over the coming years.

CHAPTER TWO

SEASONAL CHANGES IN THE COMPOSITIONAL CHARACTERISTICS OF NEW ZEALAND GRAPEFRUIT JUICE OF IMPORTANCE TO CITRUS PROCESSORS

SECTION I

COMPOSITIONAL CHARACTERISTICS OF NEW ZEALAND GRAPEFRUIT JUICE OF IMPORTANCE TO CITRUS PROCESSORS

Much of the information that has been reported on the general composition of citrus fruits has mainly local relevance in the growing region concerned. Kefford and Chandler (1970) list sources of routine information of this kind, the commonly reported constituents being soluble solids, acidity, ascorbic acid and juice yield. In a number of cases more complete analyses include sugars, ash constituents, nitrogen, and formol and chloramine values.

Different parties in the chain from the primary producer to the consumer are interested in the composition of citrus fruits from different points of view. The grower is interested in the yield and size of the fruit, and the yield of soluble solids in kilograms per tree or kilograms per hectare. The processor is interested in the yield of juice, the yield of soluble solids in kilograms per tonne of fruit, and in the characteristics of the fruit as they relate to consumer preferences. The consumer is interested in the palatability of the juice, in terms of flavour, colour, appearance and nutritive value.

According to Kefford and Chandler (ibid.), the major factors affecting the general composition of citrus

fruits are the horticultural and climatic conditions under which they are grown. A whimsical comment by Monselise and Turrell (1959) suggests that there are at least 3.67×10^{11} permutations and combinations of variables in citrus culture. It is not surprising therefore that it is difficult to draw general conclusions about the influence of particular factors on the composition of citrus fruits.

As mentioned previously, the compositional characteristics of citrus fruits of interest to processors are:

(i) yield of juice
(ii) flavour
(iii) colour
(iv) appearance
(v) nutritive value

(i) Yield of Juice

From the production control point of view, this is the most important factor to the citrus processor. If the processor is producing only a single strength juice (as is the case in New Zealand at the moment) then the yield can be simply calculated as the weight of juice per unit weight of fruit, expressed as a percentage. Any change in the yield is directly reflected in the profitability of the processing operation, and it is for this reason that processors attempt to maximise their yields, even if it is at times detrimental to the organoleptic quality of the juice.

When the processor is extracting juice for

subsequent concentration, it is customary to express the yield in terms of kilograms of soluble solids per tonne of fruit. This is because the juice is concentrated to a certain level of soluble solids, and the higher their initial level, the smaller the quantity of water which needs to be removed to reach the required concentration.

The two main operations in the process which affect yield are extraction and finishing. Mechanical extractors are now almost universally used for the commercial extraction of citrus juices and are capable of adjustment to vary the pressure on the fruit. Increasing the extraction pressure tends to increase the yield of juice but decrease the soluble solids and acidity because some of the juice is derived from the peel rather than from the juice vesicles. The effect of extraction pressure on juice composition is less the more spherical the fruit and thinner the peel. Therefore, because of the ovoid shape of the NZGF, extraction pressure is an important variable affecting both yield and juice composition.

In the finishing stage, the mesh size of the screen and the pressure head influences the degree of retention of the large particles in the juice, thus affecting yield. For concentrated citrus juices, excessive free and suspended pulp is termed a physical defect under United States Department of Agriculture (USDA) specifications, and a maximum level of pulp is specified. No minimum level is set, but the lower the pulp level, the lower the yield.

The effect of pulp quantity on the chemical and physical properties of citrus juices is presented by Rouse et al. (1954).

Other factors outside the control of the processor which can influence yield include the nutrient status of the tree and the rootstock. In New Zealand, Fletcher and Hollies (1965) found that trifoliate orange was consistently superior to rough lemon and sweet orange as a rootstock for several varieties of orange, giving higher juice content and soluble solids content.

Dawes (1970) found a similar trend for soluble solids from NZGF, the levels decreasing in the order trifoliata, sweet orange and citronelle. The effect of rootstock on juice composition is shown in Table X after Dawes (1971).

Table X

Effect of rootstock on composition of NZGF juice

	Rootstock		
	Trifoliata	Sweet Orange	Citronelle
Soluble Solids	16.35	15.15	12.70
Acidity	1.65	1.85	1.45
Total Sugars	12.8	11.6	9.8
Brix : acid ratio	9.9 : 1	8.2 : 1	8.8 : 1
Ascorbic Acid	37.0	37.0	32.4

Strachan (1971) reported that sweet orange rootstock produced fruit with more juice than trifoliata and citronella stock but that the total soluble solids are lower. Of the three rootstocks, the trifoliata produced the highest soluble solids.

In the present study, the yield of NZGF juice from fruit grown on trifoliata stock was determined throughout the 1973 season.

(ii) Flavour

The citrus flavour system consists of straightforward primary tastes: sweet, sour, and bitter, plus a superstructure of more than 100 volatile flavours, the respective roles of which are hardly known (Kefford and Chandler, 1970).

All grapefruit have relatively high concentrations of two extremely bitter substances, the triterpenoid limonin and the flavonoid naringen. These compounds give NZGF juice its characteristic flavour, but in excess are unacceptable.

The amount of limonin required in a juice before bitterness becomes detectable varies with the sweetness and the acidity of the juice as well as the sensitivity of the taster (Chandler and Kefford, 1966). As a general rule a juice containing less than 6 ppm of limonin is unlikely to taste bitter, but a juice with more than 9 ppm will seem bitter to most tasters. Limonin contents higher than 30 ppm are comparatively rare. The natural free juice of NZGF has

a limonin concentration of 15 ppm making it naturally bitter, and the concentration increases to 25-30 ppm as the limonin goes into solution from the rag (Strachan, 1971). Solution of limonin takes place very slowly at room temperature but increases rapidly at pasteurising temperatures. Dawes (1971) indicated that limonin contents of NZGF juice may vary from 6.8 ppm to 25.4 ppm, with bitterness apparent above 9 ppm.

Buffa and Bellenot (1962) suggested that a level of 0.03 - 0.07% naringen is required to give grapefruit juice its characteristic bitterness, juices with naringen contents below 0.03% being of poor quality. Strachan (1971) stated that naringen is tolerable to most individuals at the 0.07% level, with its concentration in NZGF juice averaging 0.05 - 0.12%.

With high concentrations of these bitter substances, NZGF juice is normally too bitter after processing unless action is taken to prevent their uptake in the juice.

Fortunately the insolubility of both bitter substances aids in their removal. If the rag is removed rapidly from the juice phase there is insufficient time for the bitter principles to go into complete solution. Low temperatures further retard solubility. Cold extraction and rapid rag removal can reduce limonin to 7 - 11 ppm and naringen to 0.04 - 0.06%, or less than half the normal level (Strachan, 1971).

Because of the importance of bitterness in consumer acceptance of many processed citrus products, considerable interest has been shown in the use of enzymes of fungal origin which completely hydrolyse naringen to non-bitter products. Thomas et al. (1958) showed that only partial hydrolysis of naringen to prunin was necessary for successful debittering. Although in most cases the cost does not warrant enzymic debittering, Kefford and Chandler (1970) mention a Russian paper which reports the use of enzyme preparations from Aspergillus niger for debittering grapefruit juices.

Excess peel oil adversely affects grapefruit flavour giving it an unpleasant terpene-like off-flavour, and is the main cause of poor flavour in the grapefruit drink currently on the New Zealand market (Strachan, 1971). Off-flavour is due to oxidised oil constituents, and the only method of eliminating the problem is to reduce the oil content to a low level by using correctly-adjusted mechanical extractors, followed by complete deaeration of the juice. The amount of peel oil present in the juice depends mainly on the method of juice extraction and little on the raw material.

In addition to a distinctive aromatic character provided by volatile flavouring materials, citrus juices must have an acceptable sugar : acid balance. This balance is expressed in terms of the Brix : acid ratio, a low ratio indicating a relatively sour juice, and a high ratio a

relatively sweet juice.

Kilburn (1958) and Kilburn and Davis (1959) demonstrated a linear relationship between the pH of grapefruit juice and the log of the free acid concentration expressed as milliequivalents per gram of soluble solids. Expressed in another way, this means that at a uniform soluble solids content (Brix), the pH of the juice is proportional to the log of the titratable acidity.

(iii) Colour

Immature citrus fruits are characteristically green because of the presence of chlorophyll in the flavedo. At a certain stage in the development of the fruit (known as the colour break), the flavedo begins to change in colour from green to yellow or orange, because chlorophyll is lost and carotenoid pigments appear.

The colour of citrus juices is due to carotenoid pigments carried in the chromoplasts, which form part of the suspended cloud. Because the pigments are not in solution, colour measurement is difficult, and the use of plastic colour standards has been written into the United States specifications for canned citrus juice, thus avoiding tedious analytical methods and expensive instrumentation.

NZGF is well endowed with colour and there is no difficulty in producing juice of good colour. In fact, high colour is an attractive attribute of NZGF juice (Dawes, 1971).

(iv) Appearance

While colour is unquestionably important to the overall appearance of citrus juice, the most important factor contributing to the desirable appearance of citrus juice is the presence of a stable cloud. Lime juice is the exception here as traditionally it has always been consumed in the clarified form.

Citrus juices are generally opaque because of the presence of a "heterogeneous mixture of cellular materials and perhaps emulsoids held in suspension by pectin", to quote Scott et al. (1965). These workers investigated the composition of this cloud in orange juices freshly extracted (on commercial juice extractors) from a range of varieties grown in Florida during several seasons. After a primary screening through a 16-mesh screen, the juices were centrifuged for 10 minutes at 600g to give fraction A. The effluents were then recentrifuged immediately for 3 minutes at 60,000g to give fraction B, and again after storage overnight to throw down the fine cloud which formed fraction C.

Fraction A, the "free and suspended pulp", resembled the albedo, rag, and pulp components in being high in cellulosic constituents and relatively low in pectin, lipids, and phosphorous. This fraction was evidently made up of fragments from mechanical disintegration of structural tissues.

Fractions B and C, the true cloud, were high in pectin, lipids, nitrogen and phosphorous, indicating that they were derived from the contents of the juice vesicles.

The mechanism of cloud stability is discussed in detail in Chapter Three. The presence of pectic enzymes in citrus juice leads to destabilisation of the cloud. Consequently, the present studies were undertaken to determine which pectic enzymes were present in NZGF juice so that processing conditions necessary to produce a stable cloud could be established.

(v) Nutritive Value

Because citrus fruits contain 85 - 92% water, they cannot be important sources of major nutrients. Instead, their place in the diet depends on the presence of minor nutrients, especially vitamin C, or on considerations other than nutritive value in the strict sense. They were used empirically for the prevention and treatment of scurvy around 1750, long before vitamin C was first isolated as a pure crystalline compound in 1932, lemon juice being used as the source. The role of citrus fruits and processed citrus products in human nutrition has been reviewed in detail by Kefford (1966).

According to Kefford (1959), the concentration of ascorbic acid in citrus juices varies from 10 to 80 mg/100 ml according to variety. Although other vitamins are present, their concentrations are so small that citrus fruits

consumed in normal amounts cannot be expected to make significant contributions to human daily requirements for any of these vitamins.

In general, citrus fruits tend to lose ascorbic acid slowly during storage but the loss is very small at optimum cool storage temperatures and is unlikely to exceed 10% under any reasonable conditions of distribution and marketing (Kefford, 1966).

Rohrer and Treadwell (1944), quoted by Kefford (1966), showed that ascorbic acid retention in orange juice on standing was not influenced by the method of extraction, whether by stationary reamer, rotating reamer, or juice press, nor by storage in open or closed containers, nor by dilution with tap water. They expressed the view that the length of time orange or grapefruit juices may be stored is limited by loss of palatability, and onset of fermentation, rather than by loss of ascorbic acid.

Lamden et al. (1960) carried out a critical study of the quality and particularly the ascorbic acid content of chilled orange juices purchased in retail markets and delivered to homes, and found that the ascorbic acid content was only 57% of that found in fresh oranges purchased at the same time. They attributed the loss to inadequate control of refrigerated storage temperatures during distribution.

To summarise, the constituents of citrus fruits other than ascorbic acid have doubtful dietary significance.

Paradoxically, however, lack of nutrients has become a positive virtue in citrus foods in the well-fed countries where consumers are increasingly weight conscious for reasons of health or feminine beauty.

SECTION II

ANALYSIS OF NZGF JUICE FOR ENZYMES

Before seasonal changes in NZGF juice could be determined, it was necessary to carry out analyses to see if enzymes which were present in certain overseas citrus varieties were present in NZGF juice. The enzymes of interest were:

(a) pectic enzymes, which if present could lead to cloud destabilisation, and

(b) ascorbic acid oxidase, which if present could reduce the ascorbic acid content of the juice.

A. PECTIC ENZYMES

1. INTRODUCTION

The most extensive investigations on enzyme activity in citrus fruits have been concerned with the occurrence and activity of pectic enzymes, particularly as these effect the undesirable changes in the appearance of citrus juices. Pectic enzymes may be divided conveniently into two main groups, the saponifying enzymes or pectin-esterases, and the depolymerising pectic enzymes (Rombouts and Pilnik, 1972).

(i) saponifying enzymes - these are specific pectin-methylesterases which split the methylester group of polygalacturonic acid. They are now commonly called pectinesterase (pectin pectyl-hydrolase,

EC 3.1.1.11) and abbreviated PE, or pectinmethylesterase (PME). Formerly they were often referred to as pectinmethoxylase, pectindemethoxylase, pectolipase, and pectase.

(ii) depolymerising enzymes - these include glycosidases and lyases (this term is now preferred to "transeliminase" by the International Union of Biochemistry) with specific activities pertaining to the degree of esterification of the substrate.

Polygalacturonase (poly-α-1,4-galacturonide glycanohydrolase, EC 3.2.1.15), abbreviated PG, is the name given to the enzyme which splits the glycosidic links between adjacent galacturonic acid molecules. Former names for this enzyme include pectinase, pectolase, and polygalacturonidase.

Polymethylgalacturonase (PMG) also splits the glycosidic links, but in contrast to PG, it is capable of acting on the completely esterified substrate polymer.

Both PG and PMG exist in the endo- form (causing random hydrolysis of α-1,4 glycosidic links) and the exo- form, where the hydrolysis is in a sequential fashion, starting from the non-reducing end of the chain. For each of the glycosidases, there exist corresponding lyases which cause cleavage of α-1,4 glycosidic links by transelimination as opposed to hydrolysis.

(a) Pectinesterase - MacDonnell et al. (1945) first reported on the isolation and characterisation of orange PE. The results of Rouse (1953) on the distribution of PE in various parts of Florida oranges showed all the PE to be completely adsorbed on water-insoluble cell tissue. These results are of fundamental importance to the citrus processor because they mean that the methods of juice extraction and juice finishing, which determine the amount of pulp suspended in the juice, also determine the enzyme activity of the juice.

In 1954 Rouse et al. reported a straight line relationship between PE activity and the pulp content of orange juice. Because all the NZGF juice used for the experimental work in this study was passed through a double layer of cheesecloth, the pulp content remained constant at around 10%, permitting direct comparisons of the PE level in NZGF juices of different backgrounds.

PE activity is usually measured by letting the enzyme-containing extract act on a synthetic pectin substrate while keeping its pH constant near neutrality by the addition of alkali. The carboxyl groups freed by enzyme action per unit of time can then be measured by the amount of alkali used.

Alternative methods include a manometric procedure which measures the carboxylic acid groups, liberated by the enzyme from pectin, by evolution of carbon dioxide from sodium bicarbonate solution (Primo

et al., 1967). A qualitative method (Kertesz, 1937) based on the colour change of methyl red was popular with many of the early workers but is not used today.

The method used in this study was based on one developed at the University of Florida Citrus Experiment Station (Rouse and Atkins, 1955) and now used almost universally by the citrus processing industry.

As the juice extraction method used throughout this study gave a relatively constant level of pulp or insoluble solids in the juice samples, and because the units used to express PE activity take into account variations in the level of soluble solids, direct comparisons of enzyme activity in juices of various backgrounds can be made.

Although it has never been supposed that PE is absent in NZGF, its presence has never been reported. Therefore, NZGF juice was analysed for the presence of PE activity.

(b) Polygalacturonase - in 1953 Pratt and Powers reported the thermal destruction times for pectin and pectic acid depolymerising enzymes, but admitted that due to problems with their analytical methods, they were unable to differentiate between PG and PMG. Of the 14 samples of grapefruit juice which they tested, only 4 showed any depolymerising enzyme activity.

However, the presence of PG in oranges was considered to be unlikely by Sinclair and Jolliffe (1958) when they were unable to find free galacturonic acid in oranges ripened in storage, and in juice allowed to stand until the cloud precipitated. Nevertheless, Primo et al. (1963) reported the presence of galacturonic acid in Spanish Valencia juice.

Further evidence for the absence of PG in citrus fruits was provided by Hobson (1962) who failed to detect any activity in the flesh of the tangerine although he used a highly efficient method of extraction. Mannheim and Siv (1969) also found no PG activity in oranges, mandarins, and lemons, but detected slight activity in some samples of Marsh Seedless grapefruit.

On the basis of the latter paper, it was decided to analyse NZGF juice for the presence of PG activity. If its presence was confirmed, then a method for its destruction would have to be devised as its presence in the juice would assist in the destabilisation of the cloud.

Three methods are available for the determination of PG activity in citrus juices. The method of Stier et al. (1956) involves viscometry, while the method of Hobson (1962) involves colorimetry. The method in common use is a modification of the Willstalter-Schudel hypoiodite method, involving the iodometric determination of the increase in the concentration of

reducing groups due to PG activity (Methods in Enzymology, 1955). Because Mannheim and Siv (ibid.) used a variation of the latter method when they detected slight PG activity in Marsh Seedless grapefruit, it was decided to use the same basic method in these studies.

2. EXPERIMENTAL

(a) Pectinesterase

To 20 ml of a 1% solution of pectin (Sigma Chemical Company Grade 1 Citrus Pectin) in 0.1 M sodium chloride, sufficient 0.1 N sodium hydroxide was added to bring the pH to 7.5. The solution was then placed in a constant temperature water bath at 30 C. After the addition of 10 ml of the juice, the pH was readjusted to 7.5, again with 0.1 N sodium hydroxide. The pH was then maintained at 7.5 for 30 minutes by the addition of 0.02 N sodium hydroxide through an automatic titrimeter.

Pectinesterase activity was expressed as pectinesterase units per millilitre of total soluble solids (Brix) as determined by a refractometer, as this is a term commonly used and understood by the citrus industry. The formula used to compute PE activity was:

$$(\text{PEu})\text{ml TSS} = \frac{\text{ml sodium hydroxide X normality of NaOH}}{\text{volume of juice sample X 30 min. X Brix/100}}$$

For easy interpretation, the activity of the enzyme was multiplied by 10^4.

Samples of juice from both early and late

season NZGF were analysed for the presence of PE activity.

(b) Polygalacturonase

To 99.5 ml of freshly-extracted NZGF juice was added polygalacturonic acid (0.5 g) (sodium polypectate Grade II, Sigma Chemical Company) and the mixture stirred until the acid had dissolved. At the same time, an equal quantity of polygalacturonic acid was added to 99.5 ml of juice which had been heated to 100 C, held at that temperature for 5 minutes, and then cooled. This served as the blank.

The samples were incubated at 40 C for up to 96 hours and checked periodically for enzyme activity. The activity was determined by adding to 10 ml of the sample in a 250 ml conical flask, 1.8 ml of 1 M sodium carbonate solution and 10 ml of standardised 0.1 N iodine solution. The mixture was set aside in the dark for 30 minutes and then acidified by the addition of 4 ml of 2 M sulphuric acid. The solution was immediately titrated with 0.05 N sodium thiosulphate solution using starch as the indicator. The difference in thiosulphate consumption between sample and blank, prepared as outlined, yielded the increase in the concentration of reducing groups due to enzyme activity, after taking into account the differences in normality of the iodine and thiosulphate solutions. 1 ml of 0.1 N iodine is equivalent to 0.05 meq. of galacturonic acid.

Samples of juice from both early and late

season NZGF were analysed for the presence of PG activity. The above procedure was performed in duplicate for each sample.

3. RESULTS

(a) Pectinesterase - the results are presented below in Table XI.

Table XI

Pectinesterase Activity in NZGF Juice

Sample	pH	Sodium Hydroxide Consumption (mls 0.02 N solution)	PE Activity (PEu)ml TSS
Early Season (July)	2.95	6.55	41.2
Late Season (December)	3.40	16.7	89.2

(b) Polygalacturonase - the results are presented below in Tables XII and XIII.

Table XII

Polygalacturonase Activity in Early Season (July) NZGF Juice

Sample	Incubation Time (hours)	Thiosulphate Consumption (mls 0.05 N solution) $\bar{x}$	Δx	PG Activity (meq. GA per ml of juice)
Juice	24	8.9		
Blank	24	9.0	+ 0.1	0.000025
Juice	48	9.1		
Blank	48	9.1	0.0	
Juice	72	9.6		
Blank	72	9.0	- 0.6	
Juice	96	9.1		
Blank	96	9.0	- 0.1	

Table XIII

Polygalacturonase Activity in Late Season (December)

NZGF Juice

Sample	Incubation Time (hours)	Thiosulphate Consumption (mls 0.05 N solution) $\bar{x}$	Δx	PG Activity (meq. GA per ml of juice)
Juice	24	10.9		
Blank	24	11.1	+ 0.2	0.00005
Juice	48	11.3		
Blank	48	10.4	- 0.9	
Juice	72	11.9		
Blank	72	11.0	- 0.9	
Juice	96	8.9		
Blank	96	9.0	+ 0.1	0.000025

4. DISCUSSION

(a) Pectinesterase - the results presented in Table XI indicate that PE is present in NZGF juice. The significance of the levels is discussed in Section IV of this chapter, together with a comparison of published PE levels in other citrus juices.

(b) Polygalacturonase - in the early season juice, extremely low PG activity was detected after 24 hours, but this was not confirmed after 48 hours incubation. The analyses performed after 72 and 96 hours showed that the blanks had more reducing groups than the samples of juice. Thus the experimental errors are greater than the supposed levels of PG in the juice.

The late season juice showed a similar pattern, with extremely low PG activity being detected after 24 and 96 hours incubation. The blanks had more reducing groups than the samples of juice after 48 and 72 hours incubation.

Mannheim and Siv (1969), using the same method, found low levels of PG activity (0.0008 meq. GA per ml of enzyme extract) in some but not all of their extracts of Marsh Seedless grapefruit. They found no PG activity in extracts of Shamouti and Valencia oranges, mandarins and lemons. However, using the same method, they did find relatively high levels of PG activity in all tomato extracts, a fruit renowned for its PG content.

Pratt and Powers (1953) reported the presence of PG activity in 4 out of 14 samples of grapefruit juice tested, but they used different assay techniques from those used in this study and the one by Mannheim and Siv. Only one of their assay methods (a viscometric technique) indicated the presence of PG activity, the other method (determining the amount of non-degraded pectin after incubation) giving negative results for all 14 samples. They were unable to account for the difference in sensitivity of the two assay methods.

5. CONCLUSION

(a) Pectinesterase - the presence of PE activity in both early and late season NZGF juice has been confirmed.

(b) Polygalacturonase - the presence of PG activity in both early and late season NZGF juice has not been confirmed. The only two published reports of PG activity in citrus fruits are tenuous and refer to a variety of grapefruit which is quite unrelated to the NZGF.

It is therefore concluded that NZGF juice does not contain PG.

B. ASCORBIC ACID OXIDASE

1. INTRODUCTION

Ascorbic acid oxidase was first detected in 1928 and is a copper-containing protein that catalyses the aerobic oxidation of ascorbic acid to dehydroascorbic acid.

The presence of the enzyme in citrus peel was reported by Huelin and Stephen (1948) who found negligible amounts in orange juice. However, the above report was based on the ability of the enzyme preparation to oxidase ascorbic acid, and many compounds are capable of this in the absence of ascorbic acid oxidase.

It was left to Vines and Oberbacher (1963) to confirm the enzyme's presence in oranges. They found it

concentrated in the flavedo layer of the peel. It commenced its action as soon as the peel was subdivided.

The same workers (Vines and Oberbacher, 1962) surveyed ascorbic acid oxidase activity in several citrus fruits and found the order of decreasing activity to be Marsh grapefruit, Pineapple and Valencia oranges, Thompson grapefruit, Persian limes, and Villafranca lemons. On a fresh weight basis, the ascorbic acid oxidase content was highest in immature citrus fruit and decreased as the fruit matured.

As no work had been published on the presence or absence of ascorbic acid oxidase in New Zealand citrus, it was thought appropriate to test NZGF juice for the presence of this enzyme, for if it was present in the fresh juice, it would reduce the ascorbic acid content. If its presence was confirmed, then the processing conditions necessary for its inactivation would be established.

2. EXPERIMENTAL

The method used was based on the manometric method described by Vines and Oberbacher (1963). Standard Warburg manometers were used to measure oxygen uptake at 25 C.

The reaction mixture contained 30 micromoles of ascorbic acid, 33 millimoles of potassium phosphate at a pH of 5.6, and varying quantities of freshly extracted NZGF juice (1, 2 and 5 mls). A control manometer containing juice which had been heated for 5 minutes, and a thermo-

barometer, were employed in all experimental runs. A 60 minute reaction period was used.

Samples of juice from both immature and mature NZGF were tested.

3. RESULTS

In no run was there any significant or repeatable uptake of oxygen.

4. DISCUSSION

These results are not altogether unexpected in view of the previous work on this enzyme mentioned in the introduction. Not all overseas varieties of citrus contain ascorbic acid oxidase, and in view of the doubtful parentage of the NZGF there is no reason why it should contain it.

However, these results do not preclude the presence of the enzyme in whole NZGF, but do suggest that even if it is present in the fruit, only extremely low concentrations (if any) are found in the juice. (Vines and Oberbacher (1963) found ascorbic acid oxidase to be concentrated in the flavedo layer of the peel).

5. CONCLUSION

It is concluded that there is no problem presented to the citrus processor by ascorbic acid oxidase in NZGF juice.

SECTION III

SEASONAL CHANGES IN THE COMPOSITIONAL CHARACTERISTICS OF NZGF JUICE OF IMPORTANCE TO CITRUS PROCESSORS

1. INTRODUCTION

Seasonal changes in the compositional characteristics of NZGF juice were followed throughout the 1973 season. The main juice processing season in New Zealand extends from August to November, although there have been occasions when it has begun as early as June. In this study, analysis of NZGF began in July and continued until December.

The characteristics tested for were yield, soluble solids, insoluble solids, titratable acidity, pH, PE activity, and ascorbic acid content. The importance of these factors to the citrus processor has been outlined in Section I of this chapter.

The seasonal changes in these factors were followed for two reasons. Firstly, the small amount of published data on the composition of NZGF juice gives no indication of the maturity of the fruit sampled , or of seasonal variations in the composition. Secondly, no data exists in New Zealand quantifying the interrelationships between the various compositional characteristics of NZGF juice. There is a very real need by citrus processors for this data, for if

it existed, they would be able to carry out two or three simple tests on samples of fruit delivered to the factory, and from this be able to predict the quality of the resultant juice. The payment to the grower could then be related to both the quantity and the quality of the fruit, rather than just to the quantity as at present.

Consequently, the interrelationships of the compositional characteristics of the juice were examined in some detail, and recommendations made as to the most reliable and indicative tests which could be made by processors on in-coming fruit.

2. EXPERIMENTAL

(a) Raw Material - the grapefruit samples used in this study were of the Morrison's Seedless variety grown on trifoliata stock. The trees formed part of a commercial orchard at Te Puke, Bay of Plenty, and were four years old.

Twelve representative trees were selected from the orchard, and at approximately fortnightly intervals one fruit from each of the twelve trees was picked, packed in a wooden box, and sent by rail and bus to Palmerston North, a distance of approximately 255 miles.

The transportation stage generally occupied three days, and the analyses were in most cases performed on the fourth day after picking. Occasionally, analyses

were not performed until the seventh day after picking due to transportation delays. The fruit remained at ambient temperatures from the time of picking to the time of analysis.

(b) Juice Yield - after weighing, the fruit was halved and the juice removed using the reamer attachment on a domestic Kenwood mixer operating at speed one. The juice was then passed through a double layer of cheese-cloth to remove gross suspended matter, and weighed.

The yield was calculated as the weight of juice obtained from a given weight of fruit, expressed as a percentage.

(c) Insoluble Solids - as with essential oil content, the insoluble solids content of citrus juice depends on the method of extraction rather than the raw material. Consequently, random samples of the juice used throughout this study were tested for pulp content according to the standard method published by the Food Machinery Corporation (1964). In this method, pulp is defined as the portion of the suspended particles precipitated by centrifugation at 360g for 10 minutes. Pulp volume is the volume of the precipitate as read on the graduation of the centrifugation tube and expressed as a percentage of the total juice volume.

(d) Total Soluble Solids - although this includes the sugar, acid, soluble pectins and other compounds dissolved in

the juice, the term "total soluble solids" (TSS) has become virtually synonymous with "sugar content" since in practice about 90% of the TSS is sugar. Corrections can be made to take into account the other constituents present (Stevens and Baier, 1940). Although such corrections must be made with concentrates (Basker, 1966), they are seldom applied to single strength juices.

An Abbe refractometer was used to determine the TSS content of the juice. It was read directly from the instrument as degrees Brix.

(e) pH - the pH of the juice was measured using a standard Radiometer pH meter. Prior to a reading being taken, the meter was calibrated to pH 3.2 using a suitable buffer.

