THE KINETICS OF QUALITY DETERIORATION IN LEMON JUICES AND CONCENTRATES DURING STORAGE

A THESIS PRESENTED IN PARTIAL FULFILMENT
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ABSTRACT

The effects of initial dissolved oxygen content, temperature and total soluble solids content on the kinetics of the quality deterioration in lemon juices (9 °Brix) and concentrates (20-50 °Brix) during storage were determined.

The parameters used to measure quality loss were ascorbic acid retention, nonenzymic browning, and sensory quality. The suitability of furfural and hydroxymethylfurfural (HMF) as indices of quality deterioration was also investigated.

Ascorbic acid degradation and HMF formation were observed to follow a first-order reaction model while browning and furfural formation followed a zero-order model. Temperature dependence of the different reactions could be described by the linear and Arrhenius expressions over the temperature range of 10 to 36°C.

The initial dissolved oxygen content (0.41, 1.44 and 3.74 mg/L) did not significantly affect the rate of ascorbic acid degradation and furfural formation in single-strength lemon juice stored at 36°C. However, browning and HMF formation were significantly higher in the juice with 3.74 mg/L dissolved oxygen content than in the samples with the other two oxygen contents.

The total soluble solids concentration affected the rates of the different reactions but not to such a significant extent as the temperature effect. Ascorbic acid retention was observed to increase with an increase in soluble solids content.

The rate of the browning reaction generally increased with increases in soluble solids content for the 20 to 50 °Brix juice samples. The rate of furfural formation consistently increased with increases in soluble solids level at 36°C, but was not as consistent at 10 and 20°C.

The rate constants and activation energy values of the different reactions for the 9 °Brix juice were considerably higher than those for the 20 °Brix concentrate. These observations and the poor correlation obtained between ascorbic acid retention and browning, and between ascorbic acid retention and furfural formation for the higher Brix concentrates, suggested that different reactions or reaction mechanisms predominated in single-strength juice compared with concentrates.

Furfural could serve as an index of quality deterioration in single-strength lemon juice but not in concentrates (20-50 °Brix) due to its simultaneous formation and decomposition at these high soluble solids levels.

The sensory panel perceived significant changes in colour in the juices prior to changes in flavour. The browning reaction should thus be the main criterion in the determination of storage life.

Low temperature storage is essential for optimum storage stability. Over a 16-week storage period at 10°C, it is suggested that lemon juice be stored as a 50 °Brix concentrate. Some advantages of storing lemon juice at such high soluble solids levels are high retention of ascorbic acid and flavour properties, and reduction in storage and distribution costs. To extend the storage life of lemon juice concentrates beyond four months, storage temperatures lower than 10°C would be necessary so that the extent of browning would not reach unacceptable levels.

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CHAPTER 1

INTRODUCTION

Citrus fruits and their products have long been noted as excellent sources of ascorbic acid (vitamin C), which is the most abundant vitamin in citrus fruits. They are also quite rich in the mineral element potassium. The increased awareness on the part of consumers about nutrition has led to an increased demand for citrus juices and products, a demand that is greater today than it has ever been (Varsel, 1980).

The most popular citrus juices, in terms of amount consumed, are orange and grapefruit juices. Lemon juice has also gained in popularity because of the technological advances that now permit the manufacture of concentrated juices and the production of a frozen concentrate for lemonade.

Commercially processed citrus juice undergoes quality changes during storage, in terms of ascorbic acid loss, nonenzymic browning, and change in flavour properties. Most studies on these aspects of citrus juice storage have been conducted on orange and grapefruit juices. Lemon juice is distinctly different in composition from other citrus, excepting limes, which makes its processing problems and uses far more varied and in some cases more complex technically (Swisher and Swisher, 1980). Due to this difference, it is possible that the reactions taking place during the storage of lemon juice would differ from other citrus juices. Hence, this study was undertaken with the objective of investigating quality deterioration in lemon juice during storage.

The importance of this study with respect to the Philippine situation has also been considered. In the Philippines, calamansi (*Citrus mitis*) is extensively grown and is the

leading citrus fruit that is used primarily for its juice. Presently, it is consumed as freshly extracted juice. Because of the increasing demand for fruit juices and for the need to maintain a constant supply of the juice even if calamansi fruits are not in season, attempts are being made to produce thermally processed calamansi juice. The chemical characteristics and colour of calamansi juice resemble those of lemon juice. The same storage problems are thus expected to occur in these two juices. A study on the quality deterioration in lemon juice will therefore be of value to the product development and shelf-life optimization of processed calamansi juice.

The specific objectives of this study are as follows:

(a) to determine the most suitable parameters and index of quality deterioration in lemon juice

(b) to determine the kinetics of ascorbic acid degradation, nonenzymic browning, and furfural and hydroxymethylfurfural (HMF) formation in lemon juice

(c) to determine changes in sensory quality (colour and flavour) in stored lemon juice

(d) to determine the effects of storage temperature, initial dissolved oxygen content, and total soluble solids content on the kinetics of the quality deterioration in lemon juice during storage.

CHAPTER 2

LITERATURE REVIEW

Although various theories have been advanced to explain the mechanism of browning in citrus products, that involving ascorbic acid degradation is thought to be the governing one (Clegg, 1964).

One of the theories proposed involves the formation of sugar-amine condensation products which, after undergoing Amadori rearrangement and a variety of secondary reactions, give rise ultimately to dark coloured 'melanoidin' compounds. In the Amadori rearrangement, only unprotonated amine can combine with sugars and therefore the step of sugar-amine condensation is favoured by high pH (Berk, 1976). Thus, it was thought unlikely that this mechanism was the major contributor to the browning of a highly acid (pH 2.5) product such as lemon juice (Clegg, 1964).

Browning in citrus products is always associated with destruction of ascorbic acid (Joslyn and Marsh, 1935; Joslyn, 1957; Clegg, 1964).

In 1964, Clegg studied the reaction taking place during the nonenzymic browning of lemon juice and comparable model systems. The results showed that ascorbic acid was the main precursor of browning. Browning was found to be proportional to the level of ascorbic acid (Clegg, 1964).

2.1 Ascorbic Acid Degradation

The exact route of ascorbic acid degradation is highly variable and dependent upon the particular system. Factors which can influence the nature of the degradation mechanism include temperature, salt and sugar concentration, pH,

oxygen, enzymes, metal catalysts, amino acids, oxidants or reductants, initial concentration of ascorbic acid, and the ratio of ascorbic acid to dehydroascorbic acid (Tannenbaum, 1976).

Reaction mechanisms and pathways have been based on both kinetic and physicochemical measurements, as well as on structural determination of isolated products. Many of the studies have been conducted in model systems at low pH's (pH 2-3) or in high concentrations of organic acids, and therefore the pathways have been reasonably assumed to occur in citrus juices.

The proposed scheme of ascorbic acid degradation by Tannenbaum (1976) is shown in Figure 2.1.

When oxygen is present in the system, ascorbic acid is degraded primarily via its monoanion (HA^-) to dehydroascorbic acid (A), the exact pathway and overall rate being a function of the concentration of metal catalysts (M^{n+}) in the system.

In the uncatalyzed oxidative pathway the ascorbate anion (HA^-) is oxidized in a rate-linking step to give first the radical anion $A^{\cdot-}$ and $HO_2^{\cdot}$, followed rapidly by formation of A and H_2O_2. Hydrolysis of the lactone follows to form 2,3-diketogulonic acid (DKG).

The scheme for anaerobic degradation shown in Figure 2.1 is speculative. Ascorbic acid is shown to react via its keto tautomer, H_2A-keto. The tautomer is in equilibrium with its anion HA^--keto, which undergoes delactonization to DKG.

Further degradation beyond DKG takes place. Evidence accumulated so far tends to indicate a major divergence of products formed, depending on whether or not decomposition has been oxidative. Xylosone (X) may be formed by