(f) Titratable Acidity - the total acidity of citrus juices is ordinarily determined by titrating the juice with sodium hydroxide using phenolphthalein as indicator and expressing the result as citric acid. This so-called titratable acidity adequately represents the organic acid content of the juice. The free acidity (as citric) can also be determined potentiometrically. According to the experiments of Bollinger (1942), the total acidities of juice and water extracts of fruits, calculated from potentiometric end points of pH 8.10, agreed closely with those determined by the phenolphthalein titration. If it is assumed that the inflexion point in a potentiometric titration juice is 8.10, then titratable acidity

will equal free acidity.

In this study, titratable acidity was determined by titrating 20 ml of the juice with 0.1 N sodium hydroxide, using phenolphthalein as an indicator. Distilled water (50 ml) was added to the juice prior to titration to reduce the orange colour and make the pink end point sharper.

The titration values were expressed as grams of citric acid per 100 ml of juice. As 1 milliequivalent of citric acid is 64 mg, then 1 ml of 0.1 N sodium hydroxide is equivalent to 0.0064 g of citric acid.

(g) Brix : Acid Ratio - this was calculated by dividing the total soluble solids reading by the titratable acidity.

(h) Pectinesterase Activity - this was determined by the method described in Section II of this chapter (see p. 36).

(i) Ascorbic Acid - this was determined using the visual titration method:

(i) a freshly-made solution of dye was prepared by dissolving 2,6-dichlorophenolindophenol (0.1 g) in water, diluting to 200 ml, and filtering. This solution was standardised with a freshly-prepared solution containing a known weight of ascorbic acid dissolved in 1% oxalic acid, thus permitting the calculation

of the mg of ascorbic acid which were equivalent to 1 ml of dye.

(ii) 10 ml of NZGF juice was added to a 250 ml flask containing 50 ml of water and a few drops of 10% v/v acetic acid.

(iii) the solution was titrated with the dye until a faint pink colour persisted for 15 seconds.

(iv) the ascorbic acid content of the juice was calculated and expressed as mg/100 ml of juice.

3. RESULTS

A summary of all the results in this section is contained in Tables XIV and XV.

Table XIV

SEASONAL CHANGES IN NZGF JUICE IN 1973

Summary of Results

Days	Date	Yield	TSS	T/A	Brix : Acid	pH	PE	Vit. C
1	3.7.73	26.9%	10.6	2.11	5.02	2.95	41.2	26.8
15	17.7.73	33.5	11.5	2.05	5.61	2.95	51.0	32.7
29	31.7.73	24.5	12.2	2.03	6.01	2.90	45.4	31.9
42	13.8.73	22.9	12.2	1.98	6.16	3.05	77.5	31.0
56	27.8.73	32.3	12.6	1.78	7.08	3.20	55.0	32.2
70	10.9.73	38.3	12.0	1.81	6.63	3.05	88.5	31.2
77	17.9.73	37.3	12.4	1.71	7.25	3.15	81.2	32.4
93	3.10.73	41.6	12.3	1.44	8.55	3.20	78.5	27.7
105	15.10.73	38.2	12.6	1.47	8.11	3.10	75.0	28.5
119	29.10.73	34.7	12.1	1.28	9.45	3.25	76.0	28.1
140	19.11.73	39.7	13.1	1.38	9.49	3.40	91.0	27.6
154	3.12.73	38.8	13.2	1.30	10.03	3.40	75.0	23.0
168	17.12.73	37.4	12.5	1.29	9.69	3.40	89.2	24.5

Table XV

RELATIONSHIPS BETWEEN VARIOUS COMPOSITIONAL CHARACTERISTICS OF NZGF JUICE THROUGHOUT 1973 SEASON

Figure	Variables (y and x respectively)	Regression Equation	Standard Error of Estimate	Correlation Coefficient	Significance of r
4	Titratable Acidity and Time	$y = -0.0057x + 2.132$	0.083	-0.9618	0.1%
5	Titratable Acidity and TSS	$y = -0.328x + 5.69$	0.215	-0.7065	1%
6	Brix : Acid Ratio and Time	$y = 0.031x + 5.0$	0.361	+0.9754	0.1%
7	Brix : Acid Ratio (log) and pH	$y = 0.525x - 0.7867$	0.037	+0.9228	1%
8	Brix : Acid Ratio and TSS	$y = 1.93x - 16.03$	1.136	+0.721	1%
9	Brix : Acid Ratio and Titratable Acidity	$y = -5.30x + 16.4$	0.288	-0.9845	0.1%
12	pH and Titratable Acidity	$y = -0.492x + 3.97$	0.075	-0.8974	0.1%

(a) Juice Yield - the change in juice yield throughout the 1973 season is shown in Figure 1 (p. 55). The yield is shown on the left-hand axis as kilograms of juice per kilogram of fruit, and on the right-hand axis as kilograms of soluble solids per tonne of fruit. The points have been joined to show more clearly the seasonal trends.

(b) Insoluble Solids - all the samples tested for insoluble solids or pulp content fell within the range 10 $\pm$ 0.5%.

(c) Total Soluble Solids - Figure 2 (p. 56) shows on the left-hand axis the change in TSS throughout the season while the right-hand axis shows the same data expressed as kilograms of total soluble solids per tonne of fruit.

(d) pH - Figure 3 (p. 57) shows the change in pH throughout the season.

(e) Titratable Acidity - the seasonal variation in titratable acidity is shown in Figure 4 (p. 58). The regression equation for the line of best fit through the points was calculated as:

$$y = -0.0057x + 2.132$$

where y = titratable acidity

and x = time in days.

The correlation coefficient for the line was -0.9618, indicating a negative correlation between the titratable acidity and time. The Student's t test showed that the observed value of the correlation coefficient was

significant at the 0.1% level. The 95% confidence limits have been plotted on Figure 4.

In Figure 5 (p. 59), the titratable acidity of the juice is plotted against the corresponding TSS, the regression equation being:

$$y = -0.328x + 5.69$$

where y = titratable acidity

and x = TSS.

The correlation coefficient was -0.7065, which is significant at the 1% level according to the Student's t test. The 95% confidence limits are drawn in.

(f) Brix : Acid Ratio - Figure 6 (p. 60) shows the change in the Brix : acid ratio throughout the season. The equation of the regression line was calculated as

$$y = 0.031x + 5.0$$

where y = Brix : acid ratio

and x = time in days.

The correlation coefficient was +0.9754 and the Student's t test indicated that this value was significant at the 0.1% level.

In Figure 7 (p. 61), the regression line showing the relationship between the Brix : acid ratio and pH is plotted. The log of the Brix : acid ratio for each sample was plotted against its corresponding pH to show the spread of values. The equation of the regression line was calculated as

$$y = 0.525x - 0.7867$$

where y = log of Brix : acid ratio

and x = pH.

The correlation coefficient for the line was +0.6979, and the Student's t test indicated that this value was significant at the 1% level.

In Figure 8 (p. 62), the Brix : acid ratio was plotted against the TSS of the sample. The regression line was calculated and has been plotted, together with the 95% confidence limits. The regression equation was:

$$y = 1.93x - 16.03$$

where y = Brix : acid ratio

and x = TSS.

The correlation coefficient of +0.721 was significant at the 1% level according to the Student's t test.

Figure 9 (p. 63) shows the Brix : acid ratio plotted against the titratable acidity. The equation for the regression line was calculated as

$$y = -5.30x + 16.4$$

where y = Brix : acid ratio

and x = titratable acidity.

The correlation coefficient was -0.9845 which is significant at the 0.1% level.

(g) Pectinesterase Activity - the variation in PE activity in NZGF juice throughout the 1973 season is shown in Figure 10 (p. 64). The points have been joined to clarify the variation.

(h) Ascorbic Acid - Figure 11 (p. 65) shows the seasonal fluctuations in the ascorbic acid content of NZGF juice.

FIGURE 1. Seasonal variation in yield of NZGF juice.

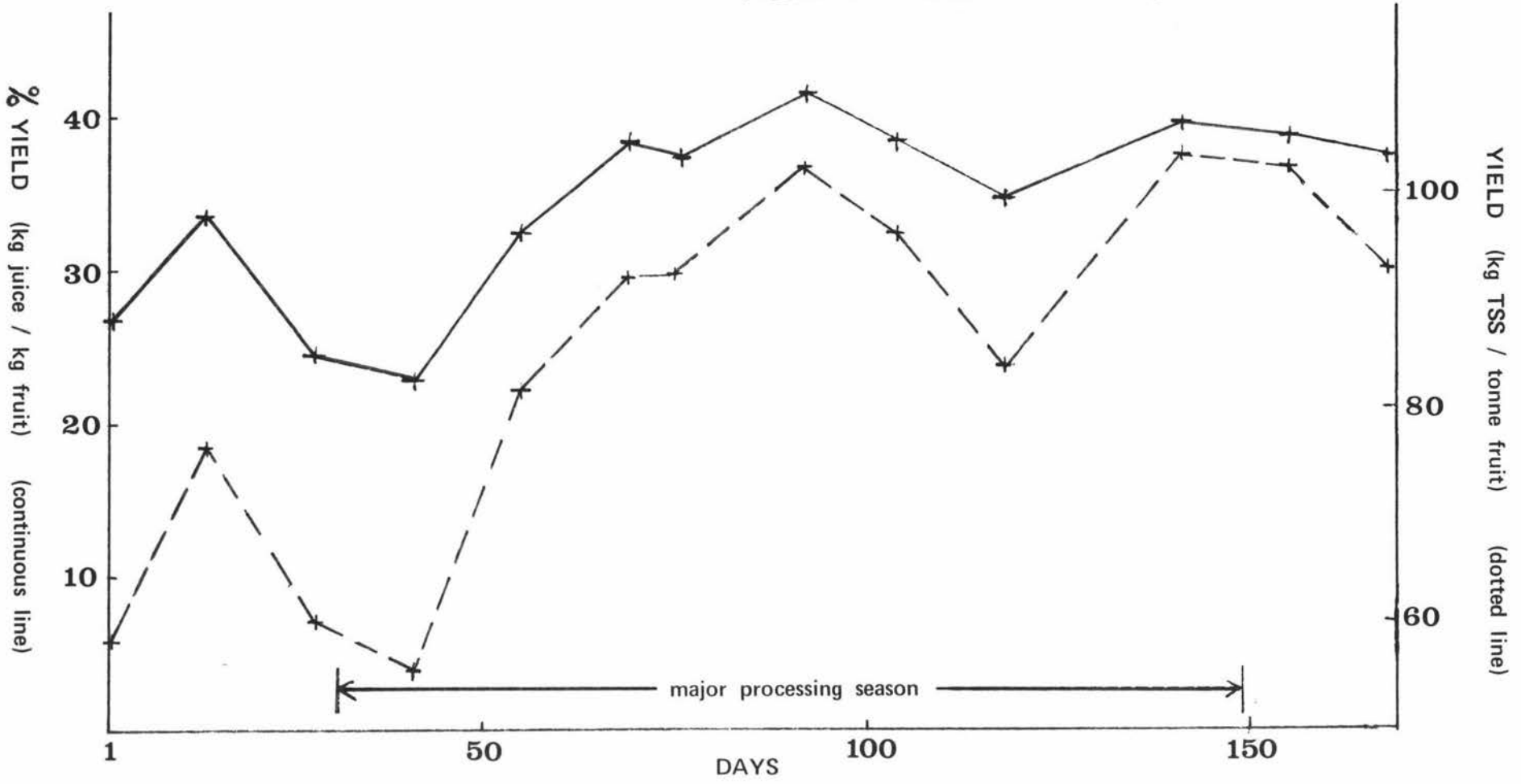


FIGURE 2. Seasonal variation in TSS and yield of NZGF juice.

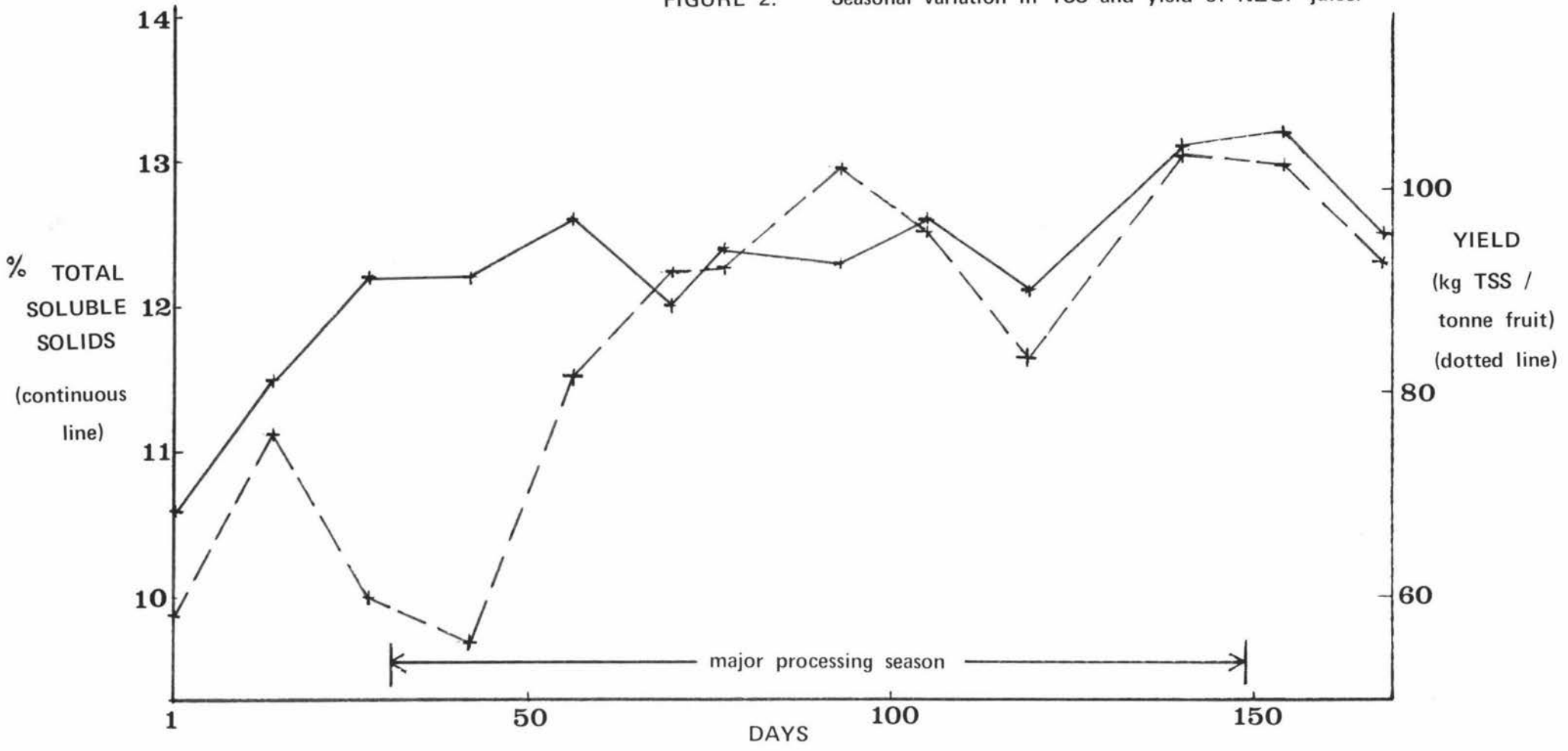
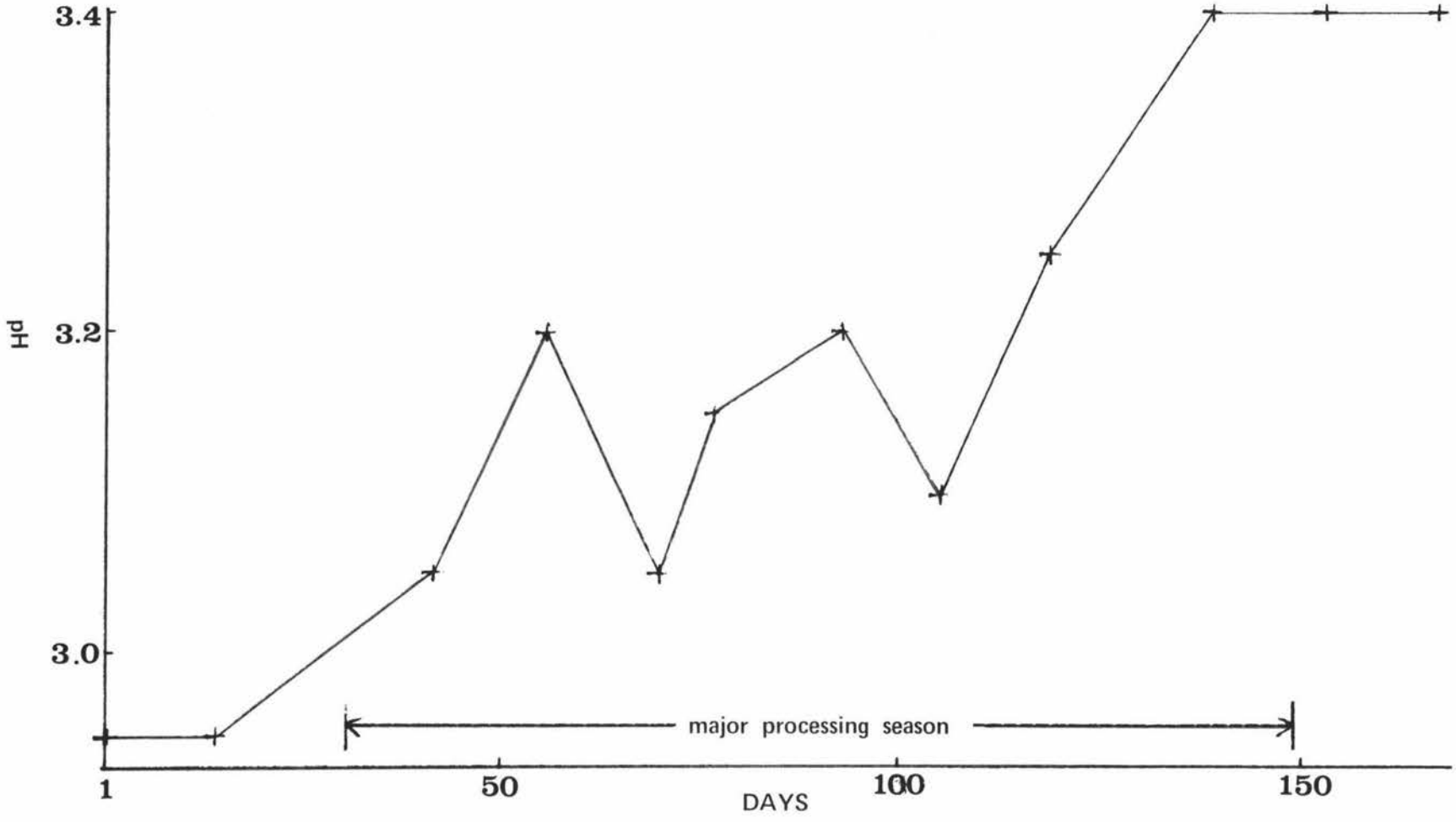


FIGURE 3. Seasonal variation in pH of NZGF juice.



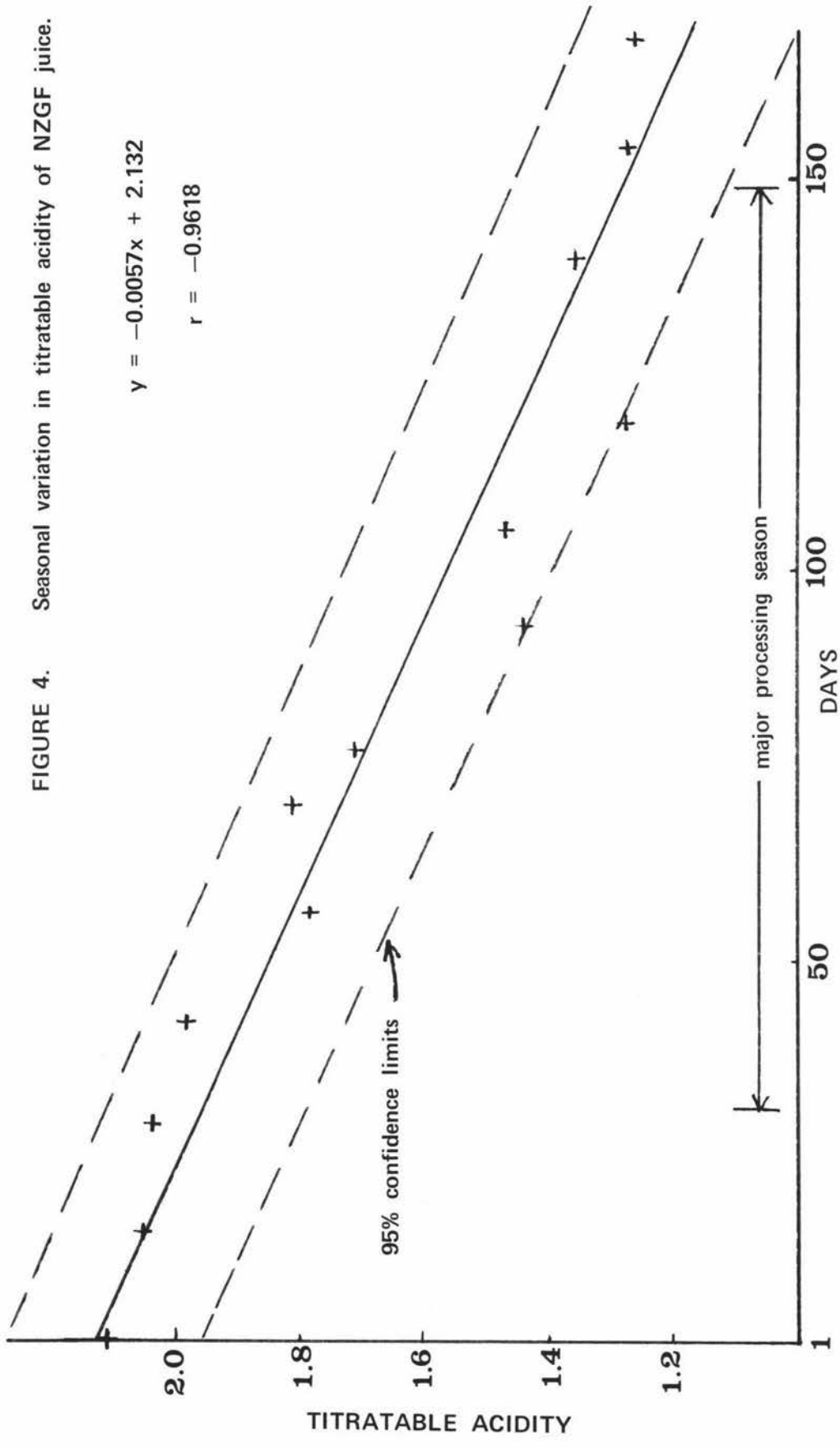


FIGURE 4. Seasonal variation in titratable acidity of NZGF juice.

FIGURE 5. Relationship between titratable acidity and TSS of NZGF juice.

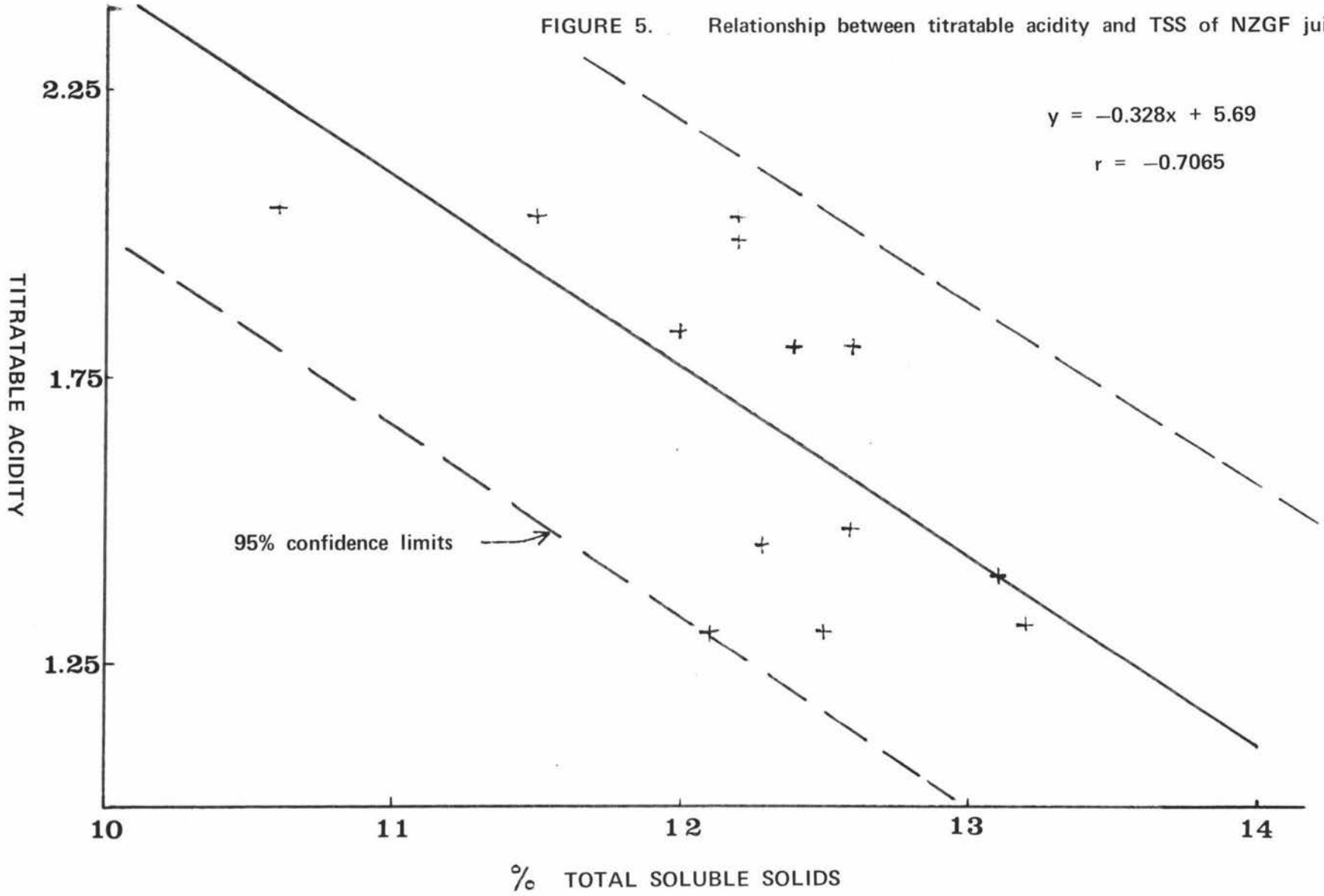


FIGURE 6. Seasonal variation in Brix : acid ratio of NZGF juice.

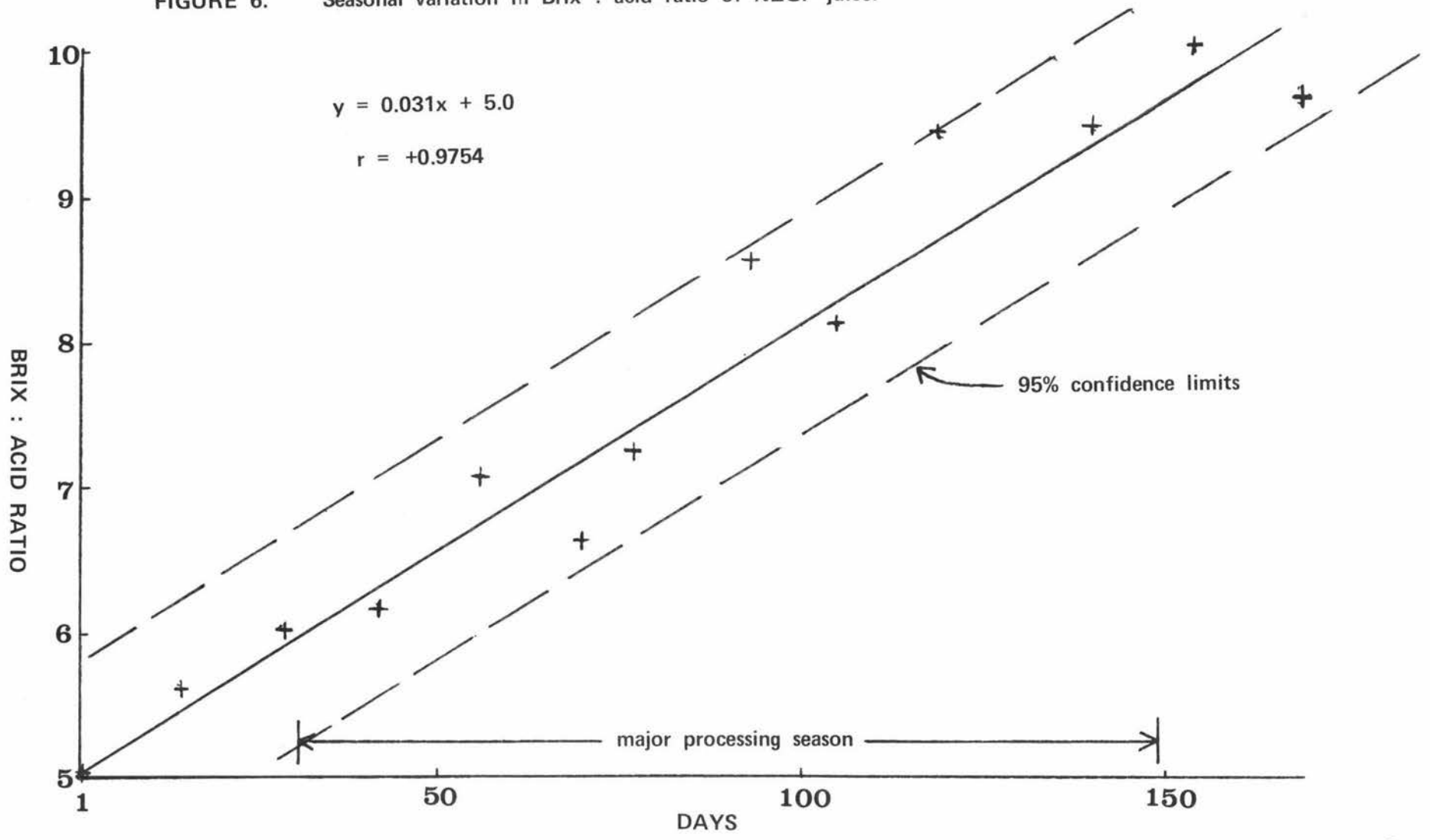


FIGURE 7. Relationship between Brix : acid ratio and pH of NZGF juice.