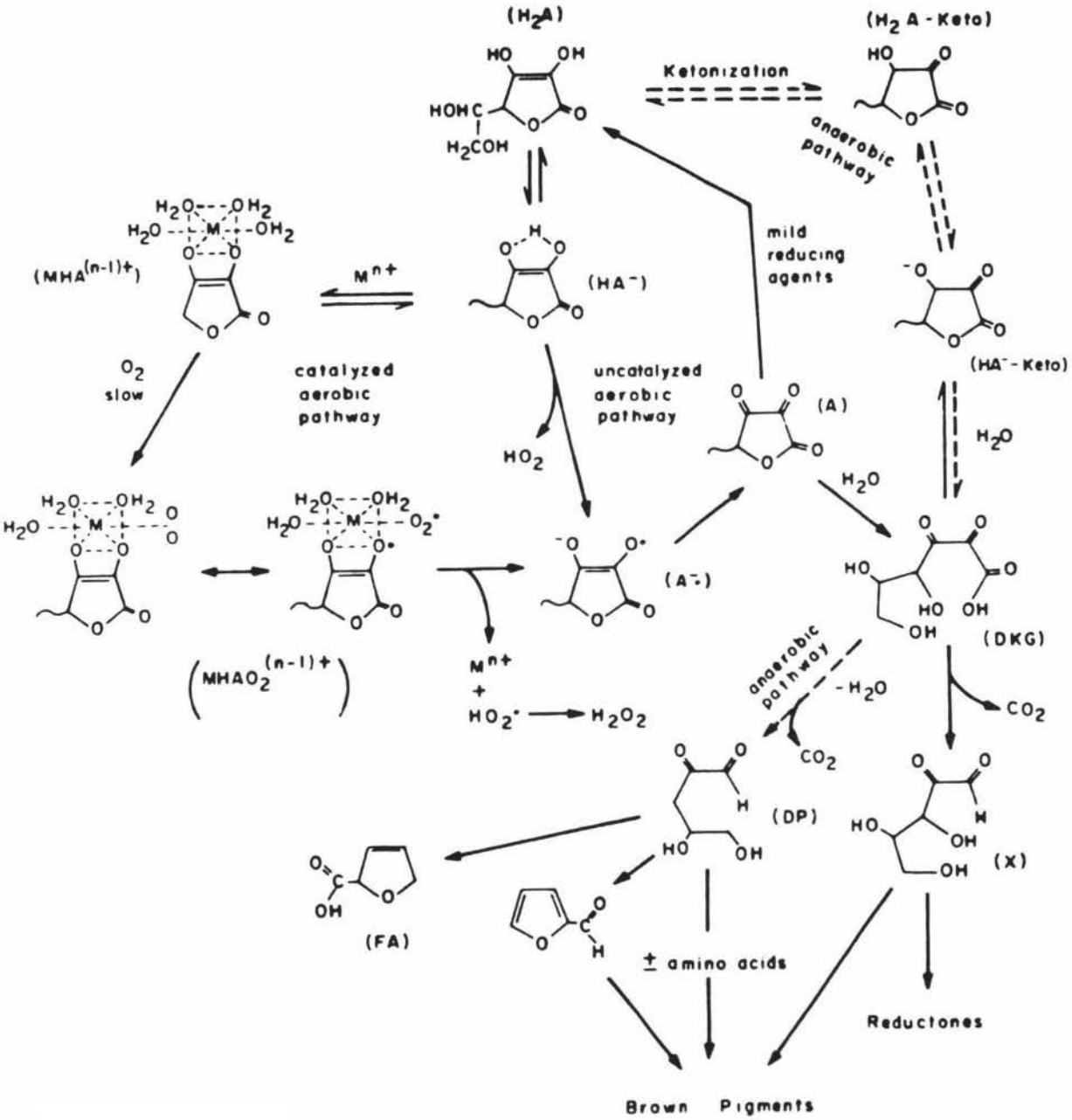

FIGURE 2.1: Degradation of ascorbic acid (From Tannenbaum, 1976).

simple decarboxylation of DKG, whereas 3-deoxypentosone (DP) is formed by β elimination at C-4 of DKG followed by decarboxylation.

Xylosone is further degraded to reductones and ethylglyoxal, while DP is degraded to furfural (F) and 2,5-dihydrofuroic acid (E). A number of these intermediate and end products have been identified in citrus products during storage.

Tannenbaum's (1976) proposed aerobic pathway is in agreement with those earlier proposed by Bauernfiend and Pinkert (1970) and Kurata and Sakurai (1967b). However, the pathway proposed by Bauernfiend and Pinkert shows hydroxymethyfurfural as one of the products of AA degradation (Figure 2.2).

Smoot and Nagy (1980) do not favour the anaerobic pathway proposed by Tannenbaum (1976) because of the speculative formation of DKG. Ascorbic acid degrades to form furfural but the intermediate steps in the anaerobic pathway are still highly speculative (Kurata and Sakurai, 1967a,b; Bauernfiend and Pinkert, 1970).

There is evidence that although furfural and hydroxymethyl-furfural are formed in the course of browning, they are not active compounds in the development of coloured complexes (Burton *et al.*, 1963b; Clegg, 1964). According to Clegg (1964), a more likely explanation is that these compounds accumulate as by-products because of their low reactivity and that early browning may be due to faster acting carbonyls.

The investigation of Clegg and Morton (1965) showed that carbonyls are the reactive components in the nonenzymic browning of an acidic product such as lemon juice. Work with the model systems confirmed that the αβ-unsaturated carbonyls are potent browning agents and also that dicarbonyls of the glyoxal type make a contribution to

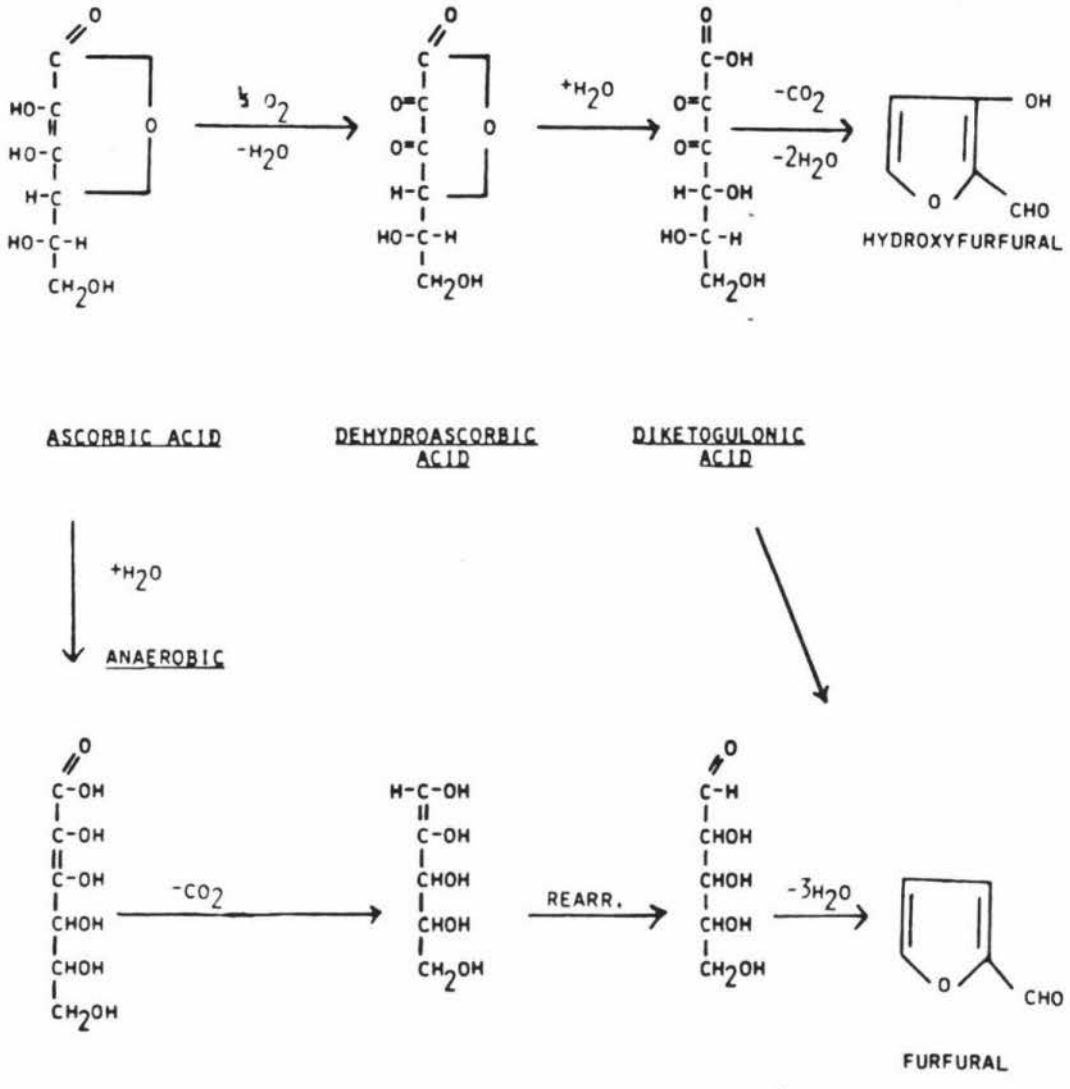

FIGURE 2.2: Possible ascorbic acid degradation pathways (From Bauernfiend and Pinkert, 1970).

the browning in the early stages. These reactive carbonyls form complexes with one another or combine with amino acids to yield brown pigments (Figure 2.1).

Both aerobic and anaerobic pathways may be operative in the presence of O_2, with oxidative pathways being dominant (Nagy, 1980). Storage studies (Kefford *et al.*, 1959; Nagy and Smoot, 1977), on the loss of vitamin C in canned, single-strength orange juice have shown an initial period (about 1-2 weeks) of rapid loss of ascorbic acid that was caused by the presence of free oxygen. After oxygen was consumed, ascorbic acid degraded anaerobically at rates lower than by the aerobic process.

2.2 Factors Affecting Ascorbic Acid Degradation and Nonenzymic Browning

2.2.1 Storage time and temperature

Loss of ascorbic acid and browning in citrus products have been shown to be related to storage time and temperature. Results of representative studies on orange, grapefruit and tangerine juices are presented in Table 2.1 as compiled by Nagy (1980).

Typical time-temperature profiles for ascorbic acid retention in canned single-strength juice are shown in Figure 2.3. As expected, the retention of ascorbic acid decreases as temperature and time of storage increase.

The effect of temperature on the rate of citrus juice reactions has been observed to follow the Arrhenius model (Equation 2-1 in Section 2.3.2.1) (Ross, 1944; Evenden and Marsh, 1948; Nagy and Smoot, 1977; Saguy *et al.*, 1978a; Smoot and Nagy, 1980). The Arrhenius relationship

Table 2.1: Studies on vitamin C retention in processed orange, tangerine, and grapefruit juices.[1]

Product[2]	storage		% retention of vitamin C	Source
	temp, °C	months		
SSOJ (canned)	9, 24, 37	12	94, 75, 17	Ross (1944)
SSOJ (canned)	10 to 26.5	24	95 to 50	Sheft *et al.* (1949)
SSOJ (canned)	4.5, 24.5	18	93, 60	Moore (1949)
SSOJ (canned)	1.7, 22.2, 37.8	12	100, 80, 5	Freed *et al.*/)1049)
SSOJ (bottled)	4.5, 24.5	18	89, 51	Moore (1949)
SSGJ (canned)	21	11	89	Lamb (1946)
SSGJ (canned)	10, 20, 30, 40, 50	3	99, 97, 90, 70, 29	Smooth and Nagy (1979)
SSGJ (canned)	10, 18, 27	18	93, 84, 62	Sheft *et d*. (1949)
SSGJ (canned)	23.9	12	83	Jones and Blanchard (1956)
FCOJ	-20, -15, -12.2	60	100, 100, 100	Kew (1957)
FCOJ	-22, -12, -7,0, 4	12	99, 98, 98, 97, 96	Huggart *et al*. (1954)
FCGJ	-22, -12, -7,0, 4	12	98, 98, 98, 98, 97	Huggart *et al*. (1954)
FCTJ	-22, -12, -7,0, 4,	12	94, 94, 91, 91, 90	Huggart *et al*. (1954)
FCTJ	-29, -18, -12, -4	3	100, 98, 95, 98	Marshall *et al.* (1955)

[1] From Nagy (1980)

[2] SSOJ = single strength orange juice; SSGJ = single-strength grapefruit juice; SSTJ = single-strength tangerine juice; FCOJ = frozen concentrated orange juice; FCGJ = frozen concentrated grapefruit juice; FCTJ = frozen concentrated tangerine juice.