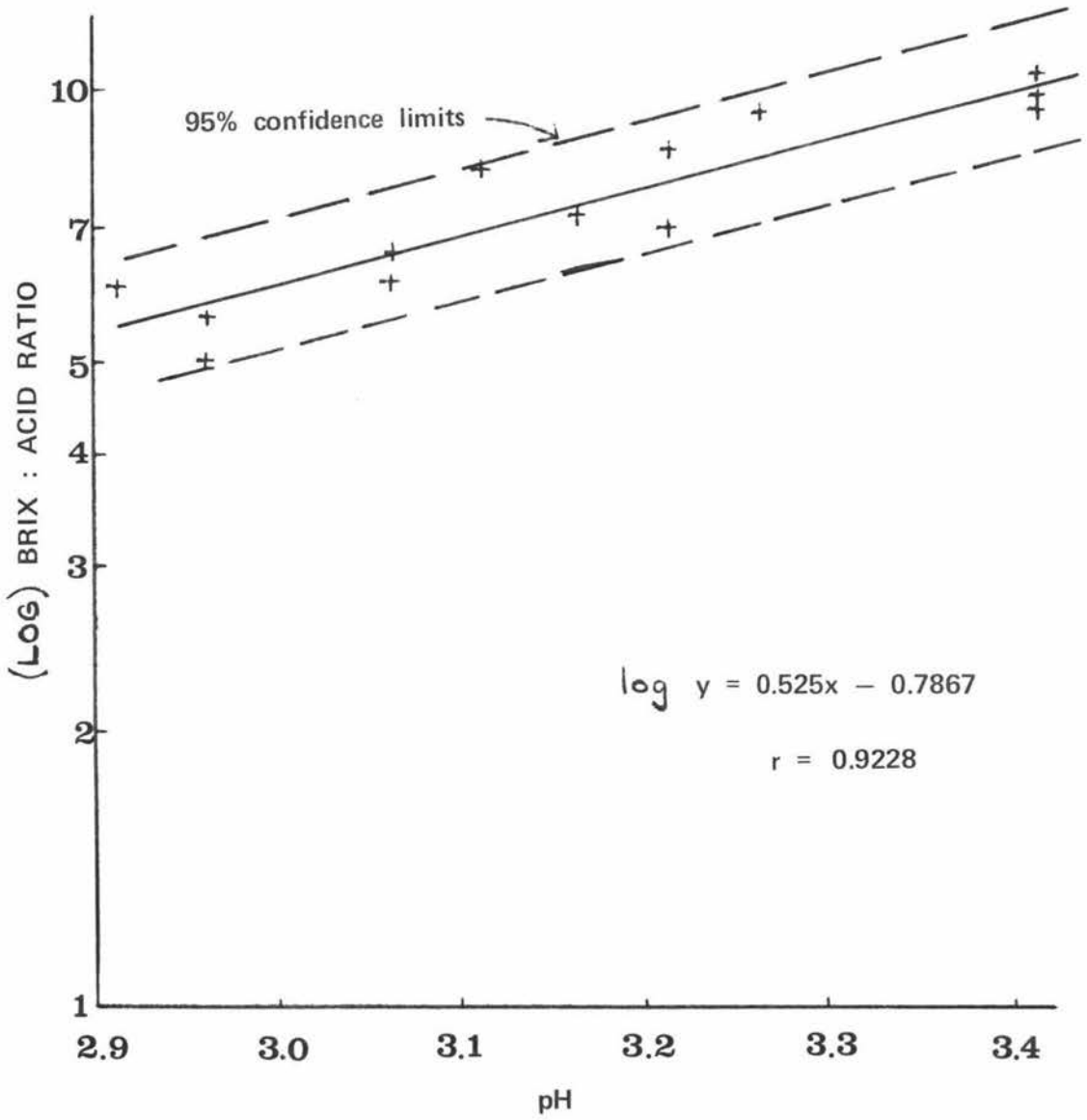
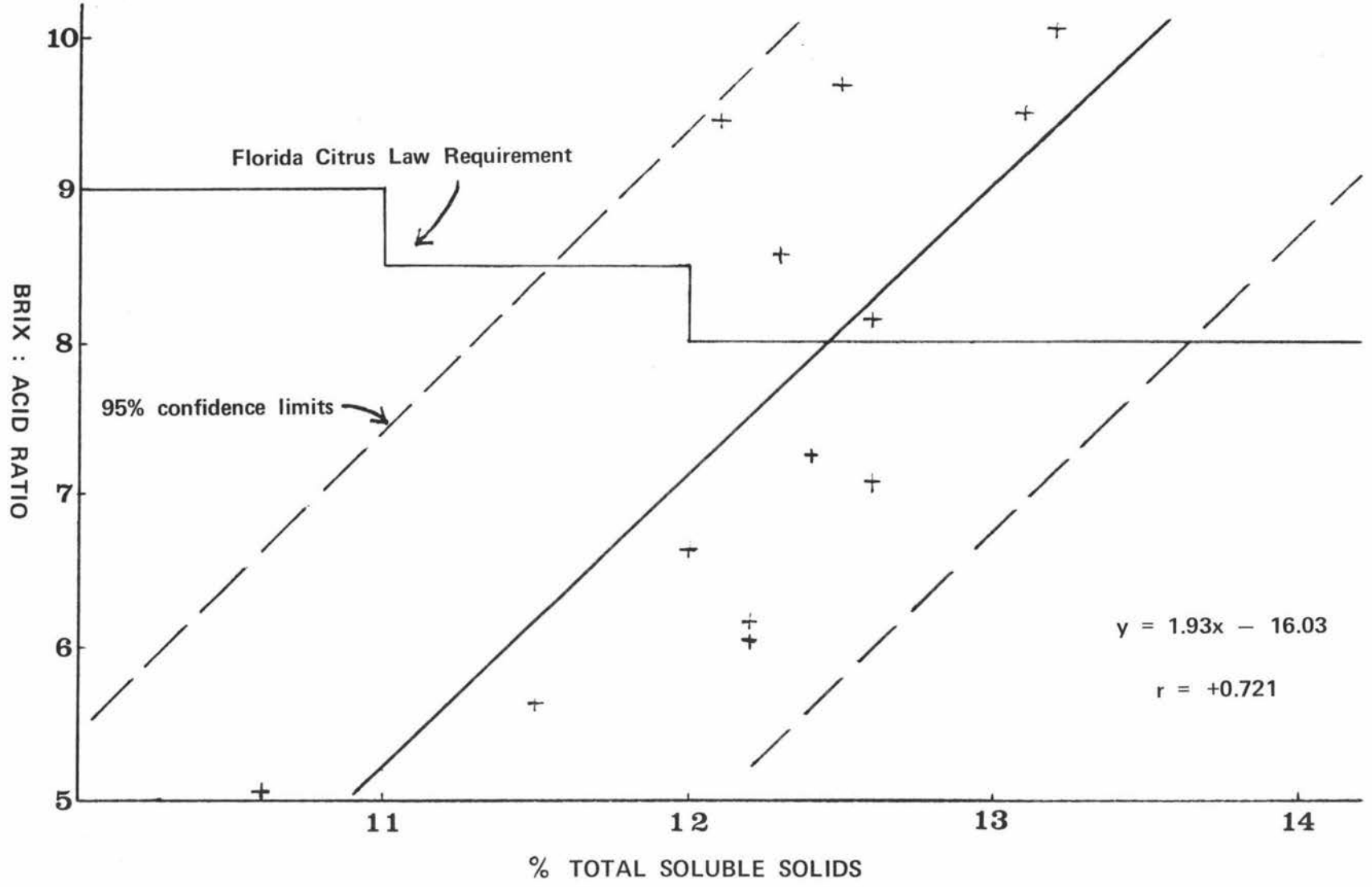


FIGURE 8. Relationship between Brix : acid ratio and TSS of NZGF juice.



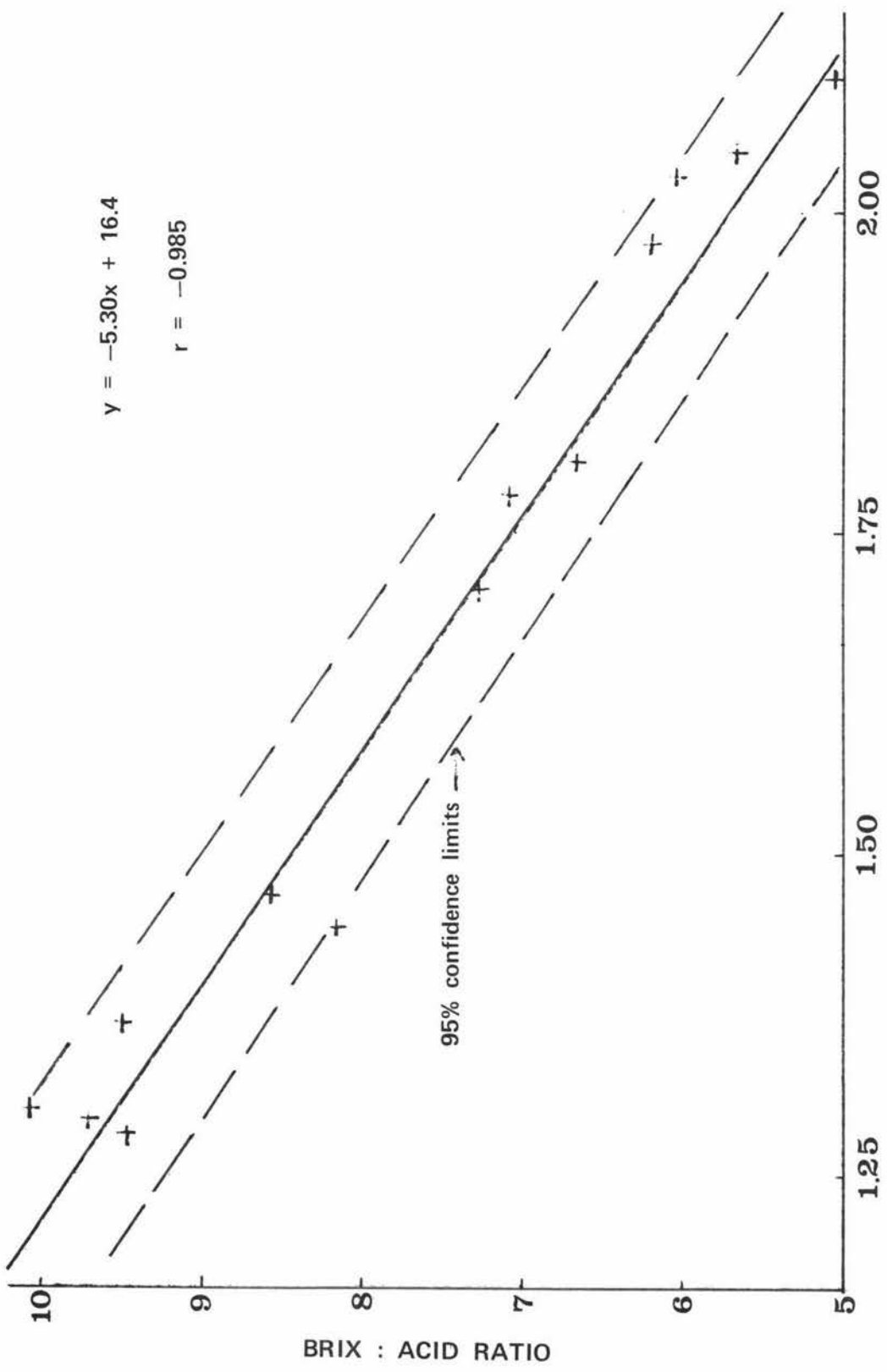


FIGURE 9. Relationship between Brix : acid ratio and titratable acidity of NZGF juice.

FIGURE 10. Seasonal variation in pectinesterase activity of NZGF juice.

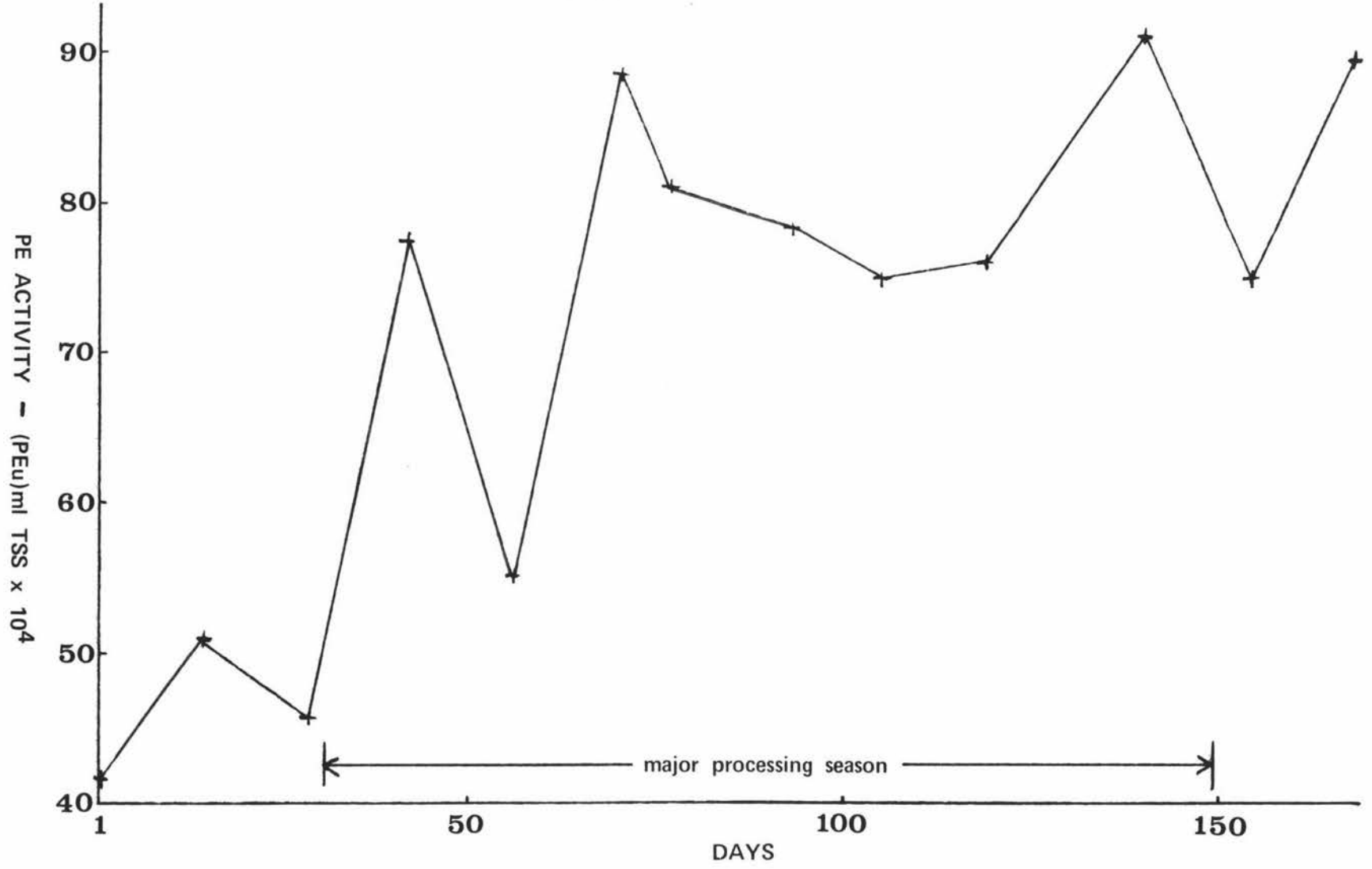


FIGURE 11. Seasonal variation in ascorbic acid content of NZGF juice.

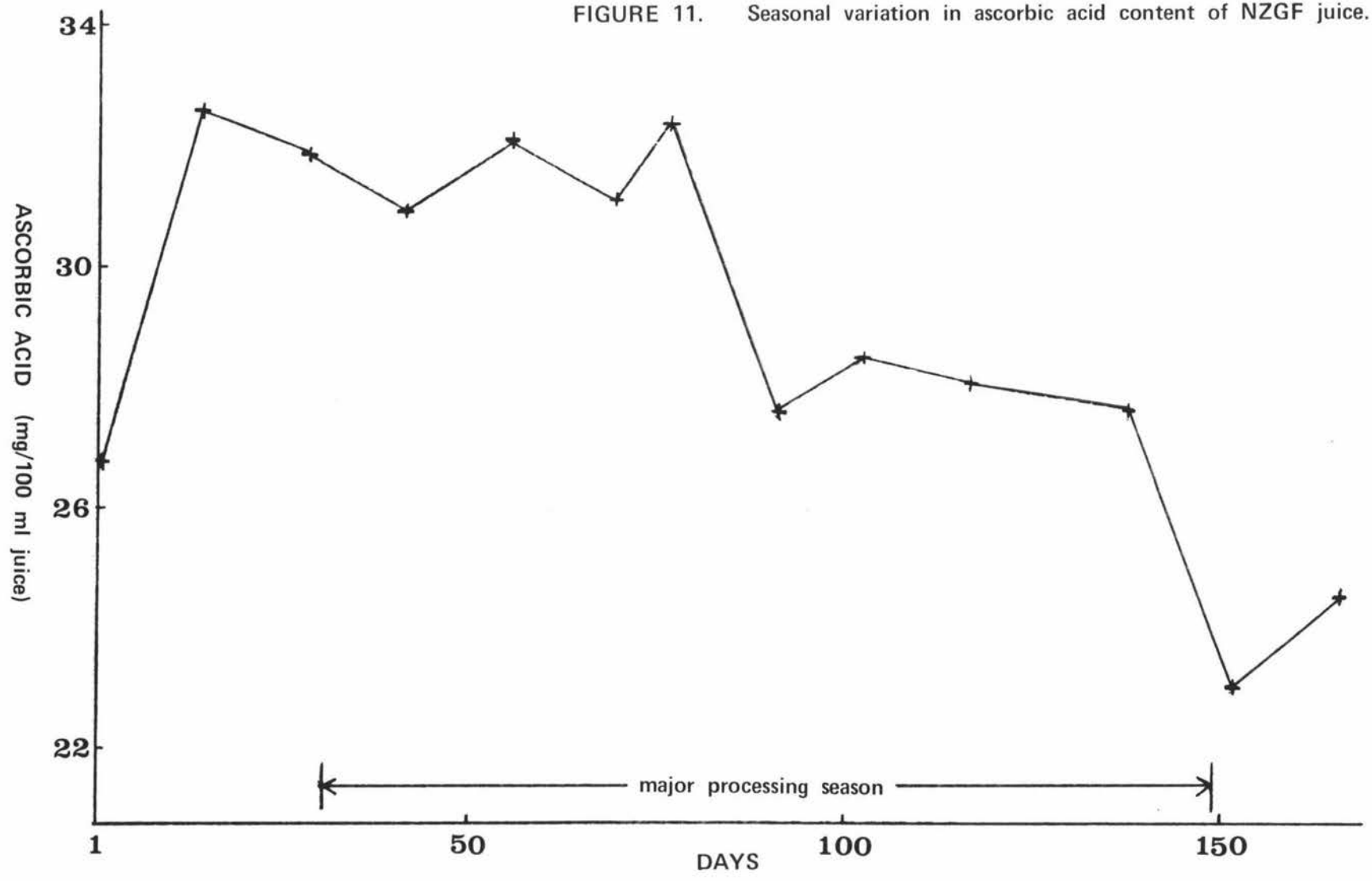


FIGURE 12. Relationship between pH and titratable acidity of NZGF juice.

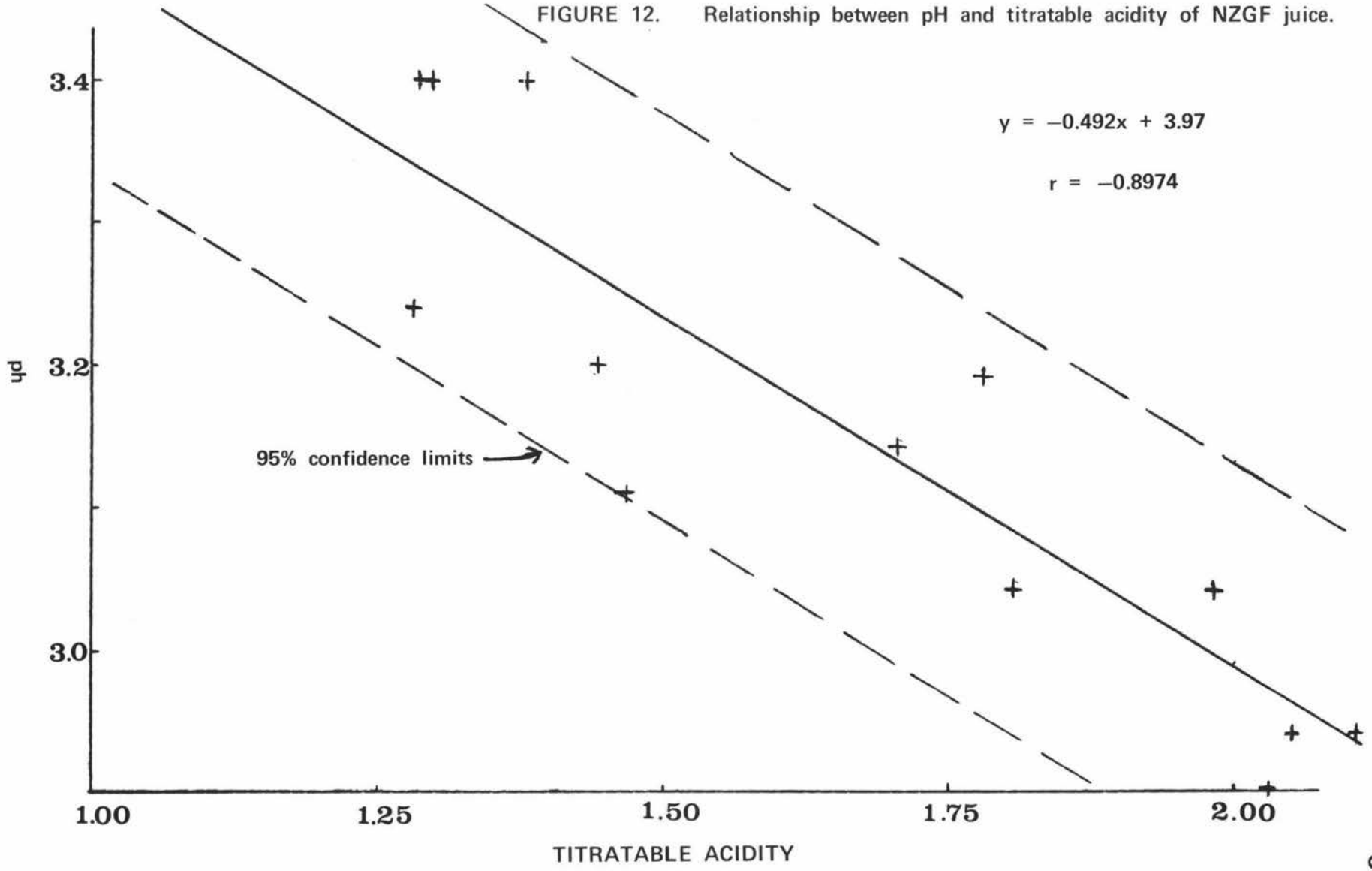
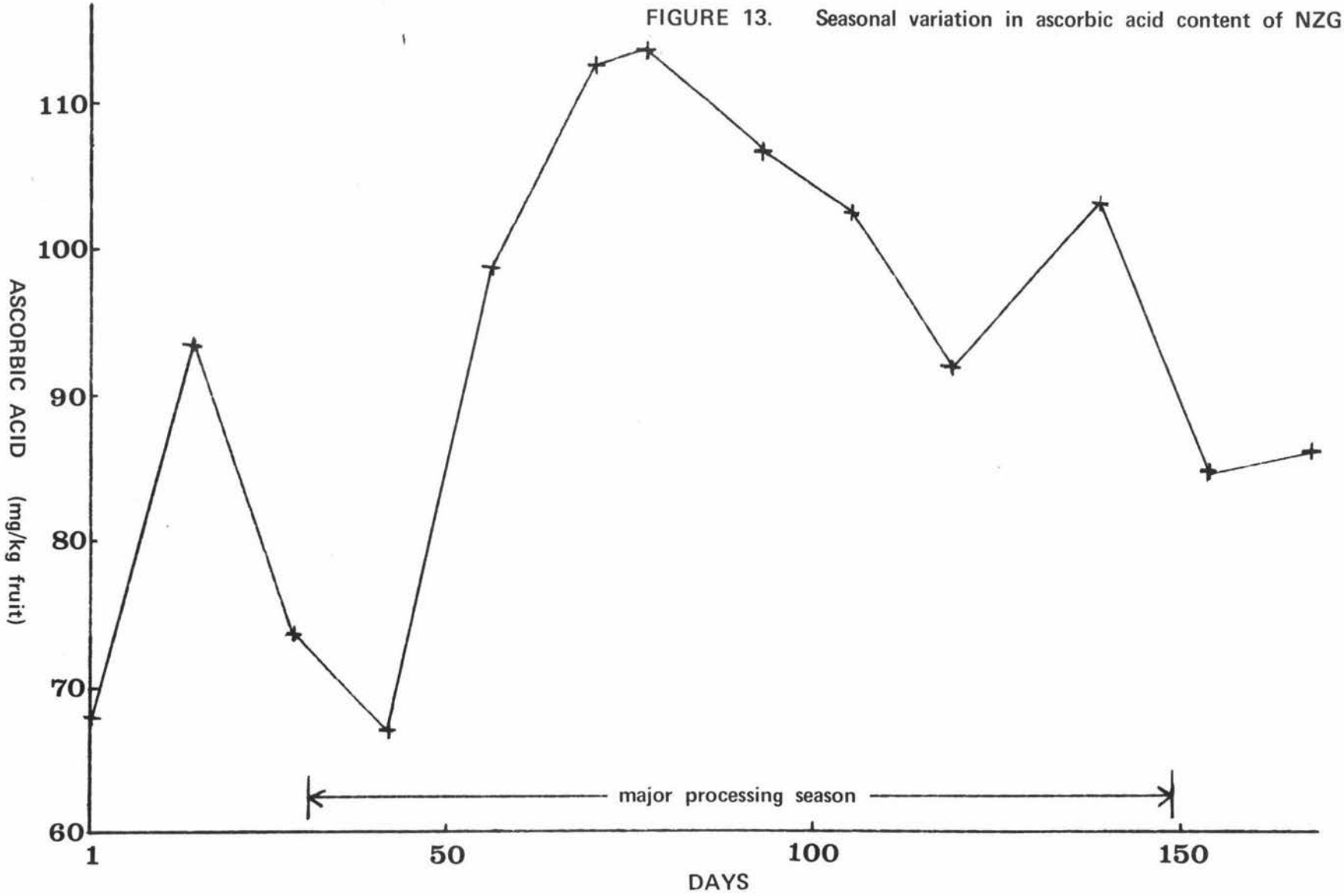


FIGURE 13. Seasonal variation in ascorbic acid content of NZGF.



4. DISCUSSION

(a) Juice Yield - Figure 1 shows that the yield of juice from NZGF increased quite considerably as the fruit matured, reaching a peak around the middle of the processing season, and declining slightly towards the end. Over the period coinciding with the major processing season the average yield was 35.6%.

It is significant that the maximum yield obtained is considerably less than that reported for grapefruit overseas, even allowing for the considerable variation from season to season, between areas, varieties, rootstocks, cultural practices, and the like. Veldhuis (1971) states that the yield of juice from grapefruit in Florida has ranged from 39.2% to 50.6% over the last 25 years, while the average yield in Texas for grapefruit juice is 47.5%.

However, direct comparisons with overseas results are tenuous for three reasons. Firstly, NZGF are different from overseas citrus fruits and therefore one would not expect them to have identical yields. Secondly, the New Zealand climate is marginal for citrus and so growth of fruit is not optimal or as good as that obtainable overseas. Thirdly, the juice in this study was extracted using a rotary reamer attachment on a household mixer, whereas the yields reported from overseas are for juice extracted commercially.

Chandler (1969) reported a trial carried out in Australia involving nine samples of Valencia fruit, three operators, and three hand reamers. There was no significant difference in the juice yields obtained for any one of the fruit samples as determined by the three operators on each of the three reamers. However, a comparison of the results of the hand-reaming method with a commercial juice extractor (FMC In-Line Extractor) showed that the yield obtained from a hand reamer could differ by as much as 8% either way from the yield obtained on a commercial extractor. If a similar discrepancy exists between a rotary reamer on a household mixer and a commercial extractor, then the maximum yield obtained in this study would still be less than those obtained overseas. Even assuming comparable yields/acre, this has the effect of making the New Zealand citrus processing operation less economic compared with overseas ones and does not augur well for export of NZGF juice.

(b) Insoluble Solids - the main variable affecting the insoluble solids content of citrus juice is the method of extraction. As the same extraction procedure was rigorously followed for all samples, it is not surprising that the insoluble solids content remained virtually constant throughout the season.

Rouse (1953) showed all the PE to be completely adsorbed on water-insoluble cell tissue and reported

(1954) a straight-line relation of PE activity with pulp in orange juice. Consequently, comparisons of juices for PE activities are valid only if the activity is defined per quantity of insoluble solids. Because the insoluble solids content of the NZGF juice samples remained virtually constant throughout the season, valid comparisons of PE activity in the juices can be made.

(c) Total Soluble Solids - there was a large increase in TSS during July (10.6% to 12.2%) but from August until the end of October, the TSS remained fairly constant in the range 12.0% to 12.6%. There was a further rise towards the end of November, with a maximum (13.2%) being reached in December, followed by a drop in the last sample.

The trend shown in Figure 2 follows that reported by Harding et al. (1940) and quoted by Sinclair (1961), for Valencia oranges. Of the 3 years of data presented, one paralleled very closely the trend shown in Figure 2, with a TSS peak late in the season, followed by a slight decline.

The change in TSS expressed as kilograms per tonne of fruit follows very closely the change in yield while following less closely the change in TSS. Figure 2 illustrates very clearly the points discussed in Section I (i) of this chapter (see p. 20) in respect of the juice requirements for a processor of citrus concentrates. Thus, for a constant juice yield (days 70 and

105), juice with a lower TSS will yield less concentrate with higher evaporating costs, while juice at a constant TSS (days 56 and 105) with a lower yield will provide less concentrate, but less water has to be removed to produce it. These facts are summarised in Table XVI.

Table XVI

Juice Yield and TSS in Concentration of Juice to 42 deg. Brix

Day	Yield (% w/w)	(Brix)	Concentrate per Tonne of Fruit (kg)	Water Removed per Tonne of Fruit (kg)
56	32.3	12.6	195.6	450.4
70	38.3	12.0	218.8	547.2
105	38.2	12.6	229.9	534.8

From Table XVI it is obvious why concentrate processors place so much importance on the TSS per tonne of fruit which they receive for juice manufacture. When citrus processors in New Zealand switch from single strength to concentrate production, it would be prudent for them to change their basis of payment to growers from kilograms of fruit to kilograms of TSS delivered to the factory.

According to the New Zealand Food and Drug Regulations (1973), regulation 197 (5a), grapefruit juice shall contain not less than 9.5g of soluble solids per 100 ml of juice. Figure 2 indicates that this

requirement would be met at any time throughout the season.

(d) pH - as reference to Figure 3 shows, the pH increased throughout the season from 2.95 to 3.40, and in doing so followed the general trend reported for citrus fruits by other workers (e.g. Sinclair, 1961).

In Figure 12, the pH has been plotted against the corresponding titratable acidity. While there is a definite relationship between the titratable acidity and pH if compared over a wide range of acid concentrations (the correlation coefficient of -0.8974 is significant at the 0.1% level), the relationship is not definite over shorter ranges, as indicated by the standard error estimate of pH, being $\pm$ 0.15 at the 95% confidence limits. Under these conditions, the pH value does not indicate at all accurately the amount of free acid present, or vice-versa.

Reasons for the variations in this relationship become clearer if the variables that may affect it are considered. The dilution effect of water in the juice is an important variable. Sinclair (1961) showed that the percentage of free acid in the juice decreased as the fruit matured because the water content of the fruit increased while the free acid per fruit remained relatively constant.

The pH tends to increase as the fruit matures,

but according to Sinclair (ibid.), the change in pH is largely independent of increases in the water content of the fruit. The variables that may affect the pH value are indicated by the following equation (Gladstone and Lewis, 1963), which is approximate for calculating the pH of weak acids. Since the citric acid system in grapefruit juice can be classed as weak acid, this equation may be applied.

$$pH = pK_a + \log(C_s/C_a)$$

where $pK_a = - \log K_a$

K_a = dissociation constant of the acid

C_s = concentration of salt

C_a = concentration of acid

Pure solutions of weak acids can undergo considerable dilution without any great change in pH, provided the dissociation constant (K_a) is small and the undissociated residue is sufficiently high to keep K_a constant. It has been shown (Sinclair (ibid.)) that in citrus juice systems the variation of pK_a with concentration is very slight and may be assumed to be a constant. Therefore the factor log (salt/acid) is the only one that may vary the pH. The dilution of the juice with water will not change the value of this factor because the salt and acid will be diluted in the same proportions.

Sinclair (ibid.) has shown that within a given

variety of citrus fruit grown under uniform conditions, increases in pH (due to increases in the salt : free acid ratio) and decreases in the concentration of free acid (due to increased water content of the fruit) take place fairly synchronously but nevertheless independently of each other. This explains why, in Figure 12, samples of juice with the same pH (3.20) differed considerably in titratable acidity (1.44 and 1.78). i.e. because of differences in the water content of the fruits. Similarly, samples with appreciably identical levels of titratable acidity (1.80) gave pH readings of 3.05 and 3.20, because of variation in the value of the factor log (salt/acid). i.e. the juice with the higher pH had a greater salt concentration.

(e) Titratable Acidity - Figure 4 indicates a steady decline in titratable acidity throughout the season, a trend which has been observed by many other workers (e.g. Harding et al., 1940). As stated above in (d), the decrease in titratable acidity is due primarily to an increase in the water content of the fruit.

The New Zealand Food and Drug Regulations (1973), regulation 197 (5b), state that 100 ml of grapefruit juice should contain not less than 1.0 g and not more than 2.0 g of acid, calculated as anhydrous citric acid. Reference to Figure 4 indicates that this requirement would be met within $\pm$ 2.5% throughout the processing season, although there is the possibility

that the upper limit could be exceeded at the beginning of the processing season in certain years or with certain crops.

(f) Brix : Acid Ratio - of considerable interest to the processor is the ratio of the TSS to the titratable acidity, i.e. the Brix : acid ratio. This is because the TSS content can mask the acid taste of a juice to a remarkable extent, so that at the same acid content a juice with a low TSS content will taste much tarter than a juice with a high TSS content, and thus will be less suitable for processing.

The Brix : acid ratio is particularly important in consumer acceptance of citrus juices, and it is common practice for processors to add sugar to the juice to obtain a standard ratio. This practice is permissible under the New Zealand Food and Drug Regulations (1973), provided the package of fruit juice shows the percentage of added sugar. Although a properly constituted taste-panel would be required to determine what New Zealand consumers regard as an acceptable ratio for NZGF juice, comments from tasters who regularly sampled the juices used in this study indicated that once the Brix : acid ratio reached 9.5 the juice was acceptable to the palate. From Figure 6 it is seen that the processor would need to add variable amounts of sugar for approximately three-quarters of the processing season in order to reach this ratio. This

presents problems with the labelling of the juice, for the percentage of added sugar would be changing continually, thus preventing the preprinting of the percentage of added sugar on the juice label. The commercial solution to this problem has been to add a constant amount of sugar (5% w/v) throughout the season, but this results in juice of variable quality.

The New Zealand Food and Drug Regulations (1973) do not specify a Brix : acid ratio as such, but one can be calculated from the requirements for soluble solids and acidity. Not less than 9.5% TSS and not more than 2.0 g citric acid is equivalent to a Brix : acid ratio of 4.75. The author suggests that such a low ratio would be quite unacceptable to the majority of consumers. The palatability of such a juice is improved by processors who add 5% w/v of sugar, thus raising the Brix : acid ratio to 7.25, but this is still lower than that considered desirable by many consumers.

However, the Brix : acid ratio by itself is not a sufficient criterion of acceptability. As mentioned previously, the TSS content can mask the acid. i.e. two juices with widely different acid levels and TSS contents can have the same Brix : acid ratio. This fact has been recognised by the State of Florida citrus law which operates on a sliding scale, so that fruit with higher solids is permitted to have disproportionately higher acid. i.e. to have a lower ratio than juice with

lower solids. Typical values from the specification illustrate this and are shown in Table XVII.

Table XVII

Required Ratios of TSS to Acid

Solids not less than %	Required Ratio
8.0	10.00 : 1
9.0	9.50 : 1
10.0	9.00 : 1
11.0	8.50 : 1
12.0 or above	8.00 : 1

Source: State of Florida Citrus Law, Section 601.17, 1957.

Figure 8 is a plot of the Brix : acid ratio against TSS content. The Florida citrus law requirement is shown as a solid line - to be acceptable, juices must be above this line. Under the Florida law, only NZGF juice processed from October onwards would be acceptable, unless sugar was added to increase the ratio. While it is not recommended that the Florida citrus law be inserted unchanged into the New Zealand Food and Drug Regulations, it is suggested that a similar type of regulation be introduced here, taking into account the unique nature of NZGF juice and the preferences of the New Zealand palate.

Because the correlation coefficient for the relationship between the Brix : acid ratio and TSS is not extremely significant in statistical terms, and because of the relatively high standard error of estimate ($\pm$ 2.27 with 95% confidence), it is not possible to predict to any useful degree of certainty the Brix : acid ratio from the TSS. This is unfortunate, if not unexpected, for if it were possible, then a processor could predict the quality of the extracted juice by doing a refractometric reading on samples of the in-coming fruit.

The data plotted in Figure 9 shows that the standard error in predicting the Brix : acid ratio from the titratable acidity with 95% confidence is $\pm$ 0.58. If the number of samples tested to obtain this relationship was greatly increased, it could be possible to decrease the standard error to a level which would make this test useful for routine examination of raw material. However, such a procedure by itself is of little value, for once a titration has been performed, it is a simple matter to do a refractometric reading and calculate the Brix : acid ratio directly.

Figure 7, showing the logarithm of the Brix : acid ratio plotted against pH, has too large a standard error to be of use in predicting the ratio from the pH of the juice. However, this graph does conform to the pattern presented by Kilburn (1958). From several thousand determinations made over three seasons, he

obtained a plot in which 75% of the points fell within ± 0.15 pH units of the regression line. A significant feature of the relationship which he demonstrated was the validity of the system over the entire range for each species of citrus. i.e. data from limes and lemons fell around the lower portion of the regression line, with grapefruit, tangerine and oranges occupying successively higher positions on the line.

Although Kilburn (ibid.) does not give the equation for his regression line, points taken from his graph fall within the 95% confidence limits of Figure 7. Considering the varietal differences and the much smaller sample size used in this study, this is an encouraging coincidence. One is tempted to surmise that if data were obtained from all the citrus varieties around the world, they may all be united on the one regression line between their Brix : acid ratio and pH!

The relationship demonstrated in Figure 7 can be expressed in another way - namely, that at a uniform soluble solids content (Brix) the pH of citrus juices is proportional to the log of the titratable acidity. Although such a relationship is of little assistance to the processor of single strength juice, it is valuable to the processor of concentrate which has a very uniform soluble solids content. In fact, in view of the difficulties in getting an accurate refractometric reading of citrus juice concentrate, the use of pH to

obtain the Brix : acid ratio has much to commend it.

(g) Pectinesterase Activity - Figure 10 indicates an irregular increase in PE activity throughout the season, reaching a peak of 88.5 units after 70 days, followed by values in the 70s until reaching a new peak of 91.0 units after 140 days. Then follows a decrease to 75.0 and an increase to 89.2 for the final sample. The overall error, including sampling technique, in PE determination has been reported by Rouse (1952) to be about ± 10%. This explains in part the variation shown in Figure 10.

Information in the literature concerning seasonal changes in PE activity is limited. However, Rouse and his co-workers have published data on the occurrence of PE activity in the component parts of all common USA citrus fruits, and a summary of the results is presented in Table XVIII.

Table XVIII

PE Activity in Various Citrus Juices

Fruit	(PEu)g TSS x 10^4	Pulp Content	Reference
Florida lemons	54 - 288	0.5%	Rouse and Knorr (1969)
Pineapple oranges	193 - 198	10-12%	Rouse and Atkins (1953)
Pineapple oranges	1263 - 1944	45%	Rouse and Atkins (1953)
Valencia oranges	9 - 36	0.5%	Rouse et al. (1962)
Silver Cluster grapefruit	4 - 36	0.5%	Rouse et al. (1965b)
Marsh Seedless grapefruit	20	0.5%	Rouse (1953)
Duncan grapefruit	30	0.5%	Rouse (1953)

The very low pulp content of some of the juices in Table XVIII is due to the fact that the fruit was separated by hand into peel, membrane, juice sacs, seeds and juice. Thus, what is referred to as juice in these cases is not the same as that obtained commercially or in this study by reaming grapefruit halves. However, when allowance is made for the pulp content of NZGF juice being 10%, it is seen that the level of PE activity is comparable to that of the overseas varieties. For direct comparisons, allowance would have to be made for the fact that the PE activity in Table XVIII is expressed on a weight basis, as compared to a volume basis in this study. For single strength juice of pulp contents up to 12% this does not involve a very large correction (approximately 6%) but with concentrates the difference would be significant.

Rouse et al. (1965a and 1967) found that in oranges from trees only 3 and 5 years old, the PE activity in all component parts except the segment walls was less than in fruit from mature trees. As the NZGF used in this study were from 4 year old trees, one would expect to find higher levels of PE activity than reported here in fruit from more mature trees.

Rouse et al. (1965b) and Rouse and Knorr (1969) found the changes with maturity were somewhat irregular but PE activity was generally greatest when the Brix : acid ratio was highest. This finding was not

confirmed in this study; in fact, the reverse was the case near the end of the season, with the decrease in PE activity coinciding with an increase in the Brix : acid ratio.

(h) Ascorbic Acid - reference to Figure 11 indicates that over the season the ascorbic acid level in the juice declined. This agrees with the only published data on seasonal changes in ascorbic acid content - that of Harding et al. (1940), quoted by Sinclair (1961). They followed the ascorbic acid change in Valencia oranges over two seasons, the change in one season being from 50 to 30 mg/100 ml juice, while in the other from 40 to 33 mg/100 ml juice, thus indicating the variable nature of the change in different seasons.

Harding et al. (ibid.) found that when the values were expressed as amounts of ascorbic acid per fruit rather than amounts per ml of juice, the ascorbic acid content increased with an increase in juice volume and fruit size. Late in the season when the fruit began to dry out, a dimunition of juice occurred along with a decrease in the concentration of ascorbic acid (mg/100 ml juice), thus causing a reduction in the total ascorbic acid per fruit. Figure 13 is a similar type of plot, showing the seasonal variation in ascorbic acid calculated as mg per kilogram of fruit. When this graph is compared to the graph showing the seasonal variation in yield (Figure 1), it is seen that they both follow

the same pattern very closely, thus confirming the observations of Harding.

In comparing the data obtained in this study with that of Dawes (1970), it is seen that the spread of values in the latter paper for ascorbic acid in NZGF juice is greater (19.2 to 37.0) than that reported here (23.2 to 32.4). However, this difference is not significant as some of Dawes' samples were of unknown rootstock. Strachan (1967) reported values of 22 to 35 mg/100 ml for ascorbic acid from a large number of commercial good quality fruit, presumably all fully mature. In contrast, the figures of Hyatt (1936) give a range of 24 to 49. Kefford (1959) gives a range of 25 to 50 mg/100 ml for so-called "true" grapefruit juice.

Before too much is read into these differences in ascorbic acid levels, it is worth considering the results of Primo et al. (1963). With the object of specifying statistically valid sampling procedures, they examined the distribution of ascorbic acid content in the juice between oranges on individual trees of several Spanish varieties, in particular zones of the trees, and between trees in an orchard. They found that to determine ascorbic acid content with 99% confidence limits of $\pm$ 1 mg/100 ml, a sample of 326 oranges should be analysed, while 8 oranges would give a result with 95% confidence limits of $\pm$ 5 mg/100 ml. It would seem reasonable to assume that the error in the determination

of ascorbic acid in this study (where each sample contained 12 fruit) would be similar. i.e. ± 5 mg/100 ml.

5. SUMMARY

The results from this section can be summarised as follows:

(a) Juice Yield - even allowing for the considerable variation between seasons and the fact that the juice was not extracted on commercial equipment, the yield obtained from NZGF in this study was considerably less than that reported for other varieties of grapefruit overseas.

The average yield obtained over the period coinciding with the major processing season was 35.6%, compared with an average yield in Texas of 47.5%.

(b) Insoluble Solids - the insoluble solids content of the juice used in this study remained constant throughout the season at 10 ± 0.5%.

(c) Total Soluble Solids - throughout the major processing season the TSS remained fairly constant in the range 12.0 to 12.6%. At all times throughout the season, the New Zealand Food and Drug Regulations (1973) were met with respect to TSS content of the juice.

(d) pH - the pH increased throughout the season from 2.95 to 3.40. The relationship between pH and titratable

acidity was not accurate enough to permit useful predictions of one value from another.

(e) Titratable Acidity - the titratable acidity declined throughout the season. The requirements of the New Zealand Food and Drug Regulations (1973) for titratable acidity were met at all times throughout the season within ± 2.5%.

(f) Brix : Acid Ratio - this varied from 5.02 to 10.03 throughout the season and at all times exceeded the minimum requirements of the New Zealand Food and Drug Regulations (1973), viz. 4.75. It is suggested that the minimum level required in the regulations is unacceptable to the majority of consumers. A sliding scale permitting fruit with higher TSS to have disproportionately higher acid is recommended for introduction into the New Zealand regulations.

The Brix : acid ratio cannot be accurately predicted from the TSS content of the juice.

The semilogarithmic relationship between Brix : acid ratio and pH coincides to a remarkable extent with that reported for overseas citrus fruits.

(g) Pectinesterase Activity - allowing for the fact that the NZGF were from immature (4 year old) trees, the level of PE activity in the juice was comparable with that found in overseas citrus varieties. PE activity was not greatest when the Brix : acid ratio was highest,

as was expected from overseas reports.

(h) Ascorbic Acid - the ascorbic acid level in the juice declined over the season in agreement with published data from overseas. When the ascorbic acid content was expressed as mg/kg fruit instead of mg/100 ml juice, the change in ascorbic acid throughout the season followed closely the variation in yield.

6. CONCLUSIONS

The following conclusions can be drawn from the study of seasonal variations in the compositional characteristics of NZGF juice:

(i) the yield of juice is significantly lower than that reported for overseas grapefruit varieties.

(ii) the requirements of the New Zealand Food and Drug Regulations (1973) with respect to TSS and titratable acidity can be met at all times throughout the major processing season.

(iii) with respect to the factors considered in this study, and with the exception of yield, NZGF juice does not appear to differ markedly from overseas grapefruit juices.

CHAPTER THREE

CLOUD STABILISATION IN NEW ZEALAND GRAPEFRUIT JUICE

SECTION I

1. THE NATURE OF THE CLOUD IN CITRUS JUICES

The fine particles of suspended material in citrus juices are responsible for the colour, appearance and much of the flavour. Without these particles, commonly referred to as the 'cloud', citrus juices would be little more than clear, almost colourless, sour-sweet liquids of no particular value or appeal.

The retention of cloud in citrus juices during storage and distribution is of prime importance to the citrus processing industry, and failure to keep the cloud particles in suspension is often the cause of quality loss in citrus juices.

The composition of cloud in orange juices freshly extracted on commercial juice extractors from a range of varieties grown in Florida during several seasons was investigated by Scott et al. (1965). They first strained all samples through a 16-mesh stainless steel screen to remove particles too large to be properly considered as suspended matter. The juices were then centrifuged under conditions (10 min. at 600g) equivalent to those commonly used in the quality control determination of "free and suspended pulp" to give fraction A. The effluents were then recentrifuged (3 min. at 60,000g) immediately to give fraction B, and again after storage overnight to throw down the fine cloud

(fraction C). In addition, oranges were divided into the components albedo, rag, pulp, and pulp-free juice, in an attempt to identify the source of the fragments making up the cloud. Analysis of the fractions and components gave the results summarised in Table XIX (p. 91).

Fraction A, the "free and suspended pulp", resembled the albedo, rag, and pulp components in being high in cellulosic constituents and relatively low in pectin, lipids, and phosphorous. This fraction was evidently made up of fragments from mechanical disintegration of structural tissues. Fractions B and C, the true cloud, were high in pectin, lipids, nitrogen, and phosphorous, indicating that they were derived from the contents of the juice vesicles. The composition of the cloud was similar in different varieties of orange.

Baker and Bruemmer (1969) obtained a sample of the cloud components classified as "insolubles" by Scott et al. (ibid.) from the latter and subjected it to extraction for pectins and proteins. They found that the cloud insolubles were 45% protein, indicating a minimum protein value for the whole cloud of 34%. However, the value of 45% protein in the cloud insolubles could not be reconciled with a pectin content of 80% as reported by Scott et al. (ibid.). Therefore, Baker and Bruemmer (ibid.) examined the so-called pectin fraction of the cloud insolubles and found it to be 47% protein.

Table XIX

Composition of Orange Juice Cloud and Component Parts of Oranges

Fraction	Amount in Juice (% dry wt.)	Light-scattering components		Percentage composition of AIS					
		Lipids (%)	AIS (%)	Pectin (%)	Hemicellulose (%)	Cellulose (%)	N (%)	Ash (%)	P_2O_5 (%)
A (free and suspended pulp)	0.19	10	90	63	19	11	4.2	3.5	0.4
B (cloud)	0.34	25	75	83	3	2	7.6	2.6	1.3
C (cloud)	0.14	27	73	77	1	1	7.1	3.0	1.6
Albedo	-	7	93	44	13	32	0.7	3.1	trace
Rag	-	3	97	52	16	32	0.7	3.5	0.1
Pulp	-	11	89	57	19	19	2.8	4.1	0.3
Juice (pulp-free)	-	35	65	80	1	2	6.0	9.4	2.0

Source: Scott et al. (1965).

The physico-chemical characteristics of orange juice cloud were investigated by Mizrahi and Berk (1970). They found that the size of the particles making up the cloud were in the range 0.05 microns to a few hundred microns. The particles with a size below 2 microns constituted the stable cloud and consisted of chromoplastids, amorphous rag particles and oil globules attached to some of these particles. The adsorption of oil globules on the rag particles enhanced their stability in suspension by decreasing the average density of the particles and bringing it closer to that of the serum. All the cloud particles exhibited a negative charge, which decreased with decreasing pH. The carboxyl groups on the pectin were suggested as the site of the negative charges. However, it seemed that the stage of hydration of the particles rather than their electrical charge was more important with respect to cloud stabilising mechanisms. Heat treatment of the juice caused an increase in the number of fine particles at the expense of coarser ones. In the process, some extraction of pectin into the serum took place, but this had little significance on the cloudiness and cloud stability of the juice.

2. STABILISATION OF THE CLOUD IN CITRUS JUICES

It was generally accepted that citrus juice cloud could be stabilised by inactivating pectinesterase (PE), an enzyme that initiated a series of reactions leading to clarification. PE demethylated juice soluble pectin, converting it to low-methoxyl pectin which reacted with polyvalent cations to form insoluble pectates. The precipitation of these pectates occluded the cloud particles and removed them from suspension (Dietz and Rouse, 1953). Until recently, juice-soluble pectin was presumed to form a colloidal matrix that supported the particulates and therefore was necessary for citrus cloud stability.

The traditional and still the only commercial method for inactivating PE is to heat the juice in a heat exchanger with steam or hot water. This topic is reviewed in some detail by Joslyn and Pilnik (1961). Much of the work on PE inactivation was carried out by Rouse and co-workers at the Florida Citrus Research Station during the 1950's. They investigated the effect of various temperatures and holding times on PE inactivation and cloud stability, and also took into consideration the effect of variables such as the pH, pulp content, degree of concentration of the juice during heat treatment, and seasonal and varietal differences. They left the literature full of the times required at different temperatures to inactivate PE for nearly all the combinations and permutations of the variables mentioned above. Presumably the citrus processor was

expected to find the appropriate relationship holding for his juice at a particular point in time and adjust the time and temperature conditions of his process accordingly. As soon as one of the aforementioned variables changed, it would be necessary to search the literature to find the new processing conditions and adjust the process to suit. Such a situation provides a classic example of the qualitative, empirical approach to food processing.

Other methods of inactivating PE have been investigated. Copson (1954) studied the inactivation of PE in orange juice concentrate using microwave irradiation to heat the juice. Although PE inactivation was achieved, this method does not appear to have found commercial application. Lopez and Baganis (1971) studied the effect of radio-frequency (R-F) energy at 60 MHz on PE in raw apple juice and a water solution at 20 C and 70 C. No significant loss of PE activity occurred at 20 C, while a partial loss of activity at 70 C was attributed to the effect of heat.

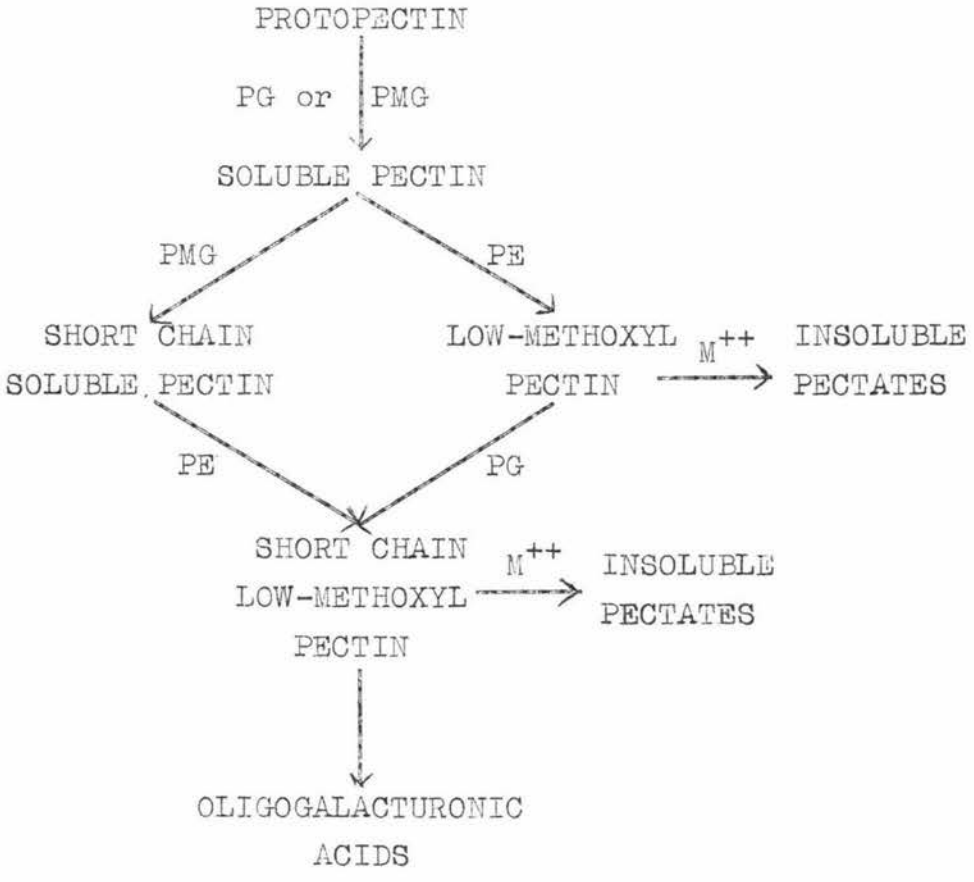
Although the inactivation of PE was not actually studied, the control of some food-related enzymes by the brief application of various inert gases at high pressures was reported by Behnke et al. (1969). This process appeared attractive to the food industry because of the availability and relative cheapness of the inert gas nitrous oxide. However, they concluded that there was little hope that the enzymes in food systems could be effectively inhibited by brief exposure to inert gases at pressures of 5000 psig or

less. Nevertheless, in their conclusion they note that "before abandoning the hope that high pressures can be used to control enzyme activity, it would be advisable to determine the extent to which the effectiveness of a pressure treatment is influenced by such variables as temperature, pH, and concentration of various constitutents." They suggested the use of higher pressures up to 75,000 psig, but at such pressures the commercial viability of the process diminshes because of the tremendous cost of pressure vessels, and the cost of a suitable inert gas, for nitrous oxide liquefies above 600 psig. No further work on the use of inert gases for enzyme inactivation in the food industry has been reported.

The most revolutionary process for the stabilisation of citrus cloud without the use of heat was reported by Baker and Bruemmer (1972). In 1969 they had shown that soluble pectin was not necessary for cloud support as a stable suspension of citrus juice particulates in water could be made. Because pectin is the source of the destabilising low-methoxyl pectin, they proposed controlled pectin degradation (using ppm levels of commercial pectolytic enzyme preparations) as an alternative to heat denaturation of PE for stabilising citrus cloud. The possible pathways for enzymic breakdown of pectin by the enzymes PE, PG, and PMG are shown in Figure 14. The proposal was supported by the stability of citrus juice particulates in centrifugally prepared citrus juice serum treated with a commercial pectinase (Baker and Bruemmer, 1969).

Figure 14

Possible Pathways for Enzymic Breakdown of Pectin by PE, PG, and PMG



Source: Baker and Bruemmer (1972)

Successful application of this method in the production of a cold pack juice would require a satisfactory cold sterilant such as diethylpyrocarbonate (DEPC) or sulphur dioxide. Modification of this method employing the minimal heat necessary to effect pasteurisation has successfully produced juices with a dense, stable juice cloud (Baker and Bruemmer, 1971). Unfortunately, no costings have been prepared to indicate how this process compares with conventional heat stabilisation of citrus juices, but it appears that it would certainly not be any cheaper.

Because of the present universal use of heat to stabilise the cloud of citrus juices, it was decided to quantitatively study the inactivation of PE in NZGF by heat.

SECTION II

1. THE HEAT INACTIVATION (DENATURATION) OF ENZYMES

An enzyme is a protein that is synthesised in a living cell and catalyses or speeds up a thermodynamically possible reaction. Whitaker (1972) defined an enzyme as a protein with catalytic properties due to its power of specific activation. When proteins, either in the dry state or in solution, are heated or treated in various other ways, they undergo certain characteristic changes. Proteins that have undergone such changes are known as denatured proteins, in contrast to fresh or 'native' proteins, and the process is known as denaturation. When the denaturation process is applied to enzymes, leading to a loss of their catalytic activity, it is then specifically referred to as inactivation or deactivation (Laidler and Bunting, 1973).