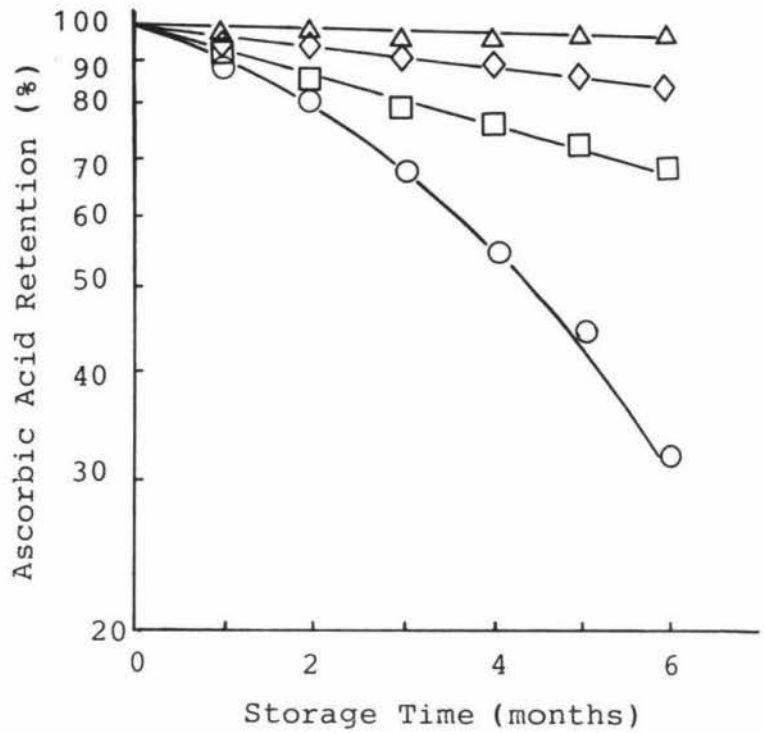

FIGURE 2.3: Percent vitamin C retention (logarithmic scale) vs. months of storage at 4°C (Δ), 24°C (◇), 32°C (□), and 37°C (O) for canned single-strength orange juice (From Nagy, 1980).

generally applies over a certain intermediate temperature range but deviations from this relationship can occur at high or low temperatures (McWeeny, 1968). The study of Nagy and Smoot (1977) and Kefford *et al*. (1959) concluded that plots between log (ascorbic acid retention) and storage time were linear for storage temperatures up to 30°C but the departures from this linear relationship were evident at temperatures above 30°C. Their data suggested two different reaction mechanisms predominating at the two temperature ranges.

Browning increases as temperature and time of storage increase (Huggart and Wenzel, 1955; Josyln, 1957; Kefford *et al* ., 1959; Mannheim and Passy, 1979). Figure 2.4 presents a typical plot of browning levels as measured by optical densities at 420 nm of grapefruit concentrate at different temperatures (Passy and Mannheim, 1979). At all storage temperatures a lag period was observed (demonstrated by a constant optical density) followed by a linear increase in optical density. The same trend was observed by Kanner *et al*. (1982) for orange juice concentrate and by Saguy *et al*. (1978b) during thermal and concentration processes of grapefruit juice.

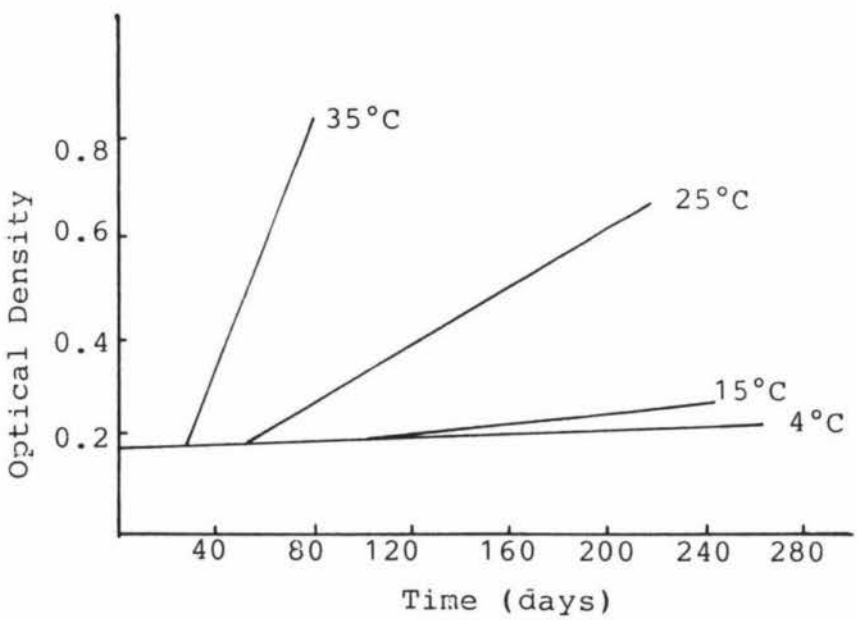

FIGURE 2.4: Changes in optical density of concentrated grapefruit juice (From Passy and Mannheim, 1979).

2.2.2 pH

The pH not only affects the rate of browning, but also determines the predominant mechanism of the process, as has been discussed earlier.

Clegg (1964) found that the degree of nonenzymic browning in lemon juice model systems was influenced by the pH value of the product and was maximal at pH 4.5. Khan and Martell (1967), in a study of the mechanism for the metal-catalyzed oxidation of ascorbic acid reported that in the pH range 2-5, ascorbic acid diminished with increasing pH. Huelin *et al.* (1971), however, found that the time for destruction of half of the ascorbic acid increased with increasing pH and reached a maximum at about pH 2.3. The time then decreased to pH 4 and increased again to pH 6. These results suggested that two reactions are concerned in the decomposition, one involving unionised ascorbic acid and catalysed by hydrogen ions, and the other involving both the unionised form and the monovalent ascorbate ion with an optimum pH of around 4.2. Thus, for a product such as lemon juice with a pH of 2.5, the former reaction occurs with furfural as a major product.

2.2.3 Soluble Solids Content

It is known that increasing the concentration of foods significantly enhances browning reactions (Labuza *et al.*, 1970).

Saguy *et al.* (1978b) reported that the extent of browning in grapefruit juice increased with solids content, with a steep change upward at concentrations above 50 °Brix. The ascorbic acid destruction rate as a function of solids concentration was generated based on another study they had conducted (Saguy *et al.*, 1978a) and the results are shown in Figure 2.5.

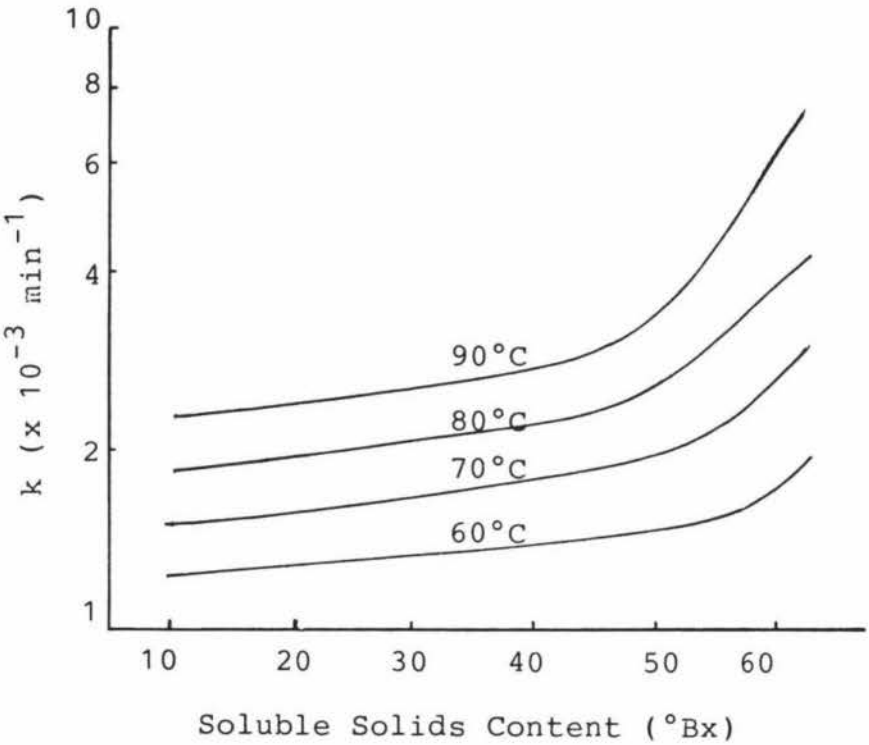

FIGURE 2.5: Effect of solids content on the rate of ascorbic acid degradation (predicted results) (From Saguy *et al.*, 1978a).

The figure indicates a sharp increase in the value of the destruction rate coefficient at high solids concentration. Similar results were obtained by Kanner *et al.* (1982) for orange juice concentrates.

The reason for such an effect is not clear. It has been attributed to the accelerating effect of fructose (Huelin, 1953) and to the increase in citric acid concentration which is known to have an important role in nonenzymic browning and ascorbic acid destruction (Lamden and Harris, 1950; Clegg, 1966).

2.2.4 Citric acid

Citric acid appeared to play an essential and characteristic role in the development of browning in the presence of ascorbic acid, irrespective of pH value, and could not be replaced to the same extent by other organic acids (Clegg, 1964). Further evidence was given by Clegg (1966) to show that the presence of citric acid made a positive contribution to the browning of acidic model systems containing ascorbic acid. Ascorbic acid itself was responsible for limited browning which was increased by the addition of citric acid, whereas no browning occurred in the citric acid systems in the absence of ascorbic acid.

Lamden and Harris (1950) reported that ascorbic acid-citric acid solutions, heated for three hours at 100°C showed an increase in browning and a decrease in ascorbic acid content with increasing concentration of the citric acid. A similar observation was made by Huggart *et al.* (1957) who reported that browning in stored cans of grapefruit sections was darkest in those to which citric acid had been added.

Clegg (1966) showed that citric acid neither acts as a catalyst nor yields reactive carbonyl compounds under aerobic incubation conditions. Thin-layer chromatographic separation of the brown pigments in different systems demonstrated a specific chromophore which occurred only when citric acid and ascorbic acid were both present. The presence of amino acids in ascorbic systems is the major contributor to the development of browning but it was suggested that citric acid, or a derivative, is incorporated into some of the brown pigments.

Contrary to this, Smoot and Nagy (1980) observed that values for pH and citric acid content of single-strength grapefruit juice samples stored at 50°C for 12 weeks showed no change during storage. No loss or breakdown of citric

acid during browning of an ascorbic acid - citric acid mixture was also reported earlier by Lamden and Harris (1950).

2.2.5 Oxygen

It is generally accepted that oxygen has a pronounced effect on the rate of browning in citrus products. Moore *et al.* (1942) showed that the rate of browning in pasteurised, bottled orange juice was correlated to the volume of air-filled headspace; as the volume of air was increased the rate of browning was also increased. It has also been reported that the presence of oxygen in the headspace of packages, dissolved in the product or occluded in its tissues is detrimental (Kefford *et al.*, 1950; Singh *et al.*, 1976).

However, it has been observed that removal of molecular oxygen did not prevent darkening or loss of ascorbic acid in orange juice (Joslyn and Marsh, 1935; Loeffler, 1941; Kefford *et al.*, 1959), or in ascorbic acid - citric acid model systems (Lamden and Harris, 1950).

Kefford *et al.* (1959) concluded that complete removal of oxygen from orange juice improves the retention of ascorbic acid and flavour during processing but has little effect on the retention of these quality factors during storage.

In order to determine the effect of the dissolved oxygen on the deterioration of grapefruit juice and concentrates, Saguy *et al.* (1978a) carried out heat treatment on de-aerated samples (2 h vacuum followed by nitrogen flushing) with a nitrogen atmosphere maintained in the heat processing unit. The additional treatment to remove oxygen had no effect on the general kinetic mechanism they had established previously for the ascorbic acid loss. Thus, it was indicated that destruction of ascorbic acid in grapefruit juice during concentration is dominated mainly by the anaerobic mechanism.

Similarly, Passy and Mannheim (1979) showed that the effect of oxygen on ascorbic acid degradation was minimal and the degradation during storage was mostly anaerobic. Anaerobic degradation was also observed by Huelin (1953).

2.3 Kinetics of Ascorbic Acid Degradation and Nonenzymic Browning

2.3.1 Reaction order

2.3.1.1 Ascorbic acid degradation

Many workers (Joslyn and Miller, 1949; Freed *et al.*, 1949; Huelin, 1953; Waletzko and Labuza, 1976; Lamden and Harris, 1950) have assumed that loss of ascorbic acid in citrus juice and intermediate aqueous systems is a first-order reaction in which log (ascorbic acid content) is linearly related to storage time.

Passy and Mannheim (1979) observed a lag period for the ascorbic acid retention in grapefruit concentrates. At the end of the lag period, the destruction of the vitamin was accelerated and could be expressed as a first-order reaction. A first-order reaction was also reported by Saguy *et al.* (1978a) during heat processing and concentration of grapefruit juice.

Kefford *et al.* (1959), however, showed that the relation between log (ascorbic acid content) and time was not linear, and when polynomial regression equations were fitted to their data, significant linear and quadratic functions of time were required to explain the degradation mode. Other studies (Smoot and Nagy, 1980; Nagy and Smoot, 1977) have provided evidence that the relation between log

ascorbic acid content and storage temperature at specific storage temperatures may not be linear.

Lin and Agalloco (1979) reported that the first-order rate equation of ascorbic acid degradation is valid only if the oxygen is present in abundance in solutions (greater than 8.5 mg/L) for aerobic degradation, or if it is totally excluded (for anaerobic degradation). In circumstances where oxygen is neither present in abundance nor totally excluded, but is present in a limited amount, then a second-order reaction may be followed (Singh *et al.*, 1976). This reaction depends on both the oxygen and ascorbic acid concentrations.

2.3.1.2 Nonenzymic browning

Saguy *et al* . (1978b) studied the kinetics of browning in grapefruit juice during thermal and concentration processes. The reaction was initially slow (lag period) and later relatively rapid (post lag period). The initial effect, also observed by Karel and Nickerson (1964), was referred to as the time during which colourless intermediates of the browning reaction are formed (Clegg and Morton, 1965). Curve fitting by Karel and Nickerson (1964) showed the browning curves in the lag period to be exponential rather than linear. Browning in the post-lag period was linear, apparently a zero-order reaction.

In 1979, a study by Passy and Mannheim gave similar results for grapefruit concentrate. At all storage temperatures a lag period was observed followed by a linear increase in optical density or darkening. The linear increase was also found to be a zero-order reaction.

2.3.2 Reaction rates

2.3.2.1 Ascorbic acid degradation

Evenden and Marsh (1948) and Freed *et al.* (1949) suggested that the rate of ascorbic acid loss in single-strength orange juice obeyed the Arrhenius equation:

$$k = A \exp(-E_a/RT) \qquad (2\text{-}1)$$

where: k = reaction rate constant
E_a = activation energy, J mol^{-1}
T = absolute temperature, K
R = gas constant, J mol^{-1} K^{-1}
A = frequency factor, $time^{-1}$

Thus, if log (reaction rate) were plotted versus the reciprocal of absolute temperature, a straight line would result.

Figure 2.6 shows plots of rate constants versus the reciprocal of absolute temperatures for canned single-strength grapefruit and orange juices (Nagy and Smoot, 1977; Smoot and Nagy, 1980).

The plot for grapefruit juice showed one linear profile for the temperature region 10-50°C. Regression analysis of the slope yielded an energy of activation (E_a) equal to 76.2 kJ mol^{-1} and also showed that a temperature rise of 10°C (Q_{10}) caused an increase in the reaction rate of about 2.7. The plot for orange juice, however, showed two distinct temperature regions. This indicated that a change in reaction kinetics occurred at a certain critical temperature (estimated at 28°C). The Arrhenius equation was obeyed within each of the two temperature regions but two different E_a's and Q_{10}'s were evident. For the region 4-28°C, regression analysis of the slope yielded an E_a value of 53.6 kJ mol^{-1} and a Q_{10} value of 2.2, whereas the region 28-50°C showed an E_a value of 102.5 kJ mol^{-1}

and a Q_{10} value of 3.7.

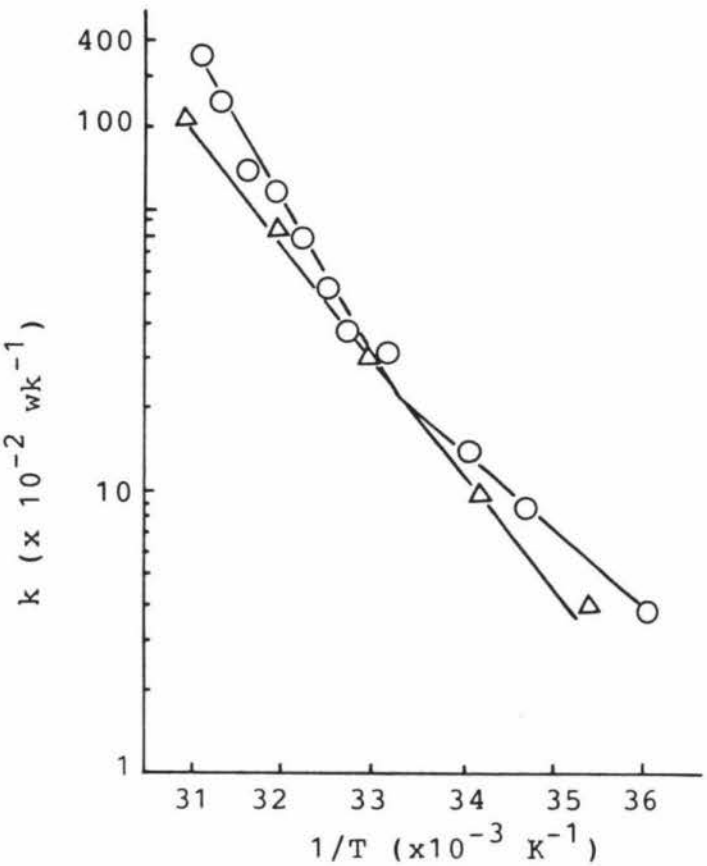

FIGURE 2.6: Arrhenius plots of log k (mg of vitamin C loss/100 mL of juice per week) vs. reciprocal of absolute storage temperature. The grapefruit (Δ) plot shows one linear profile, whereas the orange (O) plot shows two distinct profiles. The Arrhenius plots for orange indicate a change in kinetics around 28°C. (From Nagy, 1980).

A similar drastic increase in the rate of reaction at elevated temperatures was observed by Saguy *et al.* (1978a) for grapefruit juice. They reported a lower E_a ranging from 20.9 to 47.3 kJ mol^{-1} for solids contents of 11 to 62°Bx, respectively.