The term denaturation has been applied rather loosely to various types of changes occurring in proteins. According to Laidler and Bunting (ibid.) it is difficult to define denaturation in a precise manner, since it is found that different types of treatment (e.g. heat and addition of urea) may bring about different changes even in the same protein. Denaturation is frequently accompanied by a loss of biological activity of the proteins; enzymes lose their catalytic activity, hormones their physiological action, and antibodies their ability to combine with antigens. It appears

that with some proteins the overall process of denaturation occurs in several stages but the inactivation occurs only in one (sometimes the first) of these.

Joly (1965) quotes five definitions of denaturation which have been proposed over the years and then gives his own definition in the following general terms: denaturation is any modification of the secondary, tertiary or quarternary structure of the protein molecule, excluding any breaking of covalent bonds.

The secondary structure of proteins consists of helices and pleated sheets involving the backbone of the polypeptide chain, and it is stabilised by hydrogen bonds as opposed to the peptide bonds of the primary structure. The tertiary structure of the protein is folded in a complex and irregular manner to form a compact structure maintained by a number of different types of bonds including electrostatic bonds, hydrogen bonds, hydrophobic bonds, dipolar bonds, and disulphide bonds. Not every protein has all of these types of bonds involved in maintaining its tertiary structure. The quarternary structure refers to those proteins which are composed of more than one polypeptide chain. Hydrophobic and electrostatic bonds play predominant roles in maintaining the integrity of these molecules.

Tannford (1968) by inference supports Joly's definition when he remarks that the definition of denaturation, which many earlier writers found a troublesome

subject because they did not know the exact native structures of any proteins, can now be stated rather easily: it is simply a major change from the original native structure, without alteration of the amino acid sequence, i.e. without severance of any of the primary chemical bonds which join one amino acid to another. This definition is not an absolute one, however, because what one chooses to call a "major" change in conformation remains a matter of personal taste.

In the case of enzyme inactivation, however, Tannford (ibid.) prefers to make a sharp distinction between denaturation and inactivation of an enzyme. Although the definition of denaturation is in terms of physical properties relating to molecular conformation, enzymes may be inactivated as a result of minor conformational changes as well as major ones, or as a result of strong interaction between the inactivating agent and the catalytic or binding site of the enzyme.

It was originally thought that heat caused a dehydration of the protein molecule or the establishment of peptide linkages between some of the free amino and carboxyl groups. In addition, the reverse reaction, the cleavage of peptide bonds by heat, was also considered possible. However, dilatometric measurements showed that denaturation by heat was not accompanied by any noticeable change in volume. The hydration of denatured proteins in humid air was only slightly lower than that of native proteins, their

water binding being of the same order.

According to Laidler and Bunting (ibid.) what basically happens when a protein is denatured is that there are changes in the secondary and tertiary bonding existing in the protein molecules. This results in a change in the general shape of the molecule. As the catalytic effectiveness of an enzyme depends very critically on the relative positions of groups that may be on different portions of the polypeptide chains, a change in the overall structures of the protein can lead to a complete loss in enzymic activity.

There are two main types of proteins, the fibrous and the globular, most enzymes being of the latter type. The character of the denaturation process is different for the two classes of proteins, being more complicated for the globular proteins since there are both secondary and tertiary bonds. It has been suggested that reversible thermal denaturation processes involve changes in the tertiary structure alone. When changes in the secondary structure occur, the denaturation is irreversible.

2. FACTORS AFFECTING THE HEAT INACTIVATION OF ENZYMES

The four most important factors that affect the heat inactivation of enzymes are as follows:

(a) Temperature

It has long been realised that enzyme reations increase in rate with temperature and reach a maximum at the so-called "temperature optimum" of the enzyme. Above the optimum the rate decreases with further rise in temperature. The temperature optimum is not a constant for a given enzyme but varies with pH, ionic strength and nature of the buffer, the presence or absence of substrate, the enzyme concentration and purity, the presence or absence of activators and inhibitors, and the method used in measuring the rate of the catalysed reaction.

The apparent temperature optimum for the rate of enzyme reaction is explained by two independent processes being simultaneously accelerated by temperature - the catalysed reaction, and the thermal inactivation of the enzyme. At sub-optimum temperatures it is the catalysed reaction which is mainly affected while at temperatures above the optimum the inactivation of the enzyme by heat predominates.

The rate of inactivation of enzymes in solution increases rapidly with temperature and in nearly all cases inactivation is virtually instant-

aneous above 100 C, and in the majority of cases above 70 C.

In general, an enzyme is more stable to temperature in an intact tissue or in an homogenate where its structure is protected by the presence of other colloidal material (proteins, carbohydrates, pectins, etc.) than it is in a purified form.

The thermal inactivation of enzymes is usually unimolecular and increases exponentially with temperature in accordance with the Arrhenius equation over a fairly wide range, indicating that the activation energy is independent of temperature.

Vas et al. (1967) investigated the effect of temperature on the rate of enzyme deesterification of pectin, using PE's from a wide range of fruit and vegetables. They found that increasing the temperature from 30 C to 80 C resulted in ever increasing reaction rates. However, since spontaneous demethylation of pectin occurs with enhanced speed at higher temperatures, this has to be taken into account, especially when temperatures above 40 C were applied. It was found that the optimum temperature for enzymatic deesterification of pectin was around 65 C for PE from orange albedo.

(b) pH

The rate of inactivation of an enzyme at a particular temperature is highly dependent on pH.

As with temperature, there is an optimum pH at which the enzyme has greatest resistance to thermal inactivation. Either side of this optimum pH the enzyme's resistance to inactivation decreases. Neurath et al. (1944) observed that the pH of maximum thermal stability for enzymes was between their isoelectric point and neutrality. Vas et al. (ibid.) varied the pH of the reaction mixture containing orange peel PE and found that the optimum pH for deesterification of pectin by PE (as measured by the quantity of NaOH consumed in 30 minutes) was 7.5, thus confirming earlier reports (e.g. Rouse and Atkins, 1955).

(c) Time

The longer an enzyme is held at a temperature above its temperature optimum, the greater is the extent of inactivation of the enzyme. At constant temperature, the inactivation of an enzyme proceeds exponentially with time.

(d) Concentration

The rate of inactivation is also dependent on the concentration of the enzyme. The higher the concentration of enzyme the greater the time required at a given temperature to inactivate the enzyme. This follows from the fact that the reaction is first order.

3. METHODS FOR DETERMINING THE THERMAL INACTIVATION CONDITIONS FOR ENZYMES

The two variables manipulated during thermal inactivation studies of enzymes are time and temperature. The fact that the thermal inactivation of enzymes is like the thermal destruction of microorganisms, in that they both follow first-order kinetics, means that many of the methods used by microbiologists to determine the thermal destruction conditions for microorganisms can be used when studying the thermal inactivation of enzymes. These methods will now be briefly reviewed.

The first method used was the thermal death time (TDT) tube method, developed by Bigelow and Esty (1920). Small diameter (7 to 10 mm) test tubes containing the appropriate solution were sealed and placed in a thermostatically controlled bath for varying lengths of time. On removal from the bath the tubes were rapidly cooled. Although no expensive equipment is required for this method, filling, sealing and heating of the tubes is time consuming. A more serious objection is the appreciable heating and cooling lags in the tube contents. Procedures for evaluating these lags have been proposed by Sognefest and Benjamin (1944) but Stumbo (1953) established that these cannot give true corrections because the relative resistance of the microorganism or enzyme to the different temperatures encountered during heating and cooling is required to calculate the

correction. However, the aim of the whole TDT tube method is to calculate the relative resistance of the microorganism.

The TDT can method (American Can Company, 1943) is basically the same as the TDT tube method except that small cans (63mm by 9mm) are used instead of glass tubes. They are heated in small, accurately controlled steam retorts. This method is subject to the same objections as the tube method because it also has appreciable heating and cooling lags.

In the flask method of Levine et al. (1927), substrate is placed in a flask in an oil bath and the inoculum is added once the substrate has reached the desired temperature. Samples are then withdrawn at appropriate times. Apart from the problem of assessing the cooling lags (which can be minimised by withdrawing small samples in a syringe), this method is not suitable for in vivo inactivation studies where the enzyme is not separated from the substrate.

Use of the thermoresistometer designed by Stumbo (1948) is undoubtedly the most sophisticated method available for studies of thermal inactivation of enzymes and microorganisms. The sample is carried on a small boat through three chambers, of which the middle one can be rapidly filled and evacuated with steam. Virtually instantaneous heating and cooling of the sample is achieved, the maximum correction for thermal lags being 0.3 seconds. Timing can be extremely precise - to within 0.0005 minutes. However,

the cost of the instrument is very high (approximately $10,000) and only temperatures above 102 C can be studied. For the thermal inactivation of PE, temperatures below 100 C are used.

Rouse and co-workers in the 1950's built a continuous-flow apparatus in which to carry out enzyme inactivation studies. It consisted of a heating coil 5 m in length, composed of 1.6 mm I.D. stainless steel tubing. Hot water was circulated around the heating coil. The flow rate was adjusted to 560 ml per minute to give turbulent flow. The juice was in contact with heat in the pasteuriser for 0.8 seconds, the time required to raise the juice from room temperature to the desired temperature. To hold the juice for any given time at a definite temperature, the juice was run directly from the pasteuriser into test tubes submerged in a holding bath regulated to the desired temperature. All samples were cooled instantly by flashing under vacuum into small receptacles. This latter step changed the concentration of the juice, but this was allowed for by relating the enzyme concentration to degrees Brix. Even although the juice was heated rapidly, the heating lag was quite considerable as evidenced by the fact that when Valencia orange juice was raised to 85 C and immediately cooled (i.e. holding time was zero) 90% inactivation of PE occurred (Rouse and Atkins, 1952).

Various types of continuous-flow apparatus continue to be used for thermal inactivation studies on

enzymes, and despite some modifications to the basic apparatus outlined above, heating lags still occur. For example, Svensson and Eriksson (1972) used a continuous-flow apparatus with narrow thin-walled glass tubing (1.3 mm I.D.) that despite having a preheater, required 2.3 seconds to raise the enzyme from 3 C to the selected treatment temperature. Because the preheater gave a constant temperature-rising time irrespective of the selected heat reatment temperature, they used this as a reason for making no correction for heating lag. They introduced a further error into their studies by using liquid flows in the laminar rather than the turbulent range, which gave a parabolic velocity distribution of the solution in the tubing. This caused some superheating of the material near the periphery of the of the preheater glass tubing, resulting in an overall inactivation greater than that expected from the average temperature of the whole liquid stream.

Even if extremely high liquid flow rates were used with very narrow, thin-walled tubes, it seems unlikely that a continuous-flow apparatus could be constructed that would reduce the heating and cooling lags to insignificant levels.

Glass tubes (9 mm O.D.) containing grapefruit juice and thermocouples were used by Pratt and Powers (1953) to study the thermal destruction of pectic enzymes. After sealing, the tubes were submerged in a constant-temperature water bath for varying lengths of time and

immediately cooled in an ice-water bath at the end of the heating periods. The heating lag was calculated using the formula method of Ball (1928) and deducted from the actual heating period to yield the equivalent time at each temperature used. However, reference to Ball's paper reveals that the thermal resistance of the enzyme must be known before the heating lag can be calculated. In fact, the whole aim of the exercise is to calculate the thermal resistance. Pratt and Powers (ibid.) appear to have conveniently overlooked this problem and they give no indication in their paper that they were even aware of it.

To summarise, a review of the methods available for studying the thermal inactivation conditions for enzymes has not been able to find a wholly acceptable method presently available. With adequate time and money, it might be possible to design and build a continuous-flow apparatus in which the heating and cooling lags were negligible. However, such resources were not available in this present study and consequently it was decided to develop a new method for studying the thermal inactivation of enzymes. This method is described in the next section.

SECTION III

1. DEVELOPMENT OF A METHOD FOR STUDYING THE THERMAL INACTIVATION OF ENZYMES

(i) Introduction

A study of Section II (3) which describes the methods that have been used for determining the thermal inactivation of enzymes reveals that the major objection to most of the methods is the way in which the heating and cooling lags have been evaluated. Therefore, in developing a new, acceptable method for thermal inactivation studies, two possibilities arose. Either a system could have been devised in which the thermal lag was negligible, or a method could have been developed which accounted in a satisfactory way for the thermal lag. In this study the latter option was selected.

Before detailing the method which was developed in this study, it is necessary to define the terms and explain the concepts on which it was based. These are analogous to and are largely drawn from canning technology.

(ii) Methods for determining the lethality of a thermal process.

Two common methods are available for expressing analytically the lethality or lethal effect of a thermal process in which the temperature varies with time. The

first, extensively used by Ball and Olsen (1957) and Stumbo (1965 and 1973), is based on the classical experimental results of Esty and Meyer (1922). In this method the lethality of a process, F (defined below), is related to the processing temperature, T, by the empirical equation:

$$F = t_R \, 10^{(T-180)/z} \qquad (1)$$

where F is the equivalent time of the thermal process in minutes at some reference temperature (in this case 180 F), and t_R is the total processing time in minutes. The temperature difference z (defined below) appears in the usual form of equation (1) as degrees Fahrenheit.

For a process in which the temperature varies with time, the lethality can be calculated by using a modified form of equation (1):

$$F = \int_0^{t_R} 10^{(T-180)/z} \, . \, dt \qquad (2)$$

The problem then is to specify the processing temperature (T) as a function of the time (t) and to determine the value of F in terms of the total processing time (t_R). This can only be done if the value of z is known.

A convenient measure of the heat resistance of enzymes is the decimal reduction time, D, or the time (in minutes) of heating at constant temperature required to reduce the concentration of undenatured enzyme to one-tenth of its initial concentration.

If the logarithm of the D values for the thermal

destruction of an enzyme are plotted against the corresponding temperatures, a straight line is generally obtained, as shown in Figure 15. This is known as the thermal destruction (TD) curve and is specific for a particular enzyme system. The term z, which appears in equation (1), is employed in process calculation methods to account for the relative resistance of an enzyme to different temperatures. It is numerically equal to the number of degrees of temperature (usually but not necessarily quoted in Fahrenheit) required for the TD curve to traverse one log cycle. Considering any one such portion of the curve plotted in Figure 15, the slope of the curve may be expressed as follows:

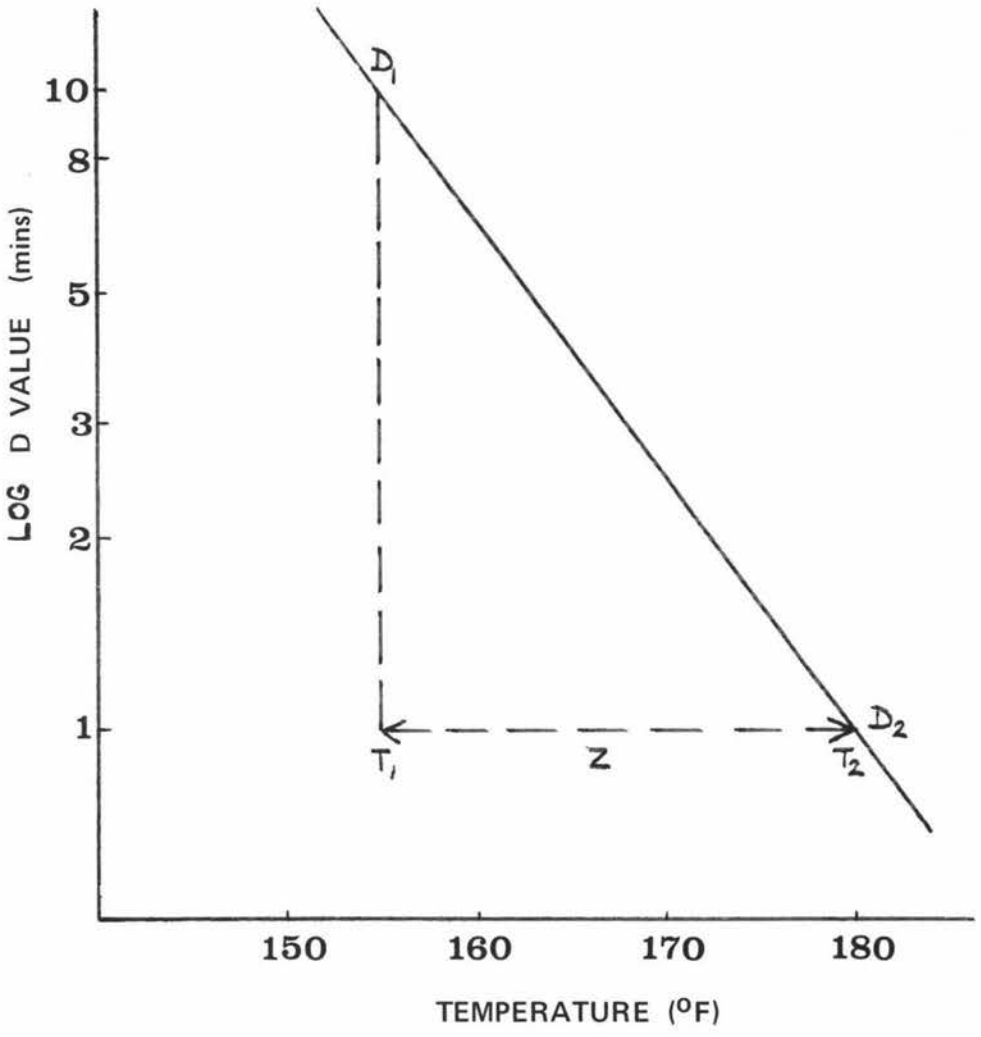
$$\frac{\log D_1 - \log D_2}{T_1 - T_2} = \frac{\log 10 - \log 1}{-z} = -\frac{1}{z} \qquad (3)$$

Stated another way, z is numerically equal to the negative reciprocal of the slope of the TD curve, or the fall in temperature necessary to increase D to ten times its former value.

All points on the TD curve represent treatments that result in equivalent enzyme destruction levels. Likewise, any line of slope $-1/z$ is a line connecting points of equivalent destruction. For reference purposes it is convenient to consider a line of slope $-1/z$, passing through the point 1 minute at 180 F. Any point on this line represents a treatment equivalent to 180 F for 1 minute. For example, from Figure 15, 2 minutes at 172 F is equiv-

FIGURE 15.

Hypothetical Thermal Destruction Curve passing through 1 minute at 180 F.



alent to 1 minute at 180 F. For the purposes of comparing the rates of destruction of the enzyme, it is useful to consider the reciprocal of the equivalent times. These are termed lethal rate values (or L values) and are numerically equal to the reciprocal of the time equivalent to 1 minute at 180 F. By definition the lethal rate value at 180 F is 1, while (again from Figure 15) the L value at 165 F is 1/4 or 0.25.

Thus it can be seen that the D value is proportional to the reciprocal of the L value. Using this relationship it is possible to rewrite equation (3) as follows:

$$\log \frac{L_2}{L_1} = \frac{T_1 - T_2}{z}$$

$$\text{and} \quad \frac{L_2}{L_1} = 10^{-(T_1-T_2)/z}$$

$$\text{so} \quad L_2 = L_1 \, 10^{-(T_1-T_2)/z} \qquad (4)$$

Rewriting equation (4) in the general form and letting T_1 be the reference temperature (180 F), and L_1 the lethal value at T_1 (by definition equal to 1), gives:

$$L_2 = 10^{-(180-T_2)/z}$$

$$\text{or} \quad L = 10^{(T-180)/z} \qquad (5)$$

Equation (5) now permits the calculation of the lethal rate at any temperature, provided that the z value is known. When equation (5) is substituted into equation (2), the following expression is obtained:

$$F = \int_0^{t_R} L \cdot dt \qquad (6)$$

Thus, once the lethal value of all the temperatures reached by a particular system during a thermal process are known, it is relatively simple to evaluate the integral in equation (6) and hence find the equivalent time of the process in minutes at 180 F.

The second method of calculation, used by Deindoerfer and Humphrey (1959) and recently by Richards (1965), expresses the rate of destruction by pseudo first order reaction kinetics, since for the majority of enzymic systems, the rate of destruction is proportional to the concentration of the enzyme in the system. The rate dependence on temperature is expressed in this method by the Arrhenius equation.

If c is the concentration of the undenatured enzyme at any time t, the rate of destruction is proportional to the decrease in concentration with time:

$$-\frac{dc}{dt} = kc$$

$$\text{or} \quad -\frac{dc}{c} = k \cdot dt \qquad (7)$$

The constant k in equation (7) is known as the rate constant or the specific reaction rate. On integration, equation (7) yields:

$$-\int_{c_0}^{c} \frac{dc}{c} = \int_{0}^{t_R} k \cdot dt$$

$$\text{and} \quad \ln \frac{c_0}{c} = \int_{0}^{t_R} k \cdot dt \qquad (8)$$

From the Arrhenius equation:

$$k = A \exp(-E/RT) \qquad (9)$$

On substituting for k in equation (8):

$$\ln \frac{c_0}{c} = \int_{0}^{t_R} A \exp(-E/RT) \cdot dt \qquad (10)$$

from which $\ln (c_0/c)$, the logarithmic reduction in the concentration of the enzyme, is calculated by integration using temperature as a function of time. However, this can only be done if the factors A and E in equation (10) are known. They must be experimentally determined, and use of equation (10) assumes that E remains constant over the temperature range studied. Deindoerfer and Humphrey (ibid.) give the analytical integration for equation (10) for varying temperature-time profiles, thus enabling the lethality of the process to be evaluated.

As Jones (1968) has pointed out, the two methods are clearly similar. It is important for the purposes of this study to examine the two methods to see whether they are identical, and if not, to decide which method is preferable.

Comparison of equations (2) and (10) shows that

evaluation of the integral of temperature varying with time is expressed in two ways. In equation (2), the term F is used. This was defined above as the equivalent time of the thermal process in minutes at 180 F. In equation (10), the term ln (c_0/c) or the logarithmic reduction in the concentration of the enzyme, is equal to the value of the integral. Although it may not seem obvious at first, these two terms are virtually equivalent provided that the time required at the reference temperature (180 F) to give a certain degree of inactivation is known. i.e. provided the D value is known. However, D does not appear as such in equation (10) but the following analysis shows that it is directly related to k.

It is obvious that D, L and k are related, since they are all connected with the rate of thermal destruction. If equation (8) is written in its general form, then:

$$\ln \frac{c_0}{c} = kt \qquad (11)$$

$$\text{or} \quad c = c_0 \, e^{-kt} \qquad (12)$$

If the concentration after time t is one-tenth of the initial concentration, then a decimal reduction in concentration has occurred and t is clearly equal to the decimal reduction time or D value. Thus by substituting into equation (11):

$$\ln \frac{10}{1} = kD$$

$$\text{and} \quad k = \frac{\ln 10}{D}$$

$$= \frac{2.303}{D} \qquad (13)$$

If k is substituted for in equation (12), then:

$$c = c_0 \, 10^{-t/D} \qquad (14)$$

Now there is quite a lot of published data on the thermal destruction of microorganisms and enzymes (e.g. Rahn, 1945; Stumbo et al., 1950) which tend to show that k is exponentially related to T, the temperature of heating, according to the general equation:

$$k = a \, e^{bT} \qquad (15)$$

where a and b are constants. It was shown above that:

$$k = \frac{\ln 10}{D}$$

Therefore, by substituting for k in equation (15):

$$\frac{\ln 10}{D} = a \, e^{bT}$$

$$\text{and} \quad D = \frac{\ln 10}{a} \cdot e^{-bT}$$

$$\ln D = \ln \left(\frac{2.303}{a}\right) - bT$$

$$\log D = \log \left(\frac{2.303}{a}\right) - bT/2.303$$

$$\text{and} \quad \log D = q - T/z \qquad (16)$$

where q and z are constants.

If D_1 is the decimal reduction time at temperature T_1, and D_2 is the decimal reduction time at temperature T_2, then from equation (16):

$$D = 10^{q - T/z}$$

$$\text{therfore} \quad \frac{D_2}{D_1} = \frac{10^{q - T_2/z}}{10^{q - T_1/z}}$$

$$\text{and} \quad D_2 = D_1 \, 10^{(T_1 - T_2)/z} \qquad (17)$$

It follows that when:

$$T_1 - T_2 = z$$

$$\text{then} \quad D_2 = 10 \, D_1$$

Consequently, if it is assumed that the reaction rate constant k varies exponentially with temperature as indicated in equation (15), then z is constant over the temperature range T_1 to T_2.

However, there is also published data on the thermal inactivation of enzymes (e.g. Svensson and Eriksson, 1972; Joffe and Ball, 1962), which suggest that k is really related to temperature according to the Arrhenius equation:

$$k = A \exp(-E/RT') \qquad (9)$$

$$\text{or} \quad \ln k = A - E/RT' \qquad (18)$$

where A = frequency factor

E = activation energy

R = Universal gas constant

and T' = absolute temperature

It was shown above that:

$$k = \frac{\ln 10}{D}$$

Therefore, by substituting for k in equation (18):

$$\frac{\ln 10}{D} = A - E/RT'$$

$$\frac{1}{D} = \frac{A - E/RT'}{2.303}$$

$$= \frac{A}{2.303} - \frac{E/RT'}{2.303}$$

$$\log \frac{1}{D} = \log \frac{A}{2.3} - \log \frac{E/RT'}{2.3}$$

$$\log D = \log \frac{E/RT'}{2.3} - \log \frac{A}{2.3}$$

$$= \log \frac{M}{T'} - b'$$

where M and b' are constants. The value of the constant M is given by:

$$M = \frac{E}{2.3R} \qquad (19)$$

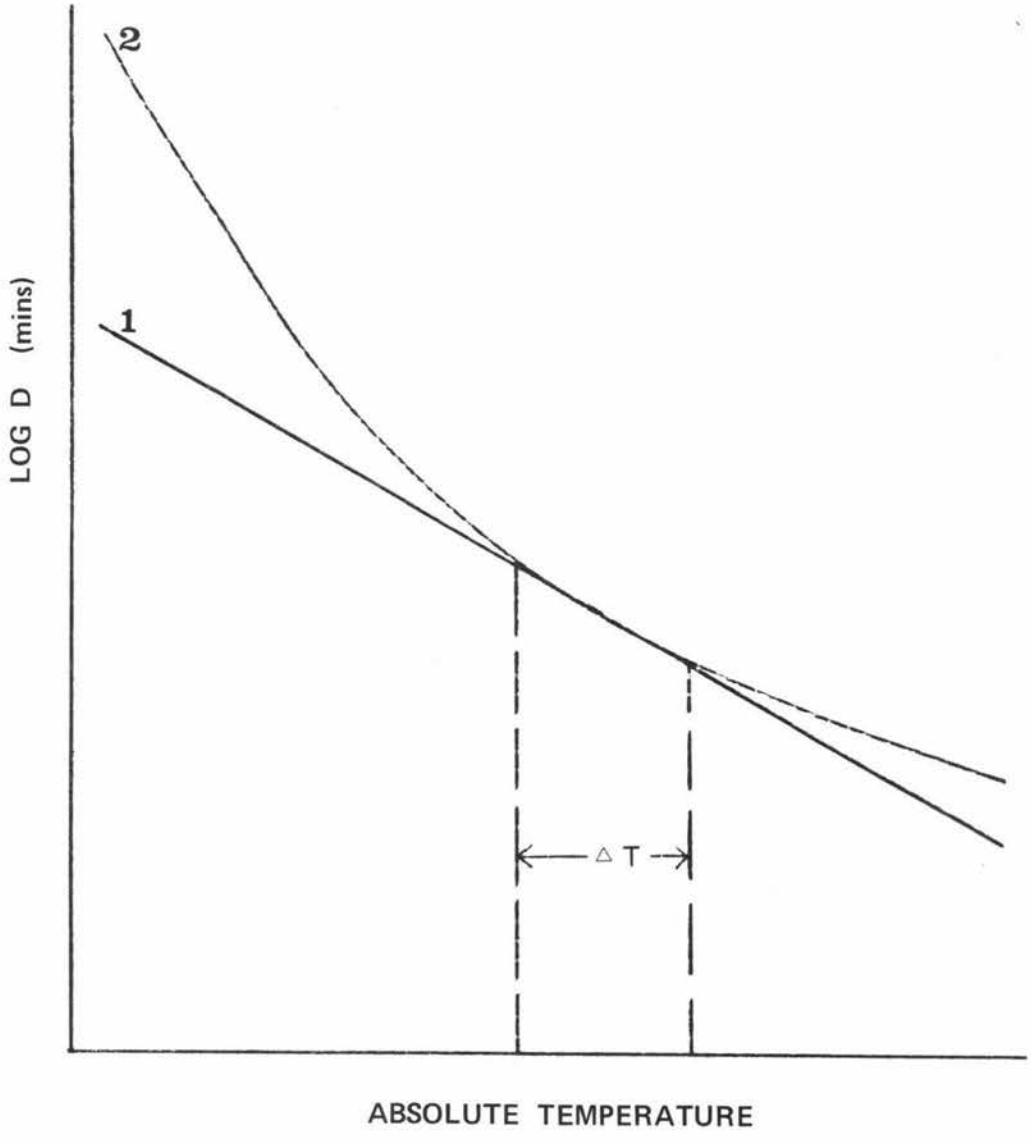
i.e. M varies directly with the activation energy (E) since R is a constant.

Therefore: $D_2 = D_1 \, 10^{M(1/T_2' - 1/T_1')}$ (20)

On comparing equations (17) and (20), the significance of the differences resulting from the two assumptions is seen. This difference is more vividly illustrated in Figure 16 (after Cowell, 1968)(p. 121) which shows a diagrammatic plot of the logarithm of the decimal reduction time as a function of absolute temperature. Curves of type (1) result from assuming that k is a linear function of absolute

FIGURE 16.