2.3.2.2 Nonenzymic browning

The effect of temperature on the rate of reaction of browning in grapefruit juice during thermal and concentration processes was found to obey the Arrhenius equation (Saguy *et al.*, 1978b). The activation energy, for a solids content of 11 to 62°Bx, ranged from 33.5 to 125.6 kJ mol^{-1} in the lag period and from 62.8 to 100.4 kJ mol^{-1} in the post-lag period.

For grapefruit concentrate, the calculated activation energies were 87.9 to 104.6 kJ mol^{-1} (Passy and Mannheim, 1979).

2.4 Sensory Quality of Citrus Juice During Storage

The sensory quality of citrus juice is known to deteriorate during storage at adverse temperatures for prolonged storage periods.

The contribution of lipid oxidative products to off-flavour development in citrus juice was reviewed by Nagy (1977). It is generally agreed that the contribution of the lipid oxidative products to the flavour deterioration of processed citrus products is relatively minor when compared to the contributions of flavouring oils and the products of non-enzymic browning (Tatum *et al.*, 1975; Maraulja *et al.*, 1973). The precise cause of flavour deterioration in citrus juice remains unclear at present.

Dinsmore and Nagy (1972) reported that the flavour change in orange juice stored at 5°C or below did not take place for approximately 1½ years and that juice stored at 30°C developed a significant flavour difference within a 2-week period.

For canned juice stored at 10°C, no significant flavour difference was observed within a 16-week storage period (Nagy and Randall, 1973). Flavour difference was detected with orange juice stored at 16, 21 and 30°C at the 10th, 8th and 2nd week, respectively.

The results of Kanner *et al*. (1982) for orange juice concentrate showed no statistically significant differences between 58°Brix concentrate stored at -18°C and those stored at 5, 12, and 17°C for 17, 10 and 8 months, respectively. After this period, off-flavour developed which was associated with a caramel-like taste. Their results suggest that flavour retention during storage is greater in concentrates compared to single-strength juice.

Johnson and Toledo (1975) reported that orange concentrate (55 °Brix) aseptically packaged in clear glass containers could be stored at 15°C for not more than 2 months without significant flavour changes.

A close correlation between furfural content and flavour changes had been demonstrated with grapefruit juice (Nagy *et al*., 1972; Maraulja *et al*., 1973) and orange juice (Nagy and Randall, 1973), but furfural *per se* did not have any flavour properties, even at a high concentration (Nagy and Randall, 1973).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Lemon juice

The lemons used in experiments 4 and 5 of this study were purchased from a Palmerston North fruit shop. Fruit that was not processed immediately was stored at 4°C.

Juice was extracted from the lemons using a domestic Kenwood mixer fitted with a reamer. The extracted juice was screened through a stainless steel sieve and two layers of cheese-cloth to separate the seeds and pulp from the juice. The juice was then deaerated in a vacuum chamber (-70 kpa), pasteurized in a plate heat exchanger at 80°C for 30 seconds and then rapidly cooled.

3.1.2 Calamansi juice

The calamansi fruits (*Citrus mitis*) were purchased in Manila, Philippines.

The fruit was soaked in 200 ppm chlorinated water for 5 minutes and then rinsed with water. They were then cut near the stem end to avoid cutting the seeds and manually squeezed (due to the small size ie.2-2.5cm diameter, of the fruit) to extract the juice. The juice was strained through a stainless steel sieve. Pasteurization was done at 80°C for 30 seconds. The juice was filled hot into 211 x 300 lacquered cans, seamed and cooled.

The cans were stored at 4°C for one week and then sent to New Zealand by air in an insulated container with ice. The cans were stored at 2°C until analysis of the juice

5 weeks later.

3.1.3 Lemon concentrate

Commercially pasteurized and frozen lemon juice was obtained from the Te Puke orchard of Schweppes (N.Z.) Ltd. The juice was thawed and then concentrated to 55 °Brix using an Alfa-Laval Model CTI B-2 centri-therm. The evaporation temperature used was 50°C and the primary steam temperature was 85°C. The feed was regulated to approximately 50 kg/h. Because a 55 °Brix concentration could not be attained in a single-pass, the concentrate was recirculated through the centri-therm until the desired concentration was reached.

3.2 Determination of Common Juice Parameters

3.2.1 Total soluble solids (TSS)

Total soluble solids were determined using an Abbe refractometer at room temperature (20°C) and expressed as degrees Brix (°Brix). The readings were corrected for acidity using the table in Appendix 1.

3.2.2 Titratable acidity

Juice (10 g) was diluted with 25 mL of distilled water and titrated with sodium hydroxide (.3125 N) to a pH of 8.2. Titratable acidity was calculated using the relationship, one mL of .3125 N NaOH = 0.2 g citric acid, results being expressed as g citric acid/100 g juice.

3.2.3 Brix : Acid ratio

The ratio was calculated by dividing the total soluble solids reading of the juice by the titratable acidity.

3.2.4 pH

The pH was determined using a pH meter calibrated with buffers at pH 4.0 and 7.0.

3.3 Determination of Ascorbic Acid

3.3.1 Background

The AOAC method (1980) for the determination of ascorbic acid content, using 2,6 dichlorophenol-indophenol, was followed except for the end-point determination. The end-point was determined based on the potentiometric method of Spaeth *et al.* (1962).

This method was selected because it has been reported to be more precise and reproducible than the colorimetric AOAC method, which is subject to error in end-point recognition particularly for coloured fruit juices (Nagy and Smoot, 1977).

The end-point was determined using an Orion Model 701A digital pH/mV meter (Orion Research Inc., USA) with a combination redox electrode.

To inhibit the interference of other reducing agents in the juice, the titration was performed at a pH range of 1-3 with the addition of metaphosphoric-acetic acid solution (HPO_3 - HOAc).

3.3.2 Procedure

3.3.2.1 Reagents

3.3.2.1.1 Metaphosphoric acid - acetic acid solution

Fifty grams of freshly pulverized HPO_3 stick were dissolved, with shaking, in 40 mL HOAc and 200 mL H_2O. It was diluted to 500 mL, and filtered through fluted paper into a glass bottle. (HPO_3 slowly changes to H_3PO_4; the solution can be stored under refrigeration for up to seven days).

3.3.2.1.2 Ascorbic acid standard solution

Fifty mg assayed ascorbic acid were accurately weighed and transferred to a 50 mL volumetric flask. It was diluted to volume immediately before use with the HPO_3 - HOAc solution described above.

3.3.2.1.3 Indophenol standard solution

Fifty mg 2,6-dichloroindophenol were dissolved into 50 ml H_2O and 42 mg $NaHCO_3$ with vigorous shaking. The solution was diluted to 200 mL with water and filtered through fluted paper into an amber glass bottle.

3.3.2.2 Sample preparation

The juice was mixed thoroughly by shaking to ensure a uniform sample. An aliquot of 100 mL of prepared juice was added to an equal volume of HPO_3 - HOAc.

3.3.2.3 Titration

An aliquot of sample containing approximately 2.0 mg ascorbic acid was added to a beaker containing 5 mL HPO_3 - HOAc solution. The sample was titrated rapidly against the indophenol standard until the end-point was reached.

Blanks were similarly titrated.

The indophenol was standardized against an ascorbic acid standard daily.

3.3.2.4 End-point determination

The approximate baseline potential was first determined. Approximately 70% of the dye required to reach the end-point was added. One ml aliquots of dye were then added and the potential was measured about 30 seconds after each addition until the end-point was passed. The baseline potential was the lowest mV reading obtained during the titration. The end-point was calculated to be 35 mV above the baseline potential. The standard and test solutions were titrated to ± 10 mV of this arbitrary end-point. Standard titration curves for ascorbic acid are given in Figure 3.1.

3.3.2.5 Calculation

Results were calculated based on the equation:

$$\text{mg ascorbic acid/ml sample} = (X-B) \times (F/E) \times (V/Y)$$

where:
- X = average mL for sample titration
- B = average mL for sample blank titration
- F = mg ascorbic acid equivalent to 1.0 mL indophenol solution
- E = number of mL assayed
- V = initial assay solution volume
- Y = volume of sample aliquot titrated

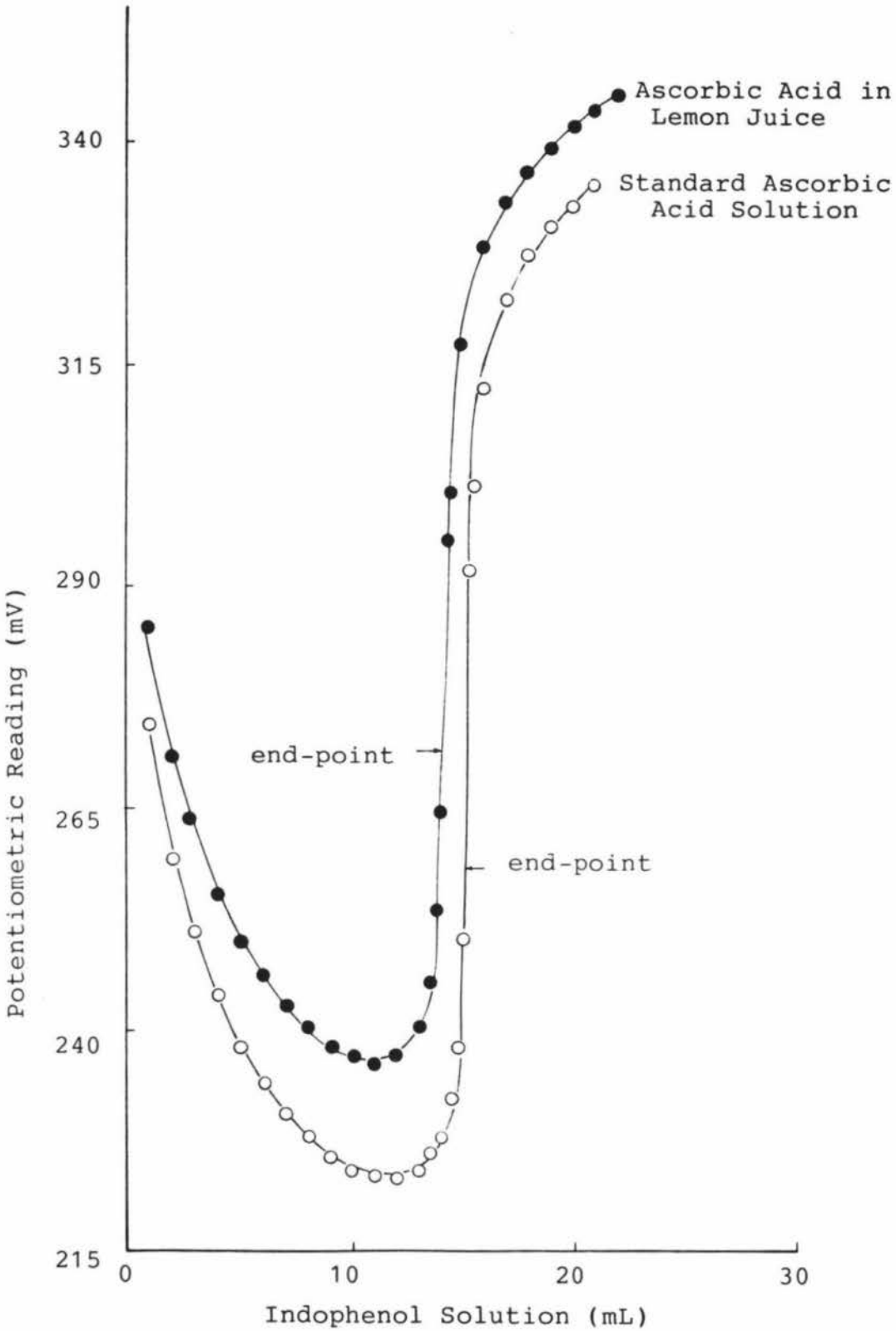

FIGURE 3.1: Standard curve for ascorbic acid.

3.3.3 Reliability of the method

The method was used to determine the ascorbic acid content of fresh lemon juice and the reliability of the method was calculated. The results are shown in Table 3.1.

TABLE 3.1: Ascorbic acid content of fresh lemon juice.

Sample No.	Ascorbic Acid (mg/100 mL)
1	45.34
2	45.34
3	45.22
4	45.34
5	45.46
6	45.22
Mean ± SD	45.32 ± 0.09
% precision	± 0.2%

3.4 Determination of Furfural

3.4.1 Background

Furfural has been recommended by several workers as an index of storage temperature abuse in commercially processed citrus juices (Dinsmore and Nagy, 1972; Maraulja *et al.*, 1973; Nagy and Randall, 1973).

Of the various known chemicals formed when juice deteriorates - peroxides, aldehydes, oxygenated terpenes and furfural - the last of these has been found to have the most satisfactory features for the desired analysis. The volatility of furfural is sufficiently high to permit its

rapid removal from juice by distillation or stripping procedures. High analytical sensitivity is available by means of direct ultraviolet spectrophotometry or by colorimetric determination (Dinsmore and Nagy, 1972).

The furfural content of fresh juice is very low, whereas large amounts have been recognized in temperature aged juice (Rymal *et al.*, 1968; Dinsmore and Nagy, 1971). A close correlation between furfural content and flavour changes has been demonstrated with grapefruit juice (Nagy *et al.*, 1972; Maraulja *et al.*, 1973) and orange juice (Nagy and Randall, 1973) but furfural *per se* did not have any flavour properties, even at a high concentration.

Furfural was determined using the improved method of Dinsmore and Nagy (1974), based on the aniline-acetic acid reaction with furfural. The measurement of absorbance at 515 nm is specific for furfural.

3.4.2 Procedure

3.4.2.1 Reagents

3.4.2.1.1 Purified aniline

At least 2 distillations in glass of aniline were done to obtain a near colourless product.

3.4.2.1.2 Stannous chloride stock solution (20%)

Two g of $SnCL_2 .2H_2O$ were dissolved in concentrated HCl (12N) to a volume of 10 mL.

3.4.2.1.3 Stannous chloride-aniline-acetic acid

One mL 20% $SnCl_2$ stock solution was added during dilution of 2 mL aniline with glacial acetic acid to 20 mL total volume.

3.4.2.2 Furfural standards

Furfural concentrations of 2, 1, and 0.5 µg/mL were obtained as calibration standards. For recovery studies samples containing 25, 50, 75 and 100 µg furfural / 200 mL were used.

3.4.2.3 Distillation

The distillation apparatus described by Scott and Veldhuis (1966) was used. Ten mL of distillate was collected from each 200 mL sample at a rate of 3 mL/min. Boiling chips and antifoam were added to the sample.

Remaining condensate in the condenser was removed after each sample analysis. The condenser was rinsed with water and methanol and dried after every use.

3.4.2.4 Colorimetry

Colour was developed in 2 mL distillate or calibration standard by adding 2 ml 95% ethanol and 1 mL aniline-acetic reagent. A vortex stirrer was used to mix the solution. Maximum colour development was obtained by 50 minutes standing. The absorbance was measured at 515 nm in Spectronic 70 (Bausch and Lomb, Inc.), using circular tubes with effective 1 cm light path.

Distillates containing greater than 2 µg furfural/mL required prior dilution with water, maintaining the ratio of distillate, ethanol and reagent (2:2:1).

A reference sample was used, in which 2 mL of distilled water was added instead of the juice distillate.

3.4.2.5 Calculation

Results were calculated using the equation:

$$\text{ppb furfural} \cong \mu\text{g furfural/L juice} = (25/RE) \times (A \text{ sample}/A \text{ cal})$$

where: RE = recovery = 35%
A sample = absorbance of sample
A cal = absorbance/µg furfural in final 5 mL colour reaction mixture = 0.142

3.4.3 Reliability of the method

The method was used to determine the furfural content of fresh lemon juice and the reliability of the method was calculated. The results are given in Table 3.2.

TABLE 3.2: Furfural content of fresh lemon juice.

Sample No.	Furfural (µg/L)
1	57.85
2	62.88
3	59.36
4	57.85
5	57.85
6	60.36
Mean ± SD	59.36 ± 2.01
% precision	± 3.39%

3.5 Determination of Hydroxymethylfurfural (HMF)

3.5.1 Background

Another chemical aside from furfural, which develops in the browning process of citrus products at levels easily detectable by chemical methods is 5-hydroxymethylfurfural (HMF). It has been proposed as an index of quality deterioration in citrus juice by Meydav and Berk (1978).

The improved colorimetric method of Meydav and Berk (1978) used in this study is based in the thiobarbituric acid (TBA) colour reaction with HMF. A colour complex stable for up to 60 min is formed in the reaction mixture. This method offers some advantages in repeatability and convenience of HMF determination, in comparison to previously reported colorimetric or TLC procedures (Berry and Tatum, 1965).

3.5.2 Procedure

3.5.2.1 Clarification

The lead acetate clarification procedure was as follows: 25 mL of single-strength juice, followed by 4 mL of $Pb(OAc)_2$ solution, was pipetted into a 50 mL volumetric flask, the volume made up with distilled water, and shaken. The precipitated juice was vacuum filtered in a Buchner funnel through two layers of Whatman No.1 filter paper. Excess $Pb(OAc)_2$ was precipitated from the clear filtrate by adding sodium oxalate crystals and filtered as above. This step was repeated once or twice for complete removal of the precipitating agent. Thorough elimination of $Pb(OAc)_2$ from the filtrate was necessary to prevent haziness during the subsequent colorimetric reaction with TBA.

3.5.2.2 Colorimetry

Two mL of clarified juice were thoroughly mixed by a vortex mixer with 2 mL of 40% w/v trichloroacetic acid (TCA) solution and 1 mL of 0.05 M thiobarbituric acid (TBA) in screw-capped test tubes. The reaction was carried out at 40 ± 0.2°C for 50 min. Then the test tubes were cooled for about 5 min in running tap water, and the colorimetric measurements were made as soon as possible.

The absorbance of all samples was measured at the 443 nm peak wavelength using a Spectronic 70 (Bausch and Lomb, Inc.).

Reference samples, in which 1 mL of distilled water was added instead of the clarified sample, were treated in the same manner.

3.5.2.3 Calculation

Results were calculated using the equation:

$$\text{HMF (mg/L)} = 16.7 \times \text{absorbance at 443 nm}$$

3.5.3 Reliability of the method

The method was used to determine the HMF content of fresh lemon juice and the reliability of the method was calculated. The results are given in Table 3.3.

TABLE 3.3: HMF content of fresh lemon juice.

Sample No.	HMF (mg/L)
1	1.30
2	1.30
3	1.25
4	1.32
5	1.30
6	1.14
Mean ± SD	1.27 ± 0.07
% precision	± 5.5%

3.6 Browning Measurements

3.6.1 Spectrophotometric method

3.6.1.1 Background

Widely used clarification methods in citrus juices and citrus products were based upon the dilution of single-strength juice with an equal volume of acetone (Curl, 1949; Joslyn, 1957) or alcohol (Joslyn, 1957; Karel and Nickerson 1964). These methods were reported to suffer from accuracy drawbacks, especially while measuring minor differences, resulting from various processing treatment of juices and concentrates. The main inaccuracy and scattering of results were caused by carotenoid interference (Meydav *et al.*, 1977).

A modified clarification procedure was proposed by Meydav *et al.* (1977), which eliminated this interference. Browning

indices obtained by this procedure were reported to be almost identical with results derived by laborious and time-consuming Millipore filtering method of Karel and Nickerson (1964). Furthermore, the procedure was found suitable for routine laboratory analysis and its repeatability as expressed by the standard deviation was significantly better, in comparison to the widely applied acetone clarification procedure (Meydav *et al.*, 1977).