Diagrammatic plot of the logarithm of the decimal reduction time as a function of the absolute temperature.



temperature and curves of type (2) from assuming that k varies with the reciprocal of the absolute temperature. Over a limited temperature range where curve (1) is a tangent to curve (2), both curves adequately describe the same data. Outside this range the curvature of the hyperbola (curve 2) results in the Arrhenius equation predicting relatively longer decimal reduction times.

The relationship between z and M is as follows:

$$\frac{D_2}{D_1} = 10^{(T_1' - T_2')/z} \qquad (17)$$

and

$$\frac{D_2}{D_1} = 10^{M(1/T_2' - 1/T_1')} \qquad (20)$$

therefore

$$\frac{T_1' - T_2'}{z} = \frac{M(T_1' - T_2')}{T_1 T_2}$$

Thus the value of z at temperature T' is given by:

$$z = (T')^2 / M \qquad (21)$$

and the mean value over a range T_1' to T_2' is given by:

$$z = T_1' T_2' / M \qquad (22)$$

Gillespy (1951) has shown that over small ranges of temperature, equation (17) gives good approximations to equation (20), but if equation (20) represents the true relationship, then M (i.e. E, as shown above) is constant and z varies with temperature. i.e. the thermal resistance varies with temperature. The value of z or M is a character-

istic of a particular enzyme and varies with the medium, the most important factor being pH.

Jones (1968) compared specimen thermal processes evaluated on the above alternative assumptions of a constant z value and a constant activation energy (E). However, his comparisons were shown to be invalid by Cowell (1968) because the z values used were calculated from one set of experiments and the activation energies from another. The experimental conditions from which the data were obtained differed so much that calculations using the same mathematical model and based on that data would not have yielded comparable results.

As no further work comparing the two models has been reported, both methods were used in this present study for evaluating the thermal inactivation of the enzyme PE.

(iii) A method for studying the thermal inactivation of PE

From the above discussion, the two common methods available for determining the lethality of a thermal process can be summarised in the following general form:

$$F = \int_0^{t_R} 10^{(T_1 - T_2)/z} \cdot dt \qquad (23)$$

$$F = \int_0^{t_R} 10^{M(1/T_2' - 1/T_1')} \cdot dt \qquad (24)$$

where F is the equivalent time of the thermal process in minutes at some reference temperature T_2 (taken as 180 F in

this study) and t_R is the total processing time in minutes. Equation (23) is based on the assumption that z remains constant over the temperature range concerned, while equation (24) assumes a constant activation energy.

The aim of the method described below was to determine values for z and M for the thermal inactivation of PE. Once these values were known, the above integrals of temperature varying with time could be solved and the equivalent time for a particular process at the reference temperature of 180 F could then be evaluated. The equivalent times or F values could then be correlated with the degree of inactivation achieved in the various processes and the conditions necessary to completely inactivate all the PE in NZGF juice could then be specified.

The method developed was as follows. Samples of NZGF juice were heated for varying lengths of time in a water bath maintained between 180 F and 200 F, the time-temperature profile of the juice being continuously recorded. The level of PE in the juice was determined both before and after the heat treatment.

For each run, the temperature of the juice was read off the chart recorder at regular intervals and fed into a digital computer, together with the initial and final concentration of PE. Using a range of values for z, the computer was programmed to calculate the F value for each run according to equation (23). The F values so obtained were then correlated with the number of decimal reductions

in PE concentration. The reason for this was that by expressing the lethaltiy of a process in terms of the F value, the process is converted to constant temperature (180 F) and F indicates the equivalent time of the process at that temperature. As mentioned above, the inactivation of enzymes follows pseudo first order reaction kinetics. i.e. over constant time intervals (and at constant temperature) the concentration of inactivated enzyme doubles. This can be illustrated by plotting the logarithm of (initial enzyme concentration/final enzyme concentration) against the F value, when a straight line is obtained. Similarly, if the logarithm of the percentage of active enzyme remaining is plotted against the F value, a straight line is also obtained, analogous to the survivor curves for microorganisms used in canning technology. Because it is usual with enzymes to refer to the amount of enzyme which has been inactivated rather than the percentage of active enzyme remaining, the former rather than the latter method of expression was used in this study.

The more precise or accurate the value assigned to z, the higher and more significant the correlation coefficient for the relationship between the decimal reductions in PE activity and the F_{180} values. The z value which resulted in the most significant correlation coefficient was taken to be the true z value for the inactivation of PE.

The above procedure was repeated using equation (24) to calculate F, thus giving rise to an optimum M value that

would be characteristic of the thermal inactivation of PE.

This method is free of the objections raised in Section II (3) to other methods for determining the thermal inactivation of enzymes. There is no requirement in this method to minimise the heating and cooling lag, as this is accounted for in a perfectly acceptable manner using the same calculations as those in canning technology. Furthermore, since both equations (23) and (24) are used, no assumptions need to be made at this stage as to how the rate of inactivation of PE varies with temperature.

The operating details of the method are described in detail in the next sub-section.

Once the optimum z or M value has been determined, it is then possible to calculate the lethal effect of different temperatures on the inactivation of PE. However, a problem arises when an attempt is made to calculate the total heating process required to completely inactivate all the PE in the juice. This problem is best discussed in terms of D values, which were defined earlier as the times (at constant temperature) required to reduce the concentration of active enzyme to one-tenth of its previous level, i.e. by 90%. Thus assuming an initial enzyme concentration of 100 (PEu)ml TSS, a 1D process will reduce the active enzyme level to 10 (PEu)ml TSS. Such a reduction can be expressed in terms of D values by taking the logarithm of the initial PE concentration over the final PE concentration:

$$\log \frac{100}{10} = \log 10 = 1D$$

For the same initial enzyme concentration, a 2D process (which will require double the time of a 1D process at the same temperature) will reduce the final concentration of enzyme to 1 (PEu)ml TSS. Similarly a 3D process will result in an enzyme concentration of 0.1 (PEu)ml TSS and a 4D process in 0.01 (PEu)ml TSS.

The problem is in deciding what is a tolerable lower limit of enzyme concentration in the juice. Accepting a final enzyme level which just fails to be detected by an analytical method is not really satisfactory, since such a criterion would change when a more precise analytical method became available.

Since the inactivation of PE is logarithmic, it is impossible to completely destroy all the enzyme present. The same problem occurs in canning technology, since microbial death is also logarithmic, and the solution is to work in terms of the probability of survival of enzymes or micro-organisms. In canning it is usual to give containers a 12D heat process; this means that there is one chance in 10^{12} that any particular container of 10^{12} containers receiving an identical thermal process should not be sterilised. This is the same as saying that one of every 10^{12} containers should not be sterilised by the process.

By applying similar reasoning to the inactivation of PE in citrus juices, if 10^{12} mls of juice, each containing 1 PEu/ml, were given a 12D thermal process, then 1 out of 10^{12} mls of juice would contain 1 PEu at the end of the process.

While there are good reasons for giving canned foods a 12D process, such severe heat treatments for citrus juices are not necessary.

From Chapter 2 it is reasonable to assume that the highest level of PE likely to be found in NZGF at any point in the season is 100 (PEu)ml TSS. The method of analysis used could detect 0.1 (PEu)ml TSS. It is possible that by using a large juice sample and a careful technique, 0.01 (PEu)ml TSS could be detected. Therefore it is not unreasonable to accept a final level of PE in the juice of 0.001 (PEu)ml TSS. If the original level of enzyme was 100 (PEu)ml TSS, a 5D thermal process would be required to give a final level of 0.001 (PEu)ml TSS. Such a level of PE would saponify 1×10^{-7} milliequivalents of ester per ml per minute (Rouse and Atkins, 1955) which can reasonably be considered insignificant. Therefore in this study a 5D reduction in PE concentration was taken as an acceptable level of inactivation.

2. EXPERIMENTAL

(i) Inactivation of PE

Cases of NZGF grown in the Bay of Plenty were bought through normal wholesale channels in August and December of 1973, these two dates being approximately the beginning and end of the NZGF processing season. The fruit was kept in a chill room at 4 C until required for testing, the total time in chill storage not exceeding 10 days.

At the commencement of each day of tests, juice was extracted from the fruit using the same procedure as that detailed in Chapter 2, Section III (2), to give an insoluble solids content in the juice of approximately 10%. The level of PE in the juice as well as the pH were determined as detailed previously. Juice not required immediately was kept in a narrow cylinder in a chill room at 4 C. The juice in the cylinder was shaken vigourously prior to withdrawing further samples to redisperse any of the insoluble solids that might have settled on standing. In no case was the extracted juice held in the chill room for more than seven hours before being used. The level of PE in the fresh juice did not change significantly after chill storage for seven hours.

Four glass test tubes 125 mm long and 15 mm O.D. were filled with juice and sealed with rubber corks held in place by a perforated brass frame. Through the centre of each cork (and held in place by an adhesive) passed a copper-

constantan thermocouple wire of sufficient length to reach the middle of the test tube. The thermocouples were wired in series and connected to a millivolt recorder, and an ice-water mixture in a thermos flask acted as the reference point. Using this system, the millivolt output was the sum of the temperatures in each of the four tubes and therefore had to be divided by four to obtain the average temperature in the tubes.

Immediately after sealing, the test tubes were placed in an ice-water bath. At zero time the four tubes were submerged for varying lengths of time in a constant temperature water bath held at various temperatures in the range 170 F to 200 F, after which they were simultaneously removed and immediately cooled in the ice-water bath. The temperatures inside the tubes were continuously recorded. When the juice in the tubes had cooled to less than 15 C, they were opened and the four samples of juice mixed in a beaker. An aliquot of juice was taken from the beaker and tested for PE activity.

Forty-one runs were made on juice extracted from the August fruit, and forty-five runs on juice from the December fruit. The pH of the juice from the two harvesting periods was measured using a Radiometer pH meter calibrated with pH 3.20 buffer.

(ii) Calculation of Results

For each run, the temperature (as millivolts) of the juice at 5 second intervals was read off the chart paper from the millvolt recorder. These readings were punched onto computer cards together with the initial and final concentrations of PE in the juice. The results from the August fruit were kept quite separate from the results of the December fruit.

Two computer programmes were written in Fortran IV for a Burroughs 6700 computer - they are reproduced in Appendix I. The programmes were written to perform the following steps:

Programme 'A'

(a) Read the initial and final concentration levels of PE for each particular run and calculate the number of decimal reductions in PE concentration as follows:

number of decimal reductions, $D = \log \frac{\text{initial PE concn.}}{\text{final PE concn.}}$

(b) Read in the millivolt readings (taken at 5 second intervals) for each run, divide them by four to get the average reading across the four tubes, and convert them from millivolts to degrees Fahrenheit.

(c) Calculate the L value at each temperature in every run using the formula:

$$L = 10^{(T - 180)/z}$$

taking values of z from 20 to 170 in increments of 0.5.

(d) Calculate the F_{180} value of every run for each value of z by summing the L values calculated above for the

temperatures at 5 second intervals. Convert the F_{180} values from units of 5 seconds to seconds by multiplying by 5.

(e) Print out (for each run) the F_{180} values obtained using the different values of z, and the number of decimal reductions in PE concentration (i.e. D values).

(f) For each value of z, calculate the correlation coefficient between the F_{180} value and the D value for each run using the following formula:

$$r^2 = \frac{(N \Sigma xy - (\Sigma x)(\Sigma y))^2}{(N \Sigma x^2 - (\Sigma x)^2)(N \Sigma y^2 - (\Sigma y)^2)}$$

Also calculate the significance of the correlation coefficient using the Student's t test calculated as follows:

$$t^2 = r^2 \frac{(N - 2)}{(1 - r^2)}$$

Print out both these values.

Programme 'B'

(a) As for programme 'A'.

(b) As for programme 'A'.

(c) Convert the temperatures from Fahrenheit to Rankine. (degrees Rankine = degrees Fahrenheit + 460)

(d) Calculate the L value at each temperature in every run using the formula:

$$L = 10^{M(1/640 - 1/T)}$$

taking values of M from 1000 to 25000 in increments of 1000.

(e), (f) and (g) - as for steps (d) to (f) in programme 'A'.

Once the approximate optimum values for M and z had been found, the programmes were re-run using values of M and z close to the approximate optimum values found in the initial runs. The optimum values were taken as those for which the relationship between the F_{180} and D values had the highest correlation coefficient and t value.

Once the optimum z and M values had been determined programmes 'A' and 'B' were modified to calculate the regression equation relating the F_{180} value at the optimum z or M value to the D value. From the regression equation it was then possible to calculate the time required at 180 F to give a 1D or 5D process. The modified forms of programmes 'A' and 'B' (hereafter referred to as programmes 'C' and 'D') are reproduced in Appendix II. The basic steps in the programmes are summarised below:

Programme 'C'

(a) As for programme 'A'.

(b) As for programme 'A'.

(c) Calculate the L value at each temperature in every run using the formula:

$$L = 10^{(T - 180)/z}$$

taking z = 160.0 for low pH juice samples

and z = 77.5 for high pH juice samples.

(d) Calculate the F_{180} value of each run by summing the L values calculated above for the temperatures at 5 second intervals. Convert the F_{180} values from units of 5 seconds to seconds by multiplying by 5.

(e) Calculate and print out the regression equation for

predicting the F_{180} value from the D value using the following formula:

$$y - \bar{y} = r \frac{y}{x} (x - \bar{x})$$

(f) Calculate and print out the standard error of estimate for the F_{180} value calculated from the regression equation using the following formula:

$$s_y^2 = \sigma_y^2 (1 - r^2)$$

Programme 'D'

(a) As for programme 'A'.

(b) As for programme 'A'.

(c) Calculate the L value at each temperature in every run using the formula:

$$L = 10^{M(1/640 - 1/T)}$$

taking M = 2200 for low pH juice samples

and M = 4100 for high pH juice samples.

(d), (e) and (f) - as for steps (d) to (f) in programme 'C'.

3. RESULTS

(i) pH of Juice

(a) August fruit - the pH of the juice extracted from the August fruit was 3.05.

(b) December fruit - the pH of the juice extracted from the December fruit was 3.40.

(ii) Heating of Juice

A typical time-temperature (in millivolts) profile of the thermal process which the juice samples were subjected to is shown in Figure 17 (p. 136). Individual time-millivolt readings for all the eighty-six runs have not been included but sample data from one run is reproduced in Appendix III.

(iii) Calculation of F_{180} values

A sample printout from programmes 'A' and 'B' showing the different F_{180} values calculated by the computer for varying values of z and M is shown in Appendix IV for one heat-treated sample of juice.

The correlation coefficients betwwen the calculated F_{180} values and the decimal reductions in PE concentration for different values of z and M, together with the significance of the correlation coefficients as expressed by Student's t test, are reproduced from computer printouts in Tables XX, XXI, XXII and XXIII (p. 137 - 140).

FIGURE 17.

Typical time-temperature (in millivolts) profile of the thermal process to which the juice samples were subjected.

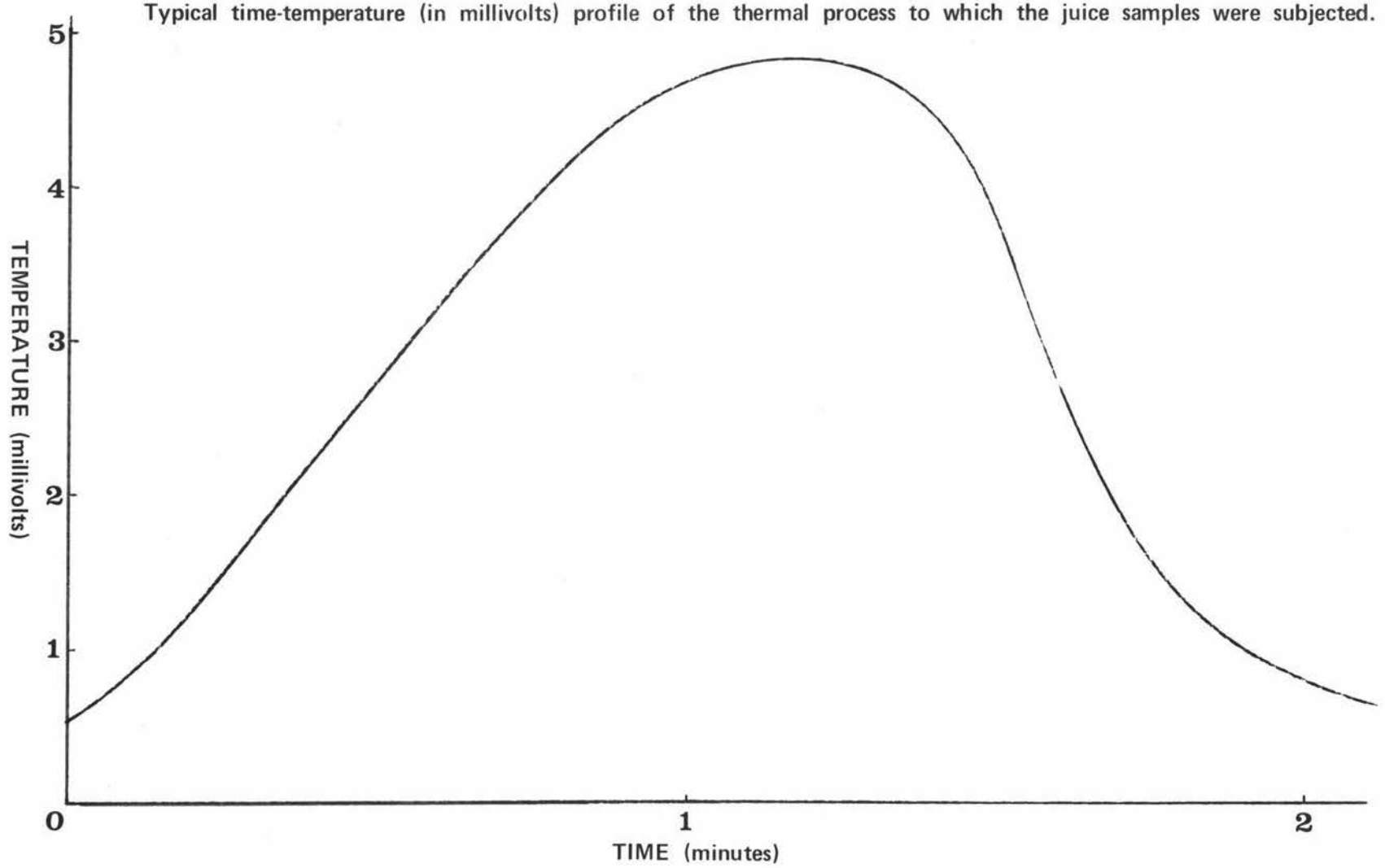


Table XX

Correlation coefficients between calculated F_{180} values and decimal reductions of PE inactivation in low pH (3.05) juice for varying values of z.

z	Correlation Coefficient	Student's t
50.0	0.787520	7.9801
60.0	0.819723	8.9377
70.0	0.841803	9.7392
80.0	0.857066	10.3888
90.0	0.867689	10.9005
100.0	0.875095	11.2924
110.0	0.880228	11.5836
120.0	0.883864	11.8008
130.0	0.886021	11.9341
140.0	0.887430	12.0232
150.0	0.888194	12.0721
158.0	0.888397	12.0852
159.0	0.888409	12.0858
160.0	0.888412	12.0862
161.0	0.888411	12.0861
162.0	0.888409	12.0860
170.0	0.888268	12.0769

optimum z value = 160.0 F

Table XXI

Correlation coefficients between calculated F_{180} values and decimal reductions of PE inactivation of high pH (3.40) juice for varying values of z.

z	Correlation Coefficient	Student's t
50.0	0.804432	8.8798
60.0	0.810926	9.0876
70.0	0.813583	9.1754
75.0	0.814008	9.1896
76.0	0.814039	9.1906
77.0	0.814054	9.1911
77.5	0.814056	9.1912
78.0	0.814054	9.1911
79.0	0.814040	9.1907
80.0	0.814012	9.1897
90.0	0.813132	9.1604
100.0	0.811489	9.1061
110.0	0.809414	9.0384
120.0	0.807114	8.9645
130.0	0.804716	8.8887

optimum z value = 77.5 F

Table XXII

Correlation coefficients between calculated F_{180} values and decimal reductions of PE inactivation in low pH (3.05) juice for varying values of M.

M	Correlation Coefficient	Student's t
1000	0.874711	11.2713
2000	0.893405	12.4191
2100	0.893632	12.4348
2200	0.893653	12.4362
2300	0.893485	12.4246
2400	0.893143	12.4011
2500	0.892639	12.3666
3000	0.888081	12.0649
4000	0.871837	11.1161
5000	0.850081	10.0802
10000	0.728375	6.6387
15000	0.636848	5.1584
20000	0.577969	4.4230
25000	0.541135	4.0186

optimum M value = 2200 R

Table XXIII

Correlation coefficients between calculated F_{180} values and decimal reductions of PE inactivation in high pH (3.40) juice for varying values of M.

M	Correlation Coefficient	Student's t
1000	0.733982	7.0867
2000	0.799272	8.7212
3000	0.811431	9.1042
3900	0.814964	9.2217
4000	0.815045	9.2244
4100	0.815075	9.2254
4200	0.815055	9.2248
4300	0.814989	9.2225
5000	0.812977	9.1552
10000	0.774830	8.0372
15000	0.729302	6.9898
20000	0.686895	6.1978
25000	0.647463	5.5711

optimum M value = 4100 R

A summary of the data in the above tables, showing the optimum values of z and M for the two juices, is presented in Table XXIV.

Table XXIV

Optimum z and M values for PE inactivation

	Low pH Juice	High pH Juice
Optimum z value	160.0 F	77.5 F
Correlation coefficient	0.888412	0.814056
Student's t	12.0862	9.1912
Optimum M value	2200 R	4100 R
Correlation coefficient	0.893653	0.815075
Student's t	12.4362	9.2254

(iv) Regression Equations for Predicting F_{180} Values from D

The regression equations calculated by programmes 'C' and 'D' are shown below in Table XXV.

Table XXV

Regression Equations for Predicting F_{180} from D

(a) For low pH juice

When z = 160.0, F_{180} = 58.9 D - 1.70

Standard error of estimate of F_{180} is 6.5%.

When M = 2200, F_{180} = 61.6 D - 3.40

Standard error of estimate of F_{180} is 6.6%.

(b) For high pH juice

When z = 77.5, F_{180} = 14.7 D - 2.47

Standard error of estimate of F_{180} is 2.4%.

When M = 4100, F_{180} = 19.0 D - 3.27

Standard error of estimate of F_{180} is 3.1%.

Using the regression equations from Table XXV, the F_{180} values (or the equivalent times at 180 F required to give 1D and 5D reductions in PE concentration) have been calculated. The 95% and 99% confidence limits for the F_{180} values have been calculated by multiplying the standard errors of estimate by 2 and 3 respectively. The results are summarised in Table XXVI.

Table XXVI

F_{180} Values for 1D and 5D Reductions in PE Concentration

(a) For low pH juice

	F_{180} for 1D	F_{180} for 5D
When z = 160.0	57.2 secs	292.8 secs
	(0.95 mins)	(4.88 mins)
95% confidence limits (±13%)	±7.44 secs	±38.06 secs
99% confidence limits (±19.5%)	±11.15 secs	±57.10 secs
When M = 2200	58.2 secs	304.6 secs
	(0.97 mins)	(5.07 mins)
95% confidence limits (±13.2%)	±7.68 secs	±40.21 secs
99% confidence limits (±19.8%)	±11.52 secs	±60.31 secs

(b) For high pH juice

	F_{180} for 1D	F_{180} for 5D
When z = 77.5	12.2 secs	71.0 secs
	(0.203 mins)	(1.18 mins)
95% confidence limits (±4.8%)	±0.58 secs	±3.41 secs
99% confidence limits (±7.2%)	±0.88 secs	±5.11 secs
When M = 4100	16.8 secs	95.0 secs
	(0.28 mins)	(1.58 mins)
95% confidence limits (±6.2%)	±1.04 secs	±5.89 secs
99% confidence limits (±9.3%)	±1.56 secs	±8.83 secs

(v) Calculation of Activation Energy

It was shown in the introduction to this section that

$$M = \frac{E}{2.3\,R} \qquad (19)$$

where E = activation energy

and R = Universal gas constant

The activation energies for destruction of PE in juices of two different pH's were calculated using optimum values of M. M was first converted from degrees Rankine to degrees Kelvin by dividing by 1.8, since M is in units of Rankine.

Thus, for low pH juice,

$$M = 2200\ R = 1222\ K$$

and for high pH juice,

$$M = 4100\ R = 2278\ K$$

Then $E = 2.3 \times 1.98 \times 1222$

$= 5565$ cal/mole for low pH juice.

and $E = 2.3 \times 1.98 \times 2278$

$= 10{,}374$ cal/mole for high pH juice.

(vi) Calculation of z from M

The relationship between z and M was shown previously to be:

$$z = \frac{(T')^2}{M} \qquad (21)$$

where T' = the absolute temperature.

By substituting for M in equation (21) using the values calculated by the computer, and taking T' from 620 to 660 R

(160 - 200 F) the results presented in Table XXVII were obtained.

Table XXVII

Values of z calculated from M

(a) Low pH juice

Temperature		z Value
133 F	593 R	160.0
160 F	620 R	174.7
170 F	630 R	180.4
180 F	640 R	186.2
190 F	650 R	192.0
200 F	660 R	198.0

(b) High pH juice

Temperature		z Value
104 F	564 R	77.5
160 F	620 R	93.8
170 F	630 R	96.8
180 F	640 R	99.9
190 F	650 R	103.0
200 F	660 R	106.2

4. DISCUSSION

(i) M values

Since M values are terms unique to canning technology, this discussion will be in terms of E values, which it has been shown previously are directly related to M values.

The E values obtained in this study are much lower than those reported in the literature for protein denaturation, as Table XXVIII indicates.

Table XXVIII

Published Energies of Inactivation (E's)

for Protein Denaturations

Enzyme	pH	E (cal/mole)
Purified Pea Lipoxygenase[1]	4.0	93,000
Purified Peroxidase[2]	7.0	25,100
Green Bean Peroxidase[3]	6.0	19,600 below 220 F
		34,400 above 220 F
Pepsin[4]	4.83	56,000 - 147,000

1 Svensson and Eriksson (1972)

2 Joffe and Ball (1962)

3 Zouell and Esselen (1959)

4 Laidler and Bunting (1973)

Continued over page

Table XXVIII continued

Energies of Activation (E's) Calculated for PE Inactivation of NZGF Juice

pH	E (cal/mole)
3.05	5,565
3.40	10,347

Generally, activation energies for protein denaturation are considerably higher than those obtained for ordinary chemical reactions occurring in the same temperature range. The fact that a reaction will occur at a measurable speed at 80 to 100 F requires the activation energy to be no higher than 18,000 cal/mole, while a value of 40,000 cal/mole or more usually means that the rate will not become appreciable until much higher temperatures have been reached (Laidler and Bunting, 1973). In this study, the temperatures used for inactivating the PE were relatively low, the temperature of the juice never exceeding 160 F during any run. However, even at these temperatures, considerable inactivation occurred in relatively short times. For example, for the run reproduced in Appendix IV, the total heating process (using z = 77.5) was equivalent to 9.1 seconds at 180 F and resulted in 0.738 decimal reductions in PE concentration, yet the maximum temperature reached by the juice was only 120.3 F. The low activation energies for PE inactivation determined in this study are confirmed by the above results. Furthermore, the low E

values indicate that the rate of inactivation of PE does not increase very much with increasing temperature over the range of temperatures used in this study.

(ii) z values

The z values obtained in this study are much higher than the only other ones reported in the literature for PE, namely by Pratt and Powers (1953). They found that z varied from 25.3 to 28.5 for the thermal inactivation of PE in true grapefruit juice. However, it is difficult to give much credence to their results since (as discussed earlier in Section I (3) of this chapter) the method that they used to account for unsteady-state heating during their inactivation studies was unsound. Also, they give no indication of the sensitivity of the method which they used to determine PE activity or the level of soluble solids and pulp in the juice samples, and whether these remained constant throughout their experimental work. Although the pH of the grapefruit used varied from 3.20 to 3.48, all their results were pooled for analysis.

They determined thermal destruction times at four different temperatures (167, 176, 185 and 194 F) and calculated F_{180} values for PE inactivation which ranged from 2.21 to 3.35 minutes. These compare with the F_{180} values calculated in this study (1.18 minutes for high pH juice and 4.88 minutes for low pH juice based on z values, and 1.58 and 5.07 minutes based on M values) necessary to

achieve complete inactivation of PE (i.e. a 5D reduction in PE concentration). The results of the two studies agree within the same order of magnitude, and the large variation in times reported by Pratt and Powers (ibid.) can be accounted for (at least in part) by their pooling of the results from samples having different pH's. This present study has shown how strongly the pH of the juice influences the F_{180} values.

Rouse and Atkins (1952) reported on the thermal inactivation of PE in Valencia orange juice with 5% and 10% pulp. Their results were expressed as the percentage inactivation of PE for different holding times at different temperatures (185, 190, 195, 200 and 205 F). No allowance was made for heating and cooling lags but some idea of the magnitude of their affect can be gauged from the fact that when the temperature of a 10% pulp juice sample was raised to 185 F and cooled immediately, 91.8% inactivation occurred. Their results were not expressed in terms of D, z or F values and they did not discuss what "end-point" concentration of PE they were aiming for in the heat-treated juice, other than to note that when their method of analysis failed to detect any PE activity it was assumed that all the PE in the juice had been destroyed.