3.6.1.2 Procedure

Single strength citrus juice was centrifuged at 800 g for 20 min to remove pulp and coarse cloud particles. The supernatant was diluted 1:1 with 95% alcohol and filtered through Whatman No. 42 filter paper, to obtain a fully clarified extract. Absorbance was measured at 420 nm wavelength using a Spectronic 70 (Bausch and Lomb, Inc.).

3.6.1.3 Reliability of the method

The method was used to measure browning in fresh lemon juice and the reliability of the method calculated. Results are given in Table 3.4.

TABLE 3.4: Browning index of fresh lemon juice.

Sample No.	Absorbance (420 nm)
1	0.080
2	0.090
3	0.085
4	0.085
5	0.085
6	0.080
7	0.085
8	0.083
9	0.083
10	0.090
Mean ± SD	0.085 ± 0.003
% precision	± 3.5%

3.6.2 Measurement with the use of the Neotec Du-Colorimeter

3.6.2.1 Background

Colour changes have traditionally been evaluated by direct visual inspection of the product, the colour grades being determined by comparing the experimental samples with a series of standard colour samples. More recently, other types of instrumental methods of colour measurement have been available (Hidalgo *et al.*, 1974).

The principles of tristimulus colorimetry were applied in a modified form when the Hunter Citrus Colorimeter was developed, principally for the measurement of citrus juice colour (Hunter, 1967). Values obtained were reported to be highly correlated with visual evaluation of colour.

The instrument used for this present study was a Du-Color Model 220 Colour Difference Meter (Neotec Corporation, Rockville, Maryland, U.S.A.) fitted with an illuminant C light source. It is a photoelectric instrument that measures and displays the absolute reflectance of the sample in CIE (Commission Internationale de l'Eclairage) tristimulus values (X, Y, Z). These three values define the colour of the sample, specifically the amount of red (X), yellow (Y) and blue (Z) in the sample.

The CIE tristimulus values as measured by this instrument were found to be highly correlated with the browning index of orange juices which had been subjected to storage temperature abuse (Robertson and Reeves, 1981). However, when the tristimulus values were converted to USDA colour scores, the level of correlation between the colour scores and browning index was too low to be useful for prediction purposes.

3.6.2.2 Procedure

The juice was filtered and clarified as in Section 3.6.1.2 prior to colorimetric measurement. These steps were thought to be necessary to avoid any errors due to the solids in the juice. Separation in the juice was observed, with the solids precipitating, during accelerated temperature storage (55°C). The precipitate appeared to become coarser as storage time increased. The particle size and distribution of solids in a sample affects both light scattering and selective absorption and hence, affects colorimetric measurement (Francis and Clydesdale, 1975).

A 30 mL sample was used for all measurements. The sample was placed in a covered, optically-clear sample holder, and a white teflon backing block fixed inside the sample cover (Neotec accessories C15 and C16) was in contact with the surface of the sample, thereby giving constant sample geometry. The tristimulus values were then read.

3.6.2.3 Reliability of the Method

The method was used to measure browning in fresh lemon juice and the reliability of the method was calculated. The results are given in Table 3.5.

TABLE 3.5: Browning measurements of fresh lemon juice.

Sample No.	Neotec - Z Readings
1	46.3
2	44.0
3	46.7
4	48.0
5	45.0
6	47.1
Mean ± SD	46.18 ± 1.46
% precision	± 3.16%

3.7 Determination of Dissolved Oxygen

3.7.1 Background

Dissolved oxygen was measured using a YSI model 54 ARC dissolved oxygen meter (Yellow Springs Instrument Co., Inc., Ohio, USA). The meter is intended for dissolved oxygen and temperature measurement in water and wastewater applications, but is also suitable for use in certain other liquids. Dissolved oxygen is indicated by a mg/L scale. The probe used was a YSI 5739 dissolved oxygen probe.

The "saturated water" method was used to calibrate the instrument for dissolved oxygen measurement (instruction manual for YSI 54 ARC D.O. meter). The meter was calibrated for temperature measurement against a mercury thermometer.

The probe was fitted with a rubber stopper that fitted the mouth of the Erlemeyer flasks used as juice containers.

3.7.2 Procedure

The probe was calibrated and the instrument zeroed before every use.

The probe was inserted into the 250 ml flask filled with lemon juice until the rubber stopper made a firm seal with the neck of the flask. Stirring at a constant speed in the flask was accomplished using a magnetic stirrer. A few minutes were allowed for the probe to stabilize to the sample temperature and dissolved oxygen. Dissolved oxygen content was then read in mg/L.

3.7.3 Reliability of the method

Samples of air saturated distilled water and lemon juice were measured for dissolved oxygen at 35°C, and the reliability of the method was calculated. The results are given in Table 3.6.

TABLE 3.6: Dissolved oxygen content (mg/L) of distilled water and lemon juice.

Sample No.	Distilled Water	Lemon Juice
1	6.45	4.25
2	6.40	4.10
3	6.80	3.75
4	7.10	3.60
5	7.20	3.80
6	6.85	4.15
Mean ± SD	6.80 ± 0.33	3.94 ± 0.26
% precision	± 4.6%	± 6.6%

3.8 Determination of Citric Acid

3.8.1 Background

Citric acid was determined by high performance liquid chromatographic (HPLC) method. The equipment used was a Water Associates Model ALC/GPC 244 liquid chromatograph with a Model 6000A solvent delivery system and a U6K septumless injector (Waters Associates, Inc., Milford, Massachusetts, U.S.A.). A μ Bondapak C_{18} reverse-phase column (4.0 mm ID x 250 mm, Bio-sil ODS-10, Bio-Rad Laboratories, Richmond, California, U.S.A.) was used. The detector was a Model R401 differential refractometer (Waters Associates). The response was recorded on a CR650 flat bed chart recorder (J.J. Lloyd Instruments Ltd, Southampton, England).

Analyses were conducted at ambient temperature (20°C). The solvent system was 2% (w/v) potassium dihydrogen orthophosphate prepared using deionized glass-distilled water and adjusted to pH 2.4 using orthophosphoric acid

(according to Coppola *et al*., 1978). The solvent flow rate was 1.3 mL/min.

3.8.2 Procedure

Single-strength lemon juice was diluted with distilled water (1:5). The diluted juice was then filtered through Whatman No. 4 filter paper to remove coarse pulp particles. Two mL of filtrate were further filtered through a micro-filter. Fifty µL of sample was injected into the chromatograph.

The amount of citric acid present was calculated by measuring the peak height of acid with reference to peak height of the standard curve. The standard curve was linear up to 10 g/L concentration (50 µL injected volume). Such a standard curve is shown in Figure 3.2.

3.9 Sensory Evaluation

A descriptive scoring method was used to evaluate the flavour of the samples. The specific parameters evaluated were the presence of fresh lemon flavour and the presence of off-flavour in the different lemonade samples (formulation for lemonade given in Section 3.9.3.1).

The method of magnitude estimation was used to estimate the degree or level of the brown colour in the stored lemon juice.

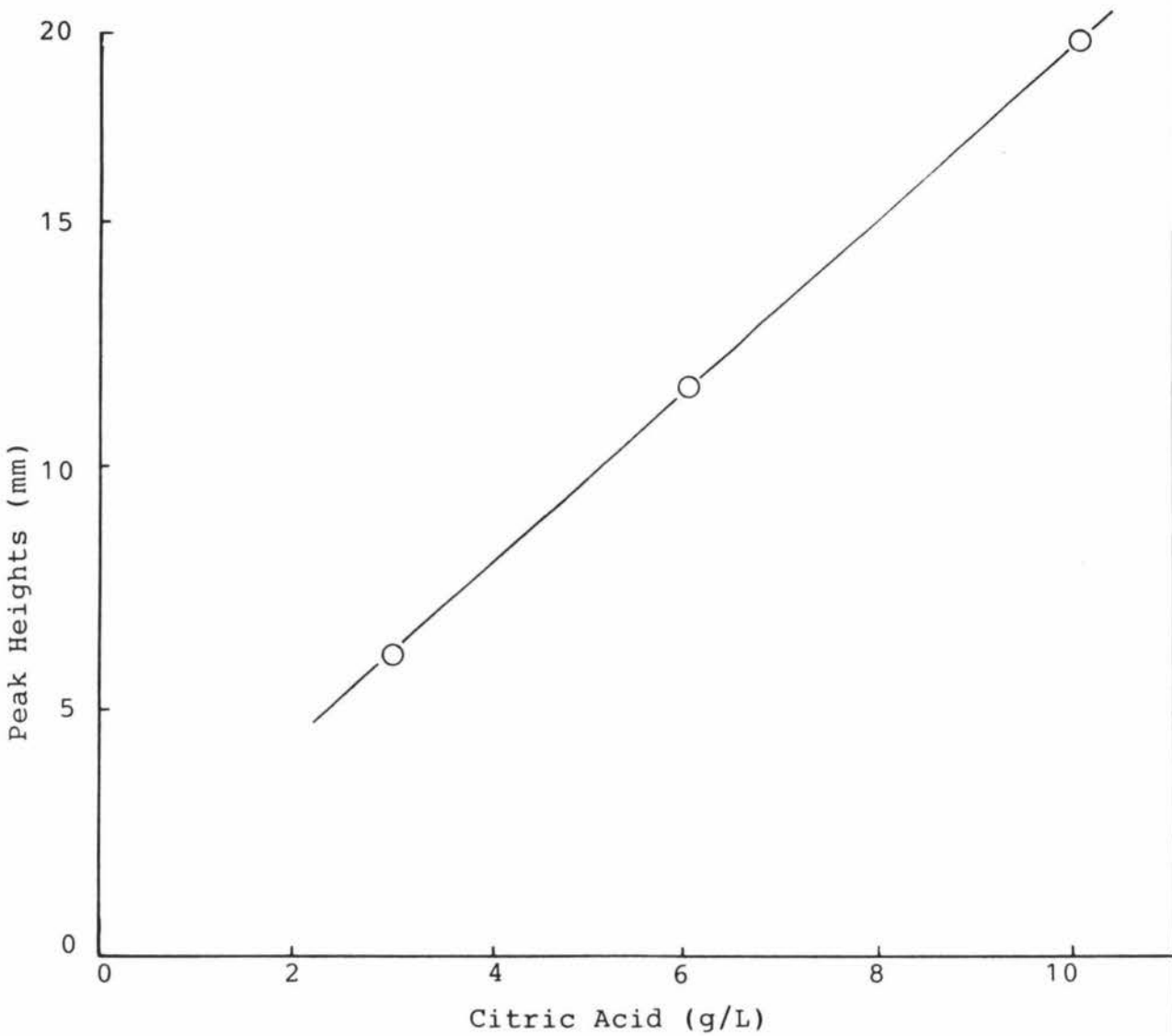

FIGURE 3.2: Standard curve for citric acid.