It is possible to convert their results from percentage inactivation of PE into decimal reductions in PE concentration for various holding times at the different temperatures. When these decimal reduction values are

plotted against their corresponding holding times at the various temperatures, the points fall around but not on a straight line. From this graph the times required to achieve a 1.5D reduction in PE concentration can be read off for the different temperatures and by multiplying by 3.33 the times for a 5D reduction in PE concentration can be determined. These times are shown in Table XXIX.

Table XXIX

Times for 5D reduction in concentration and 100% inactivation of PE in Valencia Orange Juice at pH 4.1

Temperature	Time for 5D Reduction (seconds)	Time for 100% Inactivation (seconds)
185 F	263	not given
190 F	133	120
195 F	30	60
200 F	6.6	30
205 F	1.5	10

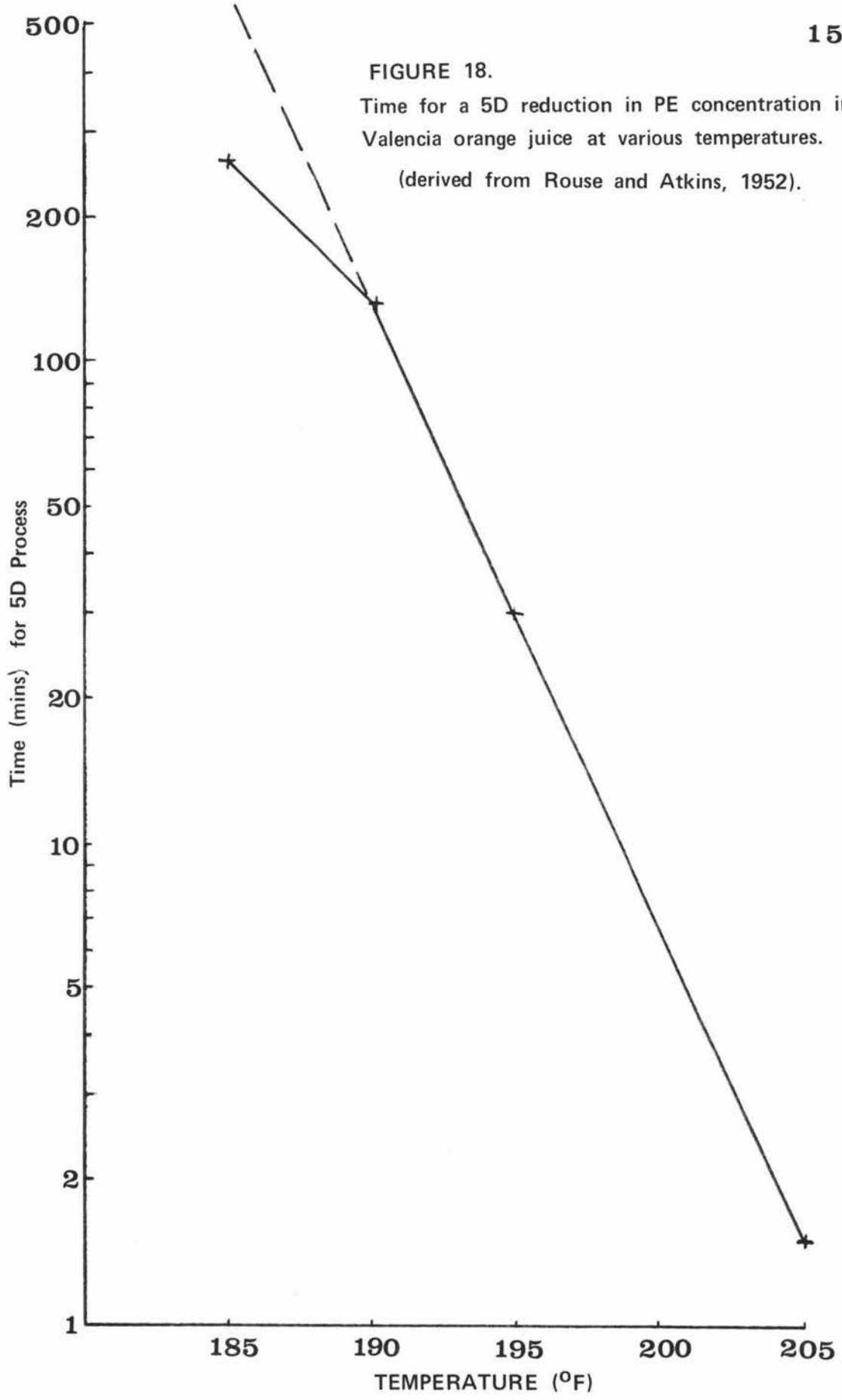
From Rouse and Atkins (1952)

A semilogarithmic plot of the times for a 5D reduction at various temperatures should yield a straight line. Figure 18 (p. 151) shows that at temperatures of 190 F and above, the points all fall on a straight line, but that the time for a 5D reduction at 185 F is less than half that expected by extending the straight line (263 seconds instead of 600 seconds). Rouse and Atkins (ibid.) gave times for

FIGURE 18.

Time for a 5D reduction in PE concentration in Valencia orange juice at various temperatures.

(derived from Rouse and Atkins, 1952).



100% inactivation of the PE and Table XXIX compares these times with those for 5D reductions derived as discussed above.

The results in Table XXIX indicate that the experimental method used by Rouse and Atkins (ibid.) does not permit (particularly at higher temperatures) accurate measurement of times for (in their terms) 100% inactivation of PE. Expressed another way, they achieved less than a 5D reduction at 190 F, but far in excess of 5D at higher temperatures, specifically 6D at 195 F, 23D at 200 F and 33D at 205 F. The particularly large decimal reductions at high temperatures resulted from the imprecise nature of their experiments.

If the results of Rouse and Atkins (ibid.) presented in Figure 18 are extrapolated back to 180 F, alternative times for a 5D reduction in PE concentration are obtained. Extrapolation of the main line gives a time of 3000 seconds (50 minutes) while extrapolation of the line joining 185 F and 190 F gives a time of 520 seconds (8.66 minutes). Both values are much higher than either those reported by Pratt and Powers (ibid.) or those found in this present study. The objections to the methodology of Rouse and Atkins discussed above, coupled with the fact that their work was carried out using Valencia orange juice at pH 4.1 possibly accounts for the large differences.

In this study it was found that at the higher pH, shorter times were required for inactivation of PE. In

contrast, Rouse and Atkins (ibid.) found that when Valencia orange juice at different pH's was heated to 180 F and immediately cooled, there was less inactivation of PE in juice at the higher pH compared to juice at the lower pH.

A detailed account of the theory of pH affects on protein denaturation has been presented by Laidler and Bunting (1973). They state that in general the rate of denaturation passes through a minimum as the pH is varied, and that the behaviour is frequently complex. They quote, for example, a very accurate study of pepsin inactivation where it was found that the rate was proportional to the reciprocal of the fifth power of the hydrogen ion concentration. From the results in this present study no detailed conclusions regarding the affect of pH on the rate of PE inactivation can be drawn, other than that the rate is greater at pH 3.40 than at pH 3.05.

(iii) Calculation of z from M

The z values, calculated from the optimum M values at each pH and presented in Table XXVII (p. 145), are higher than the optimum z values at 180 F calculated by the computer. As discussed in the introduction to Section III of this chapter, z is usually assumed to be constant over the range of temperatures studied, i.e. z is independent of temperature. The results in Table XXVII indicate that such an assumption is not valid because z, as predicted from M, varies with temperature.

Gillespy (1951) showed that over the small ranges of temperature encountered in canning (220 to 250 F), values of z predicted from M agreed with values of z calculated in the usual way from D values. However, he also showed that over a wider range, z varied with temperature while M (i.e. E, as shown above) was independent of temperature. This is the situation which exists in this present study; namely, that z cannot be considered as constant over the whole temperature range, and to be correct, should be calculated from M at each particular temperature.

Consequently, to predict the equivalent times at 180 F to achieve a given number of decimal reductions, the regression equations based on M rather than z should be used. Referring to Table XXVI (p. 143), it is seen that the F_{180} values for 5D reductions calculated using the regression equations based on M values give slightly longer times than those based on z values - 11.8 seconds longer at pH 3.05, and 24 seconds longer at pH 3.40. This agrees with the observation of Gillespy (ibid.) that M values give more conservative estimates of inactivation times than those based on z.

(iv) Equivalent times at other temperatures

One of the prime advantages of z and M values is that they enable the times required for inactivation at temperatures either above or below 180 F to be calculated.

The above discussion has indicated that M rather

than z values correctly predict the change in thermal resistance with temperature. When the F_{180} values calculated from the regression equations based on optimum M values are plotted as straight lines with slopes equal to the negative reciprocals of z values calculated from optimum M values, Figure 19 (p. 156) is obtained. The calculations on which these lines were based are outlined below:

(a) For low pH juice, the regression equation based on M is:

$$F_{180} = 61.6\ D - 3.40$$

For a 5D reduction in PE concentration,

$$F_{180} = 5.07 \text{ minutes}$$

with 99% confidence limits of ±19.8% (±1.00 minutes). Using the optimum M value of 2200, the z value at 180 F (calculated using equation (21) - see Table XXVII) is 186.2.

(b) For high pH juice, the regression equation based on M is:

$$F_{180} = 19.0\ D - 3.27$$

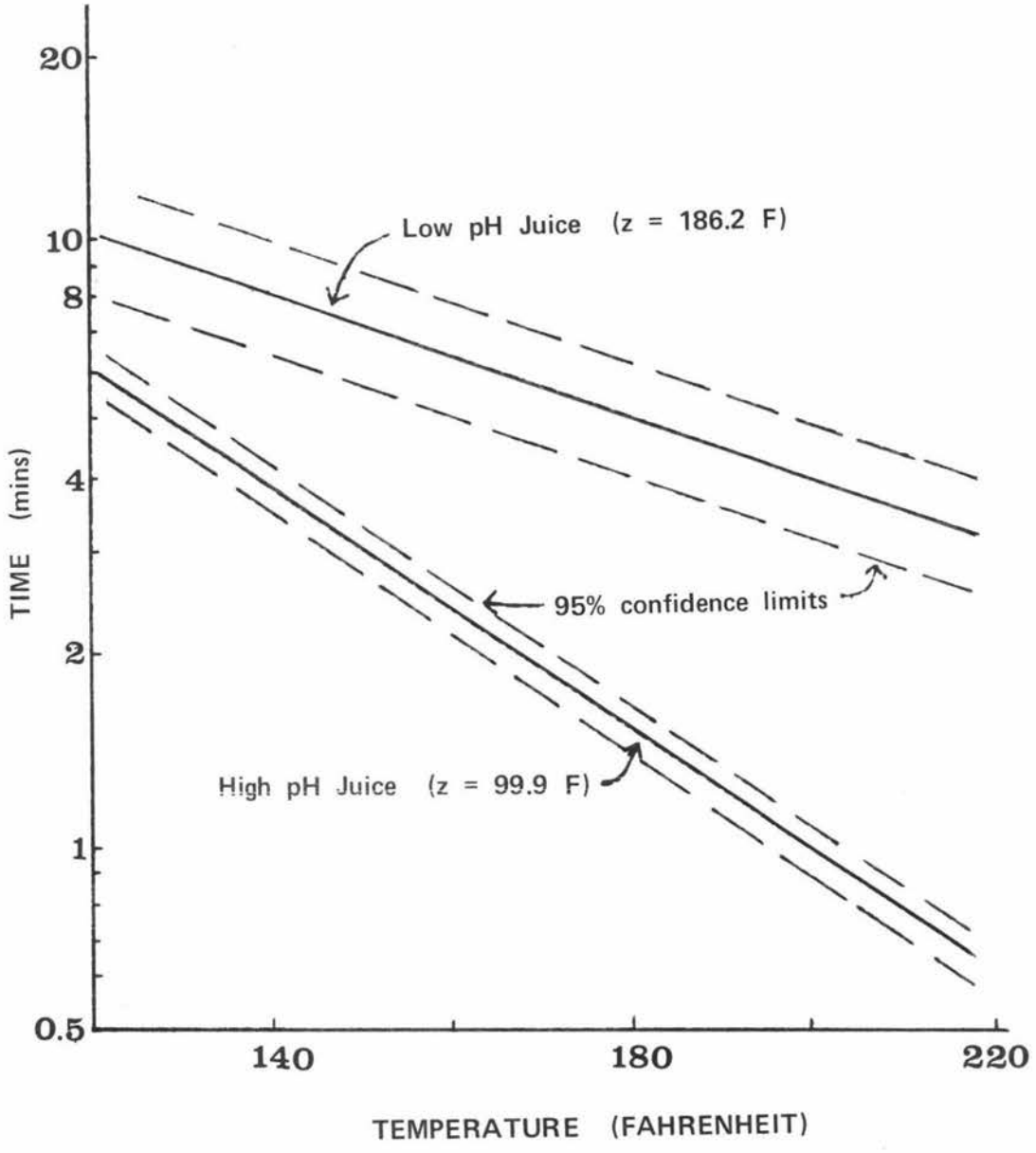
For a 5D reduction in PE concentration,

$$F_{180} = 1.58 \text{ minutes}$$

with 99% confidence limits of ±9.3% (±0.15 minutes). Using the optimum M value of 4100, the z value at 180 F (calculated using equation (21) - see Table XXVII) is 99.9.

FIGURE 19.

Logarithm of the time for a 5D reduction in PE concentration as a function of temperature for low and high pH NZGF juice.



From Figure 19 it is possible to read off the equivalent times at various temperatures to get a 5D reduction in the concentration of PE. These results are summarised below in Table XXX.

Table XXX

Equivalent times at different temperatures to achieve a 5D reduction in PE concentration

Temperature	Time	
	Low pH Juice	High pH Juice
160 F	6.5 mins.	2.4 mins.
170	5.8	1.88
180	5.07	1.58
190	4.5	1.17
200	3.95	0.93
210	3.55	0.84

Table XXX confirms results published by other workers such as Pratt and Powers (1953) and Rouse and Atkins (1952), that the higher the temperature at which citrus juices are held, the shorter the time required to inactivate the PE present in the juice.

Reference to Tables XXIX and XXX indicates that the times required to inactivate NZGF juice at 190 F are longer for the low pH juice and shorter for the high pH juice than the times reported by Rouse and Atkins (ibid.) for Valencia orange juice at pH 4.1. At 200 F the times

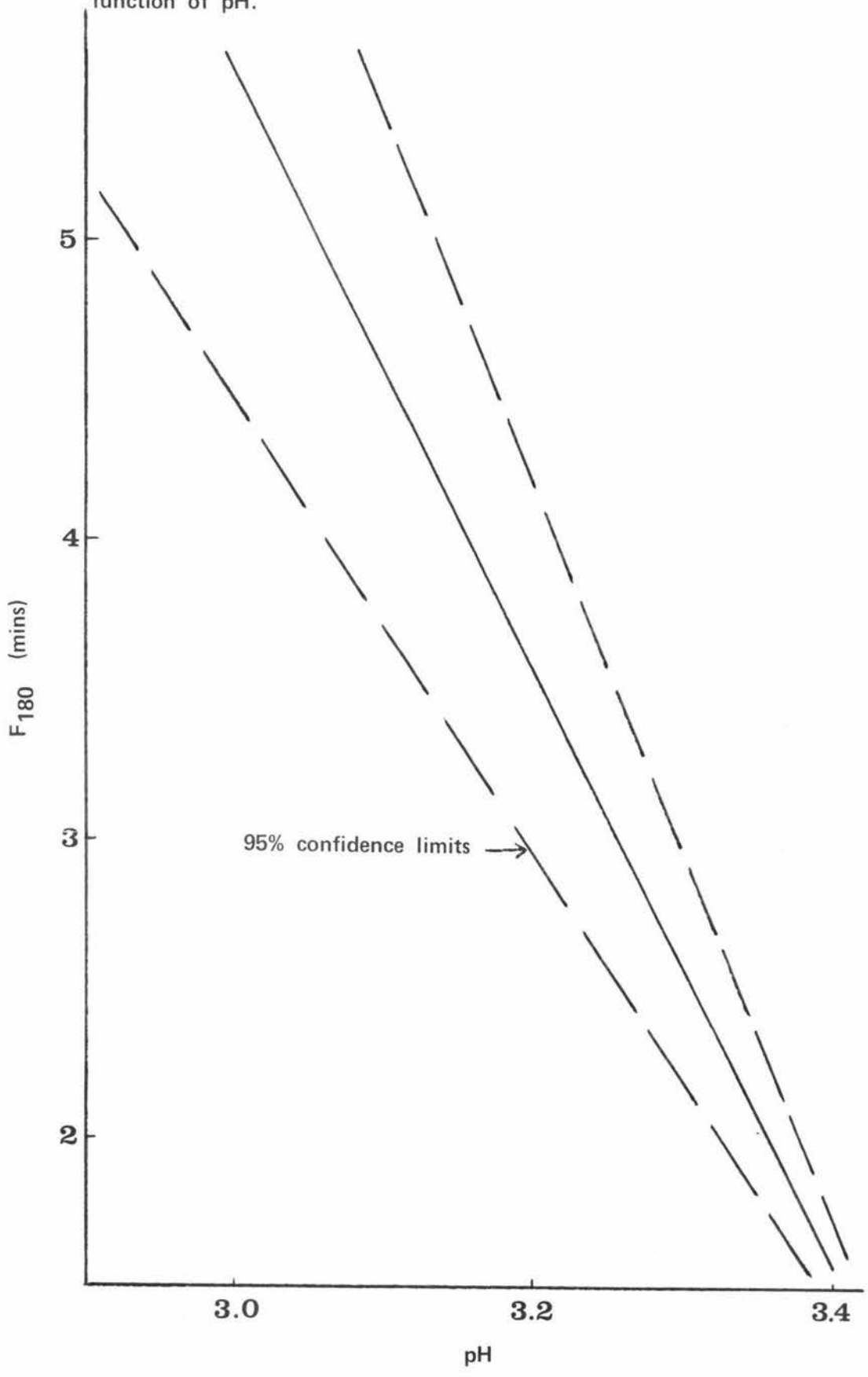
required to inactivate PE in NZGF juice are longer than in Valencia orange juice. As discussed earlier close agreement was not expected with the published results because of all the variables involved, such as different varieties of citrus fruit, dissimilar pH's, and different approaches to the handling of the heating and cooling lags.

(v) Equivalent times at different pH's

In the previous chapter the seasonal variation in the pH of NZGF juice was presented (see Figure 3 (p. 57)). Knowing the equivalent times for a 5D reduction in PE at the upper and lower limits of pH (3.40 and 3.05 respectively) it is possible to predict the equivalent times at pH's within that range. Rouse and Atkins (1953) found a linear relationship between pH and the equivalent times required to inactivate PE, and if the same linear relationship is assumed for these results, it is possible to draw graphs such as that of Figure 20 (p. 159) relating the equivalent time and pH at constant temperature. Thus as the pH of the juice increases throughout the season, the equivalent time required to reduce the PE by 5D can be decreased.

FIGURE 20.

Equivalent times at 180 F for a 5D reduction in PE in NZGF juice as a function of pH.



5. CONCLUSION

The following conclusions can be drawn from this study on the thermal inactivation of PE in NZGF juice:

(i) the activation energies for the thermal inactivation of PE in low and high pH NZGF juice are 5,565 and 10,374 cal/mole respectively. These comparatively low values indicate that the rate of inactivation proceeds at a considerable speed at relatively low temperatures, and that increasing the temperature over the range 100 F to 160 F does not greatly increase the rate of inactivation. Considerable inactivation of PE occurs at relatively low (120 F) temperatures.

(ii) the z values representing the relative thermal resistance of PE in NZGF juice are 160.0 for low pH juice and 77.5 for high pH juice when calculated on the assumption that z is independent of temperature. If this assumption is not accepted, the corresponding z values are 186.6 F and 99.9 F. These comparatively high values indicate that large increases in temperature (180 F for low pH juice and 100 F for high pH juice) are required to obtain a ten-fold change in the rate of inactivation of PE.

(iii) the thermal resistance of PE (as represented by the z values) varies with temperature, while the M value (i.e. the activation energy, E) is independent of temperature.

(iv) the equivalent time required at 180 F to inactivate the PE in low pH juice is 5.07 ± 1.00 minutes, while in high pH juice the time is 1.58 ± 0.15 minutes.

(v) the equivalent times required at various temperatures to inactivate the PE in NZGF juice are of the same order of magnitude as those reported for other citrus juices, the differences being due to the different varieties of citrus studied, the dissimilar pH's of the juices, the different approaches to the heating and cooling lags, and the treatment of the problem of deciding when all the PE in the juice had been destroyed.

SECTION IV

CLOUD STABILITY IN HEAT TREATED NEW ZEALAND GRAPEFRUIT JUICE

1. INTRODUCTION

It was stated in Section I (2) of this chapter that citrus juice cloud could be stabilised by inactivating the PE present in the juice. In the preceding section of this chapter the times and temperatures necessary to inactivate PE in NZGF juice was determined. In this present section, samples of NZGF juice containing various levels of PE were subjected to a storage trial during which the cloud stability was monitored, thus providing an opportunity to verify the earlier statement that the inactivation of PE in citrus juices leads to cloud stability.

Early investigations into cloud changes in citrus juices were confined to visual observations of change in appearance. However, Loeffler in 1941 introduced a quantitative objective method. He determined light transmittance in the supernatant after centrifugation of the juice. Loeffler's suggestion was almost universally followed, and while type of centrifuge and time and speed of centrifugation were usually indicated, proposals for standardising, or at least indicating centrifugal force, were not made until the mid-1950's when Senn et al. (1955) proposed the method

which is now in universal use. They also suggested calibrating the various colorimeters in use with bentonite suspensions, although Moore and Wenzel (1951) had already suggested barium sulphate solutions. The method used in this study was based on that of Senn et al. (ibid.).

2. EXPERIMENTAL

(i) Cloud Stability Measurement

Using the method of Senn et al. (1955), samples of juice were centrifuged for ten minutes at 470g. Turbidity of the supernatant was measured as percent transmittance using a Unicam SP 1300 Series 2 Colorimeter with filter 6 (620 mu upwards). The colorimeter was standardised using bentonite suspensions, and all results were expressed as grams of bentonite per litre.

(ii) Juice Samples

Juice was extracted from late season NZGF according to the standard method described in chapter two, to give juice with a pulp content of 10%. The pH of the juice was measured using a Radiometer pH meter standardised with buffer at 3.2. Sodium benzoate (0.1% w/v) was added to the juice to prevent any microbial growth or fermentation during the storage trial.

The PE concentration in the extracted juice was measured using the method described in Section II (A2) of chapter two. The juice was then divided into three equal

aliquots. The turbidity of sample A (which received no further treatment) was measured using the method described above. Samples B and C were subjected to heat treatments using the method outlined in Section III (2) of this chapter. The temperatures of the juice samples were continuously recorded so that the F_{180} values could be calculated using computer programme 'B' and the optimum M value for high pH juice determined in the previous chapter. The PE concentration in samples B and C was measured after heat treatment, as was the turbidity. The three samples of juice were then placed in chill storage at 0 C and their turbidity was measured at various times over a period of fourteen days.

3. RESULTS

(i) Cloud Stability Measurement

The calibration curve for the colorimeter used for turbidity measurements is shown in Figure 21 (p. 165).

(ii) Juice Samples

(a) pH of Juice - the pH of the juice was 3.40.

(b) PE Concentration -

Sample A (fresh juice)	66.7 (PEu)ml TSS
Sample B (heat treated)	12.2 (PEu)ml TSS
Sample C (heat treated)	0.0 (PEu)ml TSS

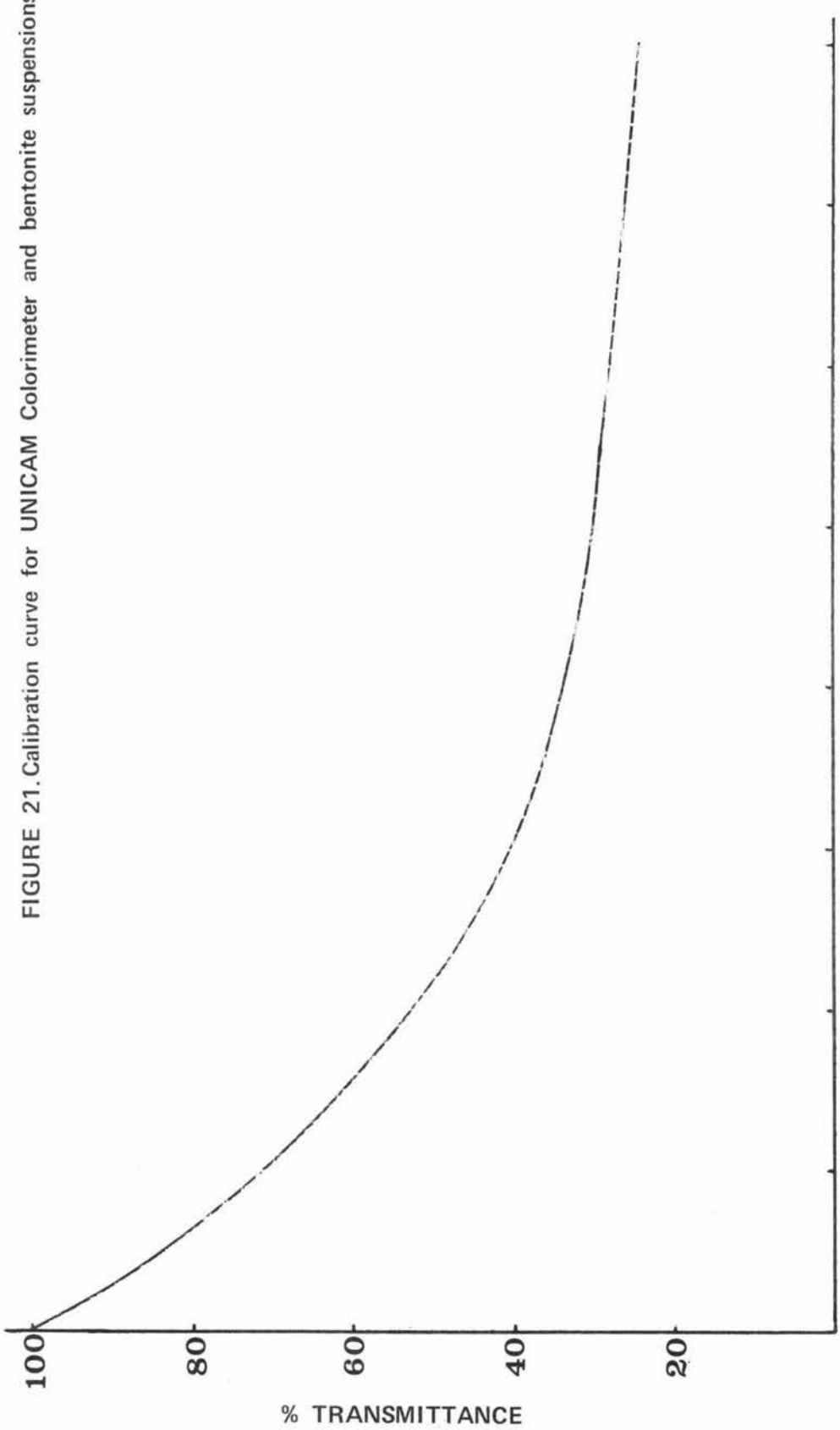


FIGURE 21. Calibration curve for UNICAM Colorimeter and bentonite suspensions.

(c) F_{180} Values -

	F_{180} value	D value
Sample A	0	0
Sample B	0.28 mins.	0.737D
Sample C	1.37 mins.	5D

(d) Turbidity -

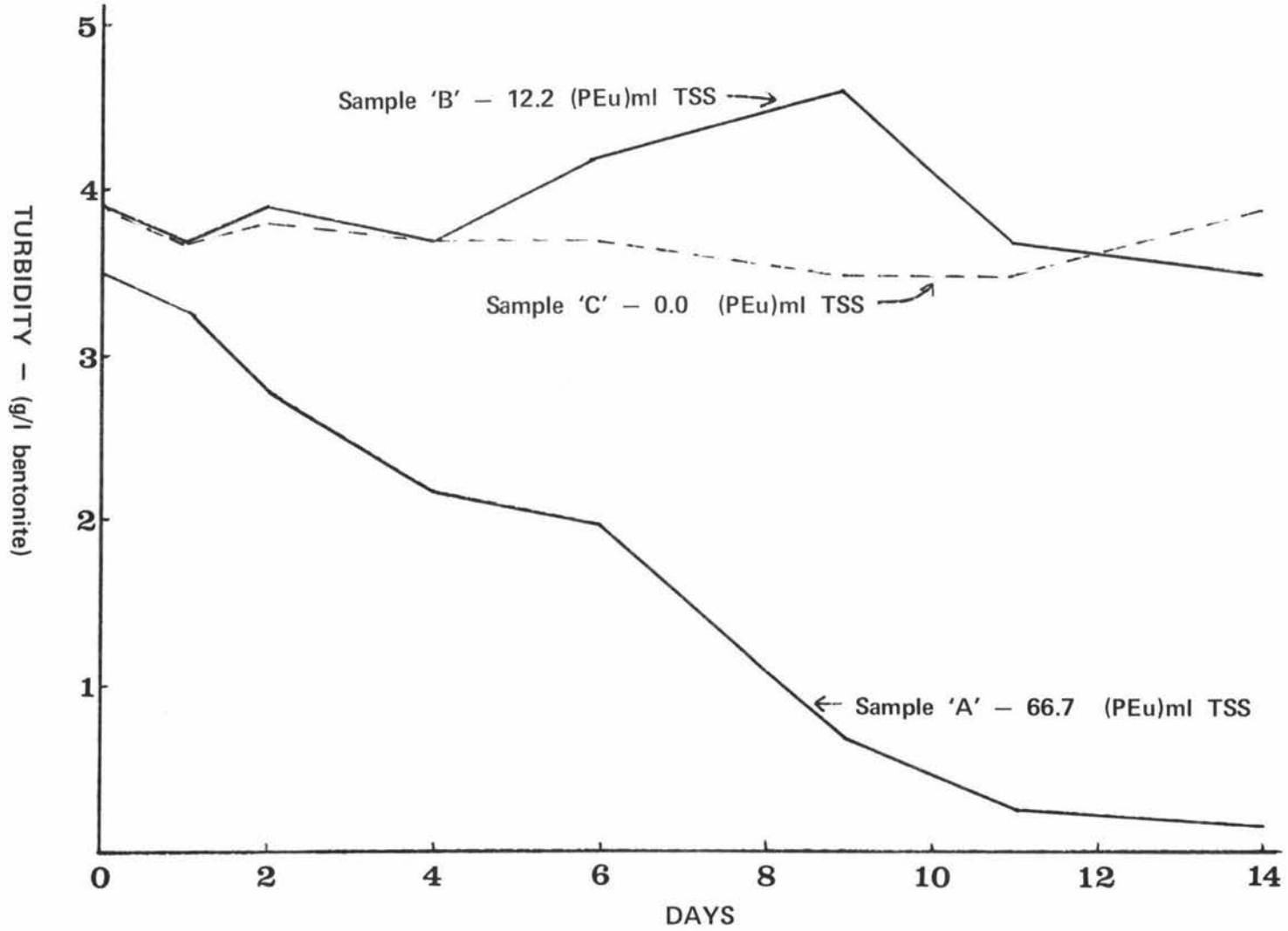
the change in turbidity of the three samples over fourteen days is shown in Figure 22 (p. 167)

4. DISCUSSION

Several important points emerge from Figure 22. First, there is the increase in turbidity of samples B and C after the heat treatment. This is in agreement with the observations made by Loeffler (1941) who reported that the cloud index increased on pasteurisation, and that this increase was apparently due to an increase in the dissolved pectins obtained from the pulp particles.

Secondly, the two samples in which PE was absent or present in relatively low concentrations maintained approximately the same level of turbidity throughout the fourteen days. There is no obvious explanation as to why sample B increased in turbidity after nine days. It is tempting to extrapolate the trend shown by the graph over the last three days and predict that sample B containing a low level of PE was beginning to show a decrease in turbidity while sample C which contained no PE was going to retain its original level of turbidness. However, such a conclusion

FIGURE 22. Turbidity of NZGF juice with varying levels of PE as a function of time.



cannot be rigorously substantiated on the data available.

The third point is that sample A which contained the high natural level of PE in fresh juice progressively lost turbidness until after fourteen days there was virtually no cloud left in suspension.

5. CONCLUSION

From this experiment it can be concluded that failure to inactivate PE in NZGF juice leads to a progressive loss in turbidity over fourteen days storage at 0 C.

CHAPTER FOUR

APPLICATION OF THESE STUDIES TO THE INDUSTRIAL PROCESSING OF NEW ZEALAND GRAPEFRUIT JUICE

In chapter one it was stated that this present study was undertaken to investigate aspects of the processing of NZGF juice and provide basic technological data that would enable citrus processors to produce a consistently high quality single strength juice. Chapter two considered seasonal changes in the compositional characteristics of NZGF juice of importance to citrus processors, and chapter three established the conditions necessary for the stabilisation of cloud in NZGF juice.

This final chapter shows how the information presented in the previous chapters can be applied by the citrus processor in the production of high quality single strength NZGF juice.

The major quality defect of NZGF juice currently marketed in New Zealand is the unstable nature of the cloud. Chapter three showed that the cloud can be stabilised by heating for certain times at specified temperatures. The citrus processor would want to carry out this heat treatment on a continuous basis and it is therefore recommended that a plate heat exchanger be used for this purpose.

A plate heat exchanger consists of a pack of pressed, corrugated, stainless steel plates held between a frame and a pressure plate. The frame is stationary but the pressure plate can be moved across supporting bars in order to give easy access to individual plates for inspection, cleaning and repair. Corrugation of the plates

ensures turbulence of the thin layer of fluid flowing between them as well as rigidity, thus permitting a minimum plate thickness. This results in maximum heat transfer rates, minimum plate weight, and minimum cost. Sealing between the plates is by gaskets which are usually made of natural or synthetic rubber.

The advantages which a plate heat exchanger has over other heat exchangers are:

(i) all heat transfer surfaces are easily and quickly accessible for cleaning, inspection and repair. This is particularly important where liquid foods are being processed, as areas of equipment which are difficult to clean soon become breeding grounds for microorganisms, resulting in contamination of any food material which subsequently passes through the equipment.

(ii) the pack of plates can be easily added to or reduced in number to meet varying changes in duty. Consequently, if a processor wishes to increase throughput, or increase the heat treatment which the juice receives, it is simply a matter of adding additional plates to the pack.

(iii) space requirements for plate heat exchangers are small due to the compact nature of their design.

(iv) the heating, holding, cooling and regenerative sections of the heat exchanger can be assembled into one unit.

(v) large volumes of juice can be treated in one relatively small piece of equipment.

(vi) heat transfer coefficients are high compared to other heat exchangers such as the shell and tube, thus maximising the overall thermal efficiency of the heat transfer process.

The three stages involved in the heat treatment of NZGF juice to stabilise the cloud are:

(i) heating the juice to the required temperature.

(ii) holding the juice at the desired temperature for the required time.

(iii) cooling the juice to chill temperatures prior to filling into containers of some sort.

The actual temperature at which the processor operates the holding section of the heat exchanger is largely a matter of individual choice and depends on several factors, the most important being the temperature of the hot water available to the heat exchanger, and the size of the holding section of the heat exchanger in relation to the throughput. Provided there is adequate control over the temperature of the hot water supplied to the heat exchanger, it is advisable to use as high a temperature as possible as this minimises the number of plates required in the holding section.

Once the temperature of operation of the holding section has been selected, the holding time required by juice of a certain pH can then be found from graphs similar to that presented in Figure 20 (p. 159) in the previous chapter. The number of plates required to give the desired holding time is dependent on the size of the plates and the

throughput of the juice. Manufacturers of plate heat exchangers can provide this information for citrus processors.

Because of the variation in PE level in the juice throughout the season (as shown in Figure 10 (p. 64)) it may be thought that there could be some justification for altering the time of heating as the level of PE varied. However, this is not so for several reasons. First, the magnitude of the change in PE level in the juice is quite small compared with the total change in PE level occurring during the heat inactivation. Figure indicates that the level of PE varies from 42 to 91 (PEu)ml TSS throughout the season. The final level of PE aimed for in the juice is 0.001. Thus, variation of PE in the fresh juice would affect the number of decimal reductions required as follows:

Initial level of PE	100	91	42
Final level of PE	0.001	0.001	0.001
Number of decimal reductions	5	4.96	4.61

Assuming that all other factors are constant, early season juice (42 (PEu)ml TSS) would require 0.39D less reductions than late season juice. i.e. 7.8% less time at constant temperature. This is less than half the standard error of estimate of the time required at constant temperature to inactivate the juice at low pH and therefore cannot be regarded as significant.

The second reason for not altering the time of heating as the level of PE in the juice varies is because of

the irregular manner of the PE variation. If the concentration of PE is increased at an even rate throughout the season, then it could be possible to consider some alteration in holding times, but because the changes in PE level are generally unpredictable on a day to day basis, such a practice could not be justified from a technological viewpoint.

The third reason for not varying the time of heating with changes in the PE level is that the pH of the juice is increasing as the season progresses and this affects the required time of heating to a much greater extent (as Figure 20 showed) than does the increasing PE level. To summarise, the holding time required at a particular temperature to achieve a 5D reduction in PE level in the juice is determined by the pH of the juice and not the initial level of PE. As the required holding time at a particular temperature of the juice decreases throughout the season, it is a simple matter for the processor to progressively reduce the number of plates in the holding section.

It will be noted that in suggesting the residence time of the juice in the holding section of the heat exchanger, no allowance was made for the lethal effect on the PE conferred during heating and cooling. This was intentional, the idea being to provide a safety factor in the heating process. The lethal effect of the heating and cooling phases would ensure that the total lethal process which the juice received would exceed the desired time plus the

calculated error in this time at the 99% confidence level.

An additional safeguard for the citrus processor is a temperature-controlled by-pass valve on the outlet side of the heat exchanger, such as is compulsory on milk pasteurisation heat exchangers. In such a system the temperature of the water which heats the holding section of the heat exchanger is compared with the desired temperature, and if the actual temperature is lower, the by-pass valve is actuated and all juice leaving the heat exchanger is returned to the inlet feed until the desired temperature is again reached in the holding section. Although such a system results in some over-heating of the juice which is in the heat exchanger when the temperature falls, it has the advantage of ensuring that no juice receives less than adequate heat treatment.

If the above procedures are followed, the citrus processor can be assured of producing NZGF juice which will maintain a stable cloud for a considerable time after processing and thus eliminate the major consumer criticism of the juice surrently available on the market.

```
C                APPENDIX I
C
C      PROGRAMME 'A' TO CALCULATE OPTIMUM Z VALUE
C
       REAL INACT(51), INITI
       DIMENSION ZT(51),F(61,50),HMV(51)
C
C      STATEMENT FUNCTION
C
            XL(T,Z) = 10.**((T - 180.)/Z)
C
       MVAR = 50
            L = 1
C
  100  READ(2,1) N, INITI, FINAL
    1       FORMAT(6X,I2,2X,F5.1,F5.1)
C
C      CHECK TO SEE IF LAST CARD (N = 0)
C
       IF (N) 11,21,22
C
C      IF HEADER IS VALID READ DATA CARDS FOR ONE RUN
C
   22  READ(2,3)(HMV(I), I = 1,N)
    3       FORMAT(18F4.2)
C
C      CALCULATE INACTIVATION OF PECTINESTERASE
C
       INACT(L) = (ALOG(INITI/FINAL))/2.303
C
C      READ IN TEMPERATURES - CONVERT THEM FROM
C                MILLIVOLTS TO FAHRENHEIT
C
       DO 2 I = 1,N
       G =HMV(I)/4.0
       E = G*G
       D = E*G
       C = 0.01074 + 25.988*G - 0.6670*E + 0.02332*D
       DF = 1.8*C + 32.00000
    2  ZT(I) = DF
C
C      CALCULATE F VALUE
```

```
            Z = MVAR
C
            DO 20 K = 1,61
            F(K,L) = 0.
C
C                 INNER LOOP TEMP VALUES (N OF THEM)
C
                  DO 10 J = 1,N
   10              F(K,L) = F(K,L) + XL(ZT(J),Z)
C
C      INCREMENT Z BY 0.5 AND REPEAT F CALCULATIONS
   20 Z = Z + 0.5
C
      WRITE(3,450) N, INACT(L)
  450      FORMAT(1H1,//T30,'NUMBER OF READINGS IS ',
     *I2,5X,'NUMBER OF DECIMAL REDUCTIONS IS',F8.4)
C
      WRITE(3,18)
   18      FORMAT(///,6('    Z      F(Z)    '1X,)//)
      Z1 = MVAR
      Z2 = Z1 + 5
      Z3 = Z1 + 10
      Z4 = Z1 + 15
      Z5 = Z1 + 20
      Z6 = Z1 + 25
C
      DO 19 K=1,10
      WRITE(3,23) Z1,F(K,L),Z2,F(K+10,L),Z3,F(K+20,L),
     *Z4,F(K+30,L),Z5,F(K+40,L),Z6,F(K+50,L)
   23      FORMAT('0',6(F4.1,F8.4,6X))
      Z1 = Z1 + .5
      Z2 = Z2 + .5
      Z3 = Z3 + .5
      Z4 = Z4 + .5
      Z5 = Z5 + .5
   19 Z6 = Z6 + .5
C     REPEAT ABOVE CALCULATIONS FOR NEXT RUN
C
      L = L + 1
      GO TO 100
C
C     ALL DATA HAS BEEN READ AND 'F' VALUES STORED IN CORE
C
C     NOW FIND OPTIMUM Z VALUE
```

```
C
      IF (L-2) 11,11,24
   24 MC = L - 1
      Q = MVAR
C
      DO 95 K = 1,61
      XF = 0.
      XY = 0.
      XFSQ = 0.
      YINSQ = 0.
      YINAC = 0.
C
C     INNER LOOP L FROM ONE TO MC
C
      DO 40 L = 1,MC
      XF = F(K,L) + XF
      YINAC = YINAC + INACT(L)
      XY = XY + (F(K,L)*INACT(L))
      XFSQ = XFSQ + (F(K,L) * F(K,L))
   40  YINSQ = YINSQ + (INACT(L) * INACT(L))
C
      DIV = SQRT((XFSQ - (XF*XF)/MC)*(YINSQ -
     *(YINAC*YINAC)/MC))
      RCOEF = (XY - ((XF * YINAC)/MC))/DIV
C     APPLY T TEST TO CORRELATION COEFFICIENT
      R = MC-2
C     CALCULATE T VALUE FOR CORRELATION COEFFICIENT  (TZ)
      TZ = RCOEF * SQRT(R)/SQRT(1.-RCOEF*RCOEF)
C
      WRITE(3,7) Q, RCOEF, TZ
    7     FORMAT(26X,'WHEN Z IS ',F7.0,'CORRELATION'
     *'COEFFICIENT IS',F9.6,'AND ''T'' IS',F7.4,//)
C
      Q = Q + 0.5
   95 CONTINUE
      IF(MC-41) 11,30,31
C
   30 WRITE(3,6) MC
    6     FORMAT(20X,'THE SAMPLE SIZE IS ',I2,'
     *AND THE PH OF THE JUICE IS 3.05')
      GO TO 32
C
   31 WRITE(3,8) MC
    8     FORMAT(20X,'THE SAMPLE SIZE IS ',I2,
     *AND THE PH OF THE JUICE IS 3.40')
      GO TO 11
C
   32 L = 1
C
C     REPEAT CALCULATIONS FOR HIGH PH DATA
C
      GO TO 100
C
   11 CALL EXIT
      END
```

```
C                 APPENDIX I
C
C       PROGRAMME 'B' TO CALCULATE OPTIMUM M VALUE
C
        REAL INACT(51), INITI, M1,M2,M3,M4,M5,M
        DIMENSION ZT(51),F(61,50),HMV(51)
C
C       STATEMENT FUNCTION
C
            XL(T,M) = 10.**(M*((1./640.) - (1./T)))
C
        MVAR = 1000
            L = 1
C
  100   READ(2,1) N, INITI, FINAL
    1       FORMAT(6X,I2,2X,F5.1,F5.1)
C
C       CHECK TO SEE IF LAST CARD (N = 0)
C
        IF (N) 11,21,22

C       IF HEADER IS VALID READ DATA CARDS FOR ONE RUN
C
   22   READ(2,3)(HMV(I), I = 1,N)
    3       FORMAT(18F4.2)
C
C       CALCULATE INACTIVATION OF PECTINESTERASE
C
        INACT(L) = (ALOG(INITI/FINAL))/2.303
C
C       READ IN TEMPERATURES - CONVERT THEM FROM
C                 MILLIVOLTS TO RANKINE
C
        DO 2 I = 1,N
        G =HMV(I)/4.0
        E = G*G
        D = E*G
        C = 0.01074 + 25.988*G - 0.6670*E + 0.02332*D
        DF = 1.8*C + 32.00000
    2   ZT(I) = DF + 460.00000
C
C       CALCULATE F VALUE
```

```
      M = MVAR
C
      DO 20 K = 1,26
          F(K,L) = 0.
C
C                INNER LOOP TEMP VALUES (N OF THEM)
C
                 DO 10 J = 1,N
   10             F(K,L) = F(K,L) + XL(ZT(J),M)
C
C     INCREMENT M BY 1000 AND REPEAT CALCULATIONS
   20 M = M + 1000
C
      WRITE(3,450) N, INACT(L)
  450      FORMAT(1H1,//T30,'NUMBER OF READINGS IS ',
     *I2,5X,'NUMBER OF DECIMAL REDUCTIONS IS',F8.4)
C
      WRITE(3,18)
   18      FORMAT(///,5('   M        F(M)   '6X)//)
      M1 = MVAR
      M2 = M1 + 500
      M3 = M1 + 1000
      M4 = M1 + 1500
      M5 = M1 + 2000
C
      DO 19 K=1,5
      WRITE(3,23)M1,F(K,L),M2,F(K+ 5,L),
     *M3,F(K+10,L),M4,F(K+15,L),M5,F(K+20,L)
   23      FORMAT('0',5(F6.0,3X,F10.5,5X))
      M1 = M1 + 1000
      M3 = M3 + 1000
      M2 = M2 + 1000
      M4 = M4 + 1000
   19 M5 = M5 + 1000




C     REPEAT ABOVE CALCULATIONS FOR NEXT RUN
C
      L = L + 1
      GO TO 100
```

```
C
      IF (L-2) 11,11,24
   24 MC = L - 1
      Q = MVAR
C
      DO 95 K = 1,26
      XF = 0.
      XY = 0.
      XFSQ = 0.
      YINSQ = 0.
      YINAC = 0.
C
C     INNER LOOP L FROM ONE TO MC
C
      DO 40 L = 1,MC
      XF = F(K,L) + XF
      YINAC = YINAC + INACT(L)
      XY = XY + (F(K,L)*INACT(L))
      XFSQ = XFSQ + (F(K,L) * F(K,L))
   40  YINSQ = YINSQ + (INACT(L) * INACT(L))
C
      DIV = SQRT((XFSQ - (XF*XF)/MC)*(YINSQ -
     *(YINAC*YINAC)/MC))
      RCOEF = (XY - ((XF * YINAC)/MC))/DIV
C     APPLY T TEST TO CORRELATION COEFFICIENT
      R = MC-2
C     CALCULATE T VALUE FOR CORRELATION COEFFICIENT  (TZ)
      TZ = RCOEF * SQRT(R)/SQRT(1.-RCOEF*RCOEF)
C
      WRITE(3,7) Q, RCOEF, TZ
    7     FORMAT(26X,'WHEN M IS ',F7.0,'CORRELATION'
     *'COEFFICIENT IS',F9.6,'AND ''T'' IS',F7.4,//)
C
      Q = Q + 1000
   95 CONTINUE
      IF(MC-41) 11,30,31
C
   30 WRITE(3,6) MC
    6     FORMAT(20X,'THE SAMPLE SIZE IS ',I2,'
     *AND THE PH OF THE JUICE IS 3.05')
      GO TO 32
C
   31 WRITE(3,8) MC
    8     FORMAT(20X,'THE SAMPLE SIZE IS ',I2,
     *AND THE PH OF THE JUICE IS 3.40')
      GO TO 11
C
   32 L = 1
C
C     REPEAT CALCULATIONS FOR HIGH PH DATA
C
      GO TO 100
```

```
C                  APPENDIX II
C       PROGRAMME 'C' TO CALCULATE REGRESSION EQUATIONS
C       AND STANDARD ERRORS OF ESTIMATE USING
C       OPTIMUM VALUES FOR Z
C
        REAL INACT(51), INITI
        DIMENSION ZT(51),F(61,50),HMV(51)
C
C       STATEMENT FUNCTION
C
             XL(T,Z) = 10.**((T - 130.)/Z)
C
             K = 1
             L = 1
        MVAR = 77.5
C
  100 READ(2,1) N, INITI, FINAL
    1      FORMAT(6X,I2,2X,F5.1,F5.1)
C
C       CHECK TO SEE IF LAST CARD (N = 0)
C
        IF (N) 11,21,22
C
C       IF HEADER IS VALID READ DATA CARDS FOR ONE RUN
C
   22 READ(2,3)(HMV(I), I = 1,N)
    3      FORMAT(18F4.2)
C
C       CALCULATE INACTIVATION OF PECTINESTERASE
C
        INACT(L) = (ALOG(INITI/FINAL))/2.303
C
C       READ IN TEMPERATURES - CONVERT THEM FROM
C                  MILLIVOLTS TO FAHRENHEIT
C
        DO 2 I = 1,N
        G =HMV(I)/4.0
        E = G*G
        D = E*G
        C = 0.01074 + 25.988*G - 0.6670*E + 0.02332*D
        DF = 1.8*C + 32.00000
    2 ZT(I) = DF
C
             Z = MVAR
C
C       CALCULATE F VALUE
C
             F(K,L) = 0.
```

```
                DO 10 J = 1,N
   10             F(K,L) = F(K,L) + XL(ZT(J),Z)
C
C      CONVERT F VALUE TO SECONDS
C      TEMPERATURE INCREMENTS WERE FIVE SECONDS
C
       F(K,L) = F(K,L)*5.0
C
C      REPEAT ABOVE CALCULATIONS FOR NEXT RUN
C
       L = L + 1
       GO TO 100
C
C      ALL DATA HAS BEEN READ AND 'F' VALUES STORED IN CORE
C                NOW DO STATISTICAL CALCULATIONS
C
       MC = L - 1
       Q = MVAR
C
C      CALCULATE REGRESSION EQUATION
C
       XY = 0.
       XFSQ = 0.
       YINSQ = 0.
       XF = 0.
       YINAC = 0.
C
C      INNER LOOP L FROM ONE TO MC
C
       DO 40 L = 1,MC
       XF = F(K,L) + XF
       YINAC = YINAC + INACT(L)
       XY = XY + (F(K,L)*INACT(L))
       XFSQ = XFSQ + (F(K,L) * F(K,L))
   40  YINSQ = YINSQ + (INACT(L) * INACT(L))
C
       DIV = SQRT((XFSQ - (XF*XF)/MC)*(YINSQ -
      *(YINAC*YINAC)/MC))
       RCOEF = (XY - ((XF * YINAC)/MC))/DIV
       XFMEAN = XF/MC
       YINMEN = YINAC/MC
       STDEVX = SQRT(XFSQ/MC - (XFMEAN*XFMEAN))
       STDEVY = SQRT(YINSQ/MC - (YINMEN*YINMEN))
       EM = RCOEF*(STDEVX/STDEVY)
       EC = XFMEAN - (EM*YINMEN)
C
       WRITE(3,106) EM,EC
  106      FORMAT(20X,'REGRESSION EQUATION IS AS FOLLOWS',
      *//,20X,'F = ',F12.4,'D ',F12.4,////)
```

```
      STERRF = STDEVX*SQRT(1. - (RCOEF*RCOEF))
      WRITE(3,108) STERRF
  108     FORMAT(20X,'STANDARD ERROR OF F IS ',
     *F12.5,'PERCENT',//)
C
      WRITE(3,107) Q
  107     FORMAT(20X,'THE Z VALUE FOR THE ABOVE
     *CALCULATIONS WAS',F6.1,////////)
C
      IF(MC-41) 11,30,31
C
   30 WRITE(3,6) MC
    6     FORMAT(20X,'THE SAMPLE SIZE IS ',I2,'
     *AND THE PH OF THE JUICE IS 3.05')
      GO TO 32
C
   31 WRITE(3,8) MC
    8     FORMAT(20X,'THE SAMPLE SIZE IS ',I2,
     *AND THE PH OF THE JUICE IS 3.40')
      GO TO 11
C
   32 L = 1
      MVAR = 160.0
C
C     REPEAT CALCULATIONS FOR HIGH PH DATA
C
      GO TO 100
C
   11 CALL EXIT
      END
```

```
C                APPENDIX II
C       PROGRAMME 'D' TO CALCULATE REGRESSION EQUATIONS
C       AND STANDARD ERRORS OF ESTIMATE USING
C       OPTIMUM VALUES FOR M
C
        REAL INACT(51), INITI
        DIMENSION ZT(51),F(61,50),HMV(51)
C
C       STATEMENT FUNCTION
C
            XL(T,M) = 10.**(M*((1./640.) - (1./T)))
C
            K = 1
            L = 1
        MVAR = 2200
C
  100 READ(2,1) N, INITI, FINAL
    1     FORMAT(6X,I2,2X,F5.1,F5.1)
C
C       CHECK TO SEE IF LAST CARD (N = 0)
C
        IF (N) 11,21,22
C
C       IF HEADER IS VALID READ DATA CARDS FOR ONE RUN
C
   22 READ(2,3)(HMV(I), I = 1,N)
    3     FORMAT(18F4.2)
C
C       CALCULATE INACTIVATION OF PECTINESTERASE
C
        INACT(L) = (ALOG(INITI/FINAL))/2.303
C
C       READ IN TEMPERATURES - CONVERT THEM FROM
C                 MILLIVOLTS TO RANKINE
C
        DO 2 I = 1,N
        G =HMV(I)/4.0
        E = G*G
        D = E*G
        C = 0.01076 + 25.988*G - 0.6670*E + 0.02332*D
        DF = 1.8*C + 32.00000
    2   [illegible] + 460.00000
C
        M = MVAR
C
C       CALCULATE F VALUE
C
            F(K,L) = 0.
```

```
C
                DO 10 J = 1,N
   10           F(K,L) = F(K,L) + XL(ZT(J),M)
C
C      CONVERT F VALUE TO SECONDS
C      TEMPERATURE INCREMENTS WERE FIVE SECONDS
C
       F(K,L) = F(K,L)*5.0
C
C      REPEAT ABOVE CALCULATIONS FOR NEXT RUN
C
       L = L + 1
       GO TO 100
C
C      ALL DATA HAS BEEN READ AND 'F' VALUES STORED IN CORE
C                NOW DO STATISTICAL CALCULATIONS
C
       MC = L - 1
       Q = MVAR
C
C      CALCULATE REGRESSION EQUATION
C
       XY = 0.
       XFSQ = 0.
       YINSQ = 0.
       XF = 0.
       YINAC = 0.
C
C      INNER LOOP L FROM ONE TO MC
C
       DO 40 L = 1,MC
       XF = F(K,L) + XF
       YINAC = YINAC + INACT(L)
       XY = XY + (F(K,L)*INACT(L))
       XFSQ = XFSQ + (F(K,L) * F(K,L))
   40  YINSQ = YINSQ + (INACT(L) * INACT(L))
C
       DIV = SQRT((XFSQ - (XF*XF)/MC)*(YINSQ -
      *(YINAC*YINAC)/MC))
       RCOEF = (XY - ((XF * YINAC)/MC))/DIV
       XFMEAN = XF/MC
       YINMEN = YINAC/MC
       STDEVX = SQRT(XFSQ/MC - (XFMEAN*XFMEAN))
       STDEVY = SQRT(YINSQ/MC - (YINMEN*YINMEN))
       EM = RCOEF*(STDEVX/STDEVY)
       EC = XFMEAN - (EM*YINMEN)
C
       WRITE(3,106) EM,EC
  106      FORMAT(20X,'REGRESSION EQUATION IS AS FOLLOWS',
      *//,20X,'F = ',F12.4,'D ',F12.4,////)
```

```
      STERRF = STDEVX*SQRT(1. - (RCOEF*RCOEF))
      WRITE(3,108) STERRF
  108     FORMAT(20X,'STANDARD ERROR OF F IS ',
     *F12.5,'PERCENT',//)
C
      WRITE(3,107) Q
  107      FORMAT(20X,'THE M VALUE FOR THE ABOVE
     *CALCULATIONS WAS',F6.1,////////)
C
      IF(MC-41) 11,30,31
C
   30 WRITE(3,6) MC
    6     FORMAT(20X,'THE SAMPLE SIZE IS ',I2,'
     *AND THE PH OF THE JUICE IS 3.05')
      GO TO 32
C
   31 WRITE(3,8) MC
    8     FORMAT(20X,'THE SAMPLE SIZE IS ',I2,
     *AND THE PH OF THE JUICE IS 3.40')
      GO TO 11
C
   32 L = 1
      MVAR = 4100
C
C     REPEAT CALCULATIONS FOR HIGH PH DATA
C
      GO TO 100
C
   11 CALL EXIT
      END
```

APPENDIX III

SAMPLE DATA FROM ONE RUN SHOWING TIME-TEMPERATURE (IN MILLIVOLTS) READINGS

TIME (SECS)	TEMPERATURE (MILLIVOLTS)
0	0.49
5	0.62
10	0.81
15	1.04
20	1.24
25	1.44
30	1.61
35	1.76
40	1.90
45	1.98
50	1.83
55	1.64
60	1.44
65	1.31
70	1.20
75	1.11
80	1.04
85	0.97
90	0.91
95	0.85
100	0.80
105	0.76
110	0.71
115	0.67
120	0.64
125	0.61

APPENDIX IV

SAMPLE PRINTOUT FROM PROGRAMME 'A' SHOWING F180 VALUES FOR DIFFERENT Z VALUES

Z	F180	Z	F180
70.0	6.9371	77.5	9.1225
70.5	7.0767	78.0	9.2745
71.0	7.2173	78.5	9.4271
71.5	7.3588	79.0	9.5804
72.0	7.5012	79.5	9.7344
72.5	7.6445	80.0	9.8890
73.0	7.7886	80.5	10.0442
73.5	7.9337	81.0	10.2001
74.0	8.0795	81.5	10.3565
74.5	8.2262	82.0	10.5135
75.0	8.3737	82.5	10.6711
75.5	8.5219	83.0	10.8293
76.0	8.6710	83.5	10.9880
76.5	8.8208	84.0	11.1472
77	8.9713	84.5	11.3069

APPENDIX IV

SAMPLE PRINTOUT FROM PROGRAMME 'B' SHOWING F180 VALUES FOR DIFFERENT M VALUES

M	F180(SECS)	M	F180(SECS)
3000.	4.38603	4000.	2.45780
3100.	4.13460	4100.	2.32262
3200.	3.89957	4200.	2.19540
3300.	3.67693	4300.	2.07563
3400.	3.46875	4400.	1.96286
3500.	3.27317	4500.	1.85666
3600.	3.08939	4600.	1.75660
3700.	2.91664	4700.	1.66232
3800.	2.75422	4800.	1.57346
3900.	2.60148	4900.	1.48968

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