3.9.1 Background of the magnitude estimation method

Magnitude estimation was developed in the 1950s by S.S. Stevens at Harvard University in the Psychoacoustic Laboratory to assess the relation between physical intensity and the perceived magnitude of brightness and loudness.

In its simplest form, the method allows panelists to assign numbers to sensory/attitudinal stimuli, without restriction, so that the ratios of the numerical assignments reflect ratios of sensory perceptions or of attitudinal levels (Moskowitz, 1977).

One of the main benefits of this method is the data which the procedure gives the experimenter. It provides a feasible method for assigning numbers to food attributes. Statistical analyses of these numbers can be carried out using techniques, such as analysis of variance and regression analysis, with much greater confidence than can be carried out with the assumption that the scale is linear, a fact which is seldom true of sensory continua (Cooper, 1981).

Magnitude estimation has the advantage that with it, the functional relationships can be developed between objective and subjective measurements.

3.9.2 Background of the descriptive scoring method

Among the different methods of sensory evaluation, the descriptive scoring and scaling method is considered as the one most frequently utilized because of "its diversity, apparent simplicity and ease of statistical analysis" (Pangborn, 1967). The method requires the use of a scale or score which corresponds to certain descriptions of, or reactions to, a given product attribute. This involves the development of a vocabulary describing varying intensities of a sensory stimulus (Gatchalian, 1981).

In this study, a verbal scale describing varying intensities of the fresh lemon flavour and off-flavour was used (refer to Appendix 2A and 2B for the scale). The specificity of the attribute to be evaluated and the simplicity of the scale makes it easier for the panelists to relate their perception of the intensity of a certain stimulus to the scale. The use of common descriptive terms such as "recognizable" and "pronounced" makes it more possible to attain a common understanding of the terms among the panelists. It should be pointed out that evaluation was not based on preference but rather on an 'objective' assessment of product characteristics.

Numerical values or scores were assigned to the verbal scale for purposes of statistical analyses. A 5-point, equal interval numerical scale was established with "5" corresponding to a favourable response and "1" corresponding to an unfavourable response towards the attribute being evaluated.

Statistical analyses of data from scoring or scaling are based on the assumptions that scores are normally distributed, categories are equally spaced, and scores are proportional to the variation in the attribute being measured. When these assumptions are not fulfilled, there is a possibility of inaccurate statements concerning probability of significance in F-test and correlation analysis (Cloninger *et al.*, 1976).

Despite the disadvantage of a possible non-linearity of the scores assigned, this method was thought suitable for flavour evaluation, to meet the objectives of the study.

3.9.3 Experimental conditions and procedure

3.9.3.1 Samples

For the colour evaluation, single-strength lemon juice samples were placed in clear, 100 mL glass bottles.

Lemonade samples for the flavour evaluation were prepared based on the following formulation:

single-strength juice	11.5%
distilled water	79.5%
sugar	9.0%
Total	100%

The lemonade had a total soluble solids of approximately 10 °Brix.

The formulation was developed based on an informal evaluation of lemonade samples by a group of six persons. Two sets of five different lemonade formulations were presented to the group in two different days (total of 10 formulations). From the 10 samples, two of the most preferred formulations were further evaluated. The lemonade prepared using the above formulation was considered by the group to have the right balance of sugar and acid.

Fifty mL samples were served in 100 mL amber glasses for flavour evaluation.

3.9.3.2 Panelists

The sensory panel consisted of 15 students and staff members from the Faculty of Technology. The panelists were given a trial session prior to actual testing. Score cards were used to explain the magnitude estimation method (Moskowitz, 1977) and lemon juice with instrumentally determined degree of brownness were used for the trial session.

The different degrees of brownness in the lemon juice were achieved by mixing different proportions of fresh lemon juice and darkened lemon juice (temperature-abused). The browning in the lemon juice samples was measured using absorbance at 420 nm prior to sensory evaluation.

Ten to twelve panelists were used for each trial, depending on their availability.

3.9.3.3 Panel facility

Sensory evaluation was conducted in a sensory room equipped with individual testing booths, under full lighting conditions.

3.9.3.4 Time and temperature

All samples for the flavour evaluation were served at 15°C. Testing was done in the afternoon between 2:00 p.m. and 4:30 p.m.

3.9.3.5 Procedure

3.9.3.5.1 Descriptive scoring — Flavour evaluation

All samples were coded with 3-digit random numbers. They were placed randomly on a tray with a glass of water and a scoresheet, and presented to the panelists. The samples were evaluated for the intensity of the fresh lemon flavour and any off-flavour. The panelists recorded their judgements on a 5-point structured scale.

An example of the scoresheet used, showing the instruction, the verbal scale and the corresponding scores, is given in Appendix 2A.

3.9.3.5.2 Magnitude estimation - Colour evaluation

Samples were coded with 3-digit random numbers and presented one at a time in a random order. The scoresheet used is given in Appendix 2B.

Each panelist evaluated the first sample and assigned it any number. Numbers were then assigned to the remaining samples such that the ratios among the numbers reflected the ratios of the perceived magnitudes. The succeeding samples were evaluated and assigned numbers with respect to the number given the first sample, (i.e. if sample A was assigned 10 for brownness, and the second sample was seen as 5 times as brown, then the second sample was assigned a value of 50. If the sample was half as brown as the first sample, then it was assigned a value of 5).

3.9.4 Data interpretation

All magnitude estimation data were normalized prior to statistical analyses. Normalization refers to the method used to bring into common the scales that different individuals use (Moskowitz, 1977). The ratings of each panelist were transformed with the use of a multiplier so that the geometric mean for each panelist across all samples was a constant, (i.e. $\sqrt[6]{100}$).

$$\text{multiplier} = \frac{\sqrt[6]{100}}{\sqrt[6]{(a)(b)(c)(d)(e)(f)}}$$

where a - f = individual colour ratings of a panelist

By doing this, the size of numbers across all panelists were kept approximately equal. Although the absolute values of all the numbers were changed, the ratios among numbers remained unchanged.

An analysis of variance was performed to determine if significant differences existed among the samples for both flavour and colour evaluations. The Duncan's multiple range test (Gatchalian, 1981) was employed for significant results.

CHAPTER 4

PRELIMINARY EXPERIMENTS

This chapter consists of four experiments on the quality changes in lemon and calamansi juices during accelerated temperature storage at 55°C.

4.1 Quality Changes in Lemon Juice During Accelerated Temperature Storage (55°C): Objective Parameters

4.1.1 Introduction

The aim of this experiment was to observe quality deterioration in lemon juice and to determine the suitability of the different quality parameters chosen as indicators or measures of quality change. The samples were analyzed for ascorbic acid retention, browning (measured using absorbance at 420 nm and the Neotec Du-Colorimeter), and two chemical indices of quality deterioration in citrus juice, namely hydroxymethylfurfural (HMF) and furfural. It was the intention to use these quality parameters for the preliminary experiments and from the results select the best methods to use for the subsequent storage studies.

The juice was held at 55°C to accelerate the deteriorative reactions and thus obtain data on quality deterioration in a relatively short period of time.

4.1.2 Experimental

Pasteurized single-strength lemon juice in stoppered Erlenmeyer flasks was stored in a 55°C incubator. A headspace (5 cm) existed in the flasks. Sampling and analysis of

the juice were done every 11 hours up to 44 hours. Samples were analyzed for ascorbic acid retention, browning (using absorbance at 420 nm and the Neotec Du-Colorimeter), HMF and furfural using the methods described previously in Sections 3.3 to 3.6.

4.1.3 Results and discussion

The results of the analyses of the lemon juice samples are presented in Table 4.1 and shown in Figure 4.1. The values are the averages of six replicates. The lemon juice at zero time had a pH of 2.60, total soluble solids of 9.14 °Brix, titratable acidity of 6.27 mg/100 mL citric acid and 38.24 mg/100 mL ascorbic acid.

TABLE 4.1: Quality changes in lemon juice during storage (55°C).

Time (Hours)	Ascorbic Acid (%)	Browning (abs. at 420 nm)	Browning (Neotec)			HMF (mg/L)	Furfural (mg/L)
			Y	X	Z		
0	100.00	0.105	44.10	41.62	45.94	1.29	0.06
11	90.79	0.105	44.72	42.32	46.26	2.02	0.23
22	83.16	0.110	43.92	41.38	44.66	2.59	0.54
33	71.55	0.132	43.18	40.58	42.40	3.49	1.18
44	26.57	0.152	43.52	41.18	41.00	4.49	1.72

Linear correlation analyses were performed to measure relationships among the different parameters. The correlation coefficients are given in Table 4.2. The statistical significance of the correlation coefficients was determined by reference to Appendix 3. It should be pointed out that a high correlation coefficient does not necessarily mean that there is a cause and effect relationship between the two parameters; it only means that some statistical correlations exists.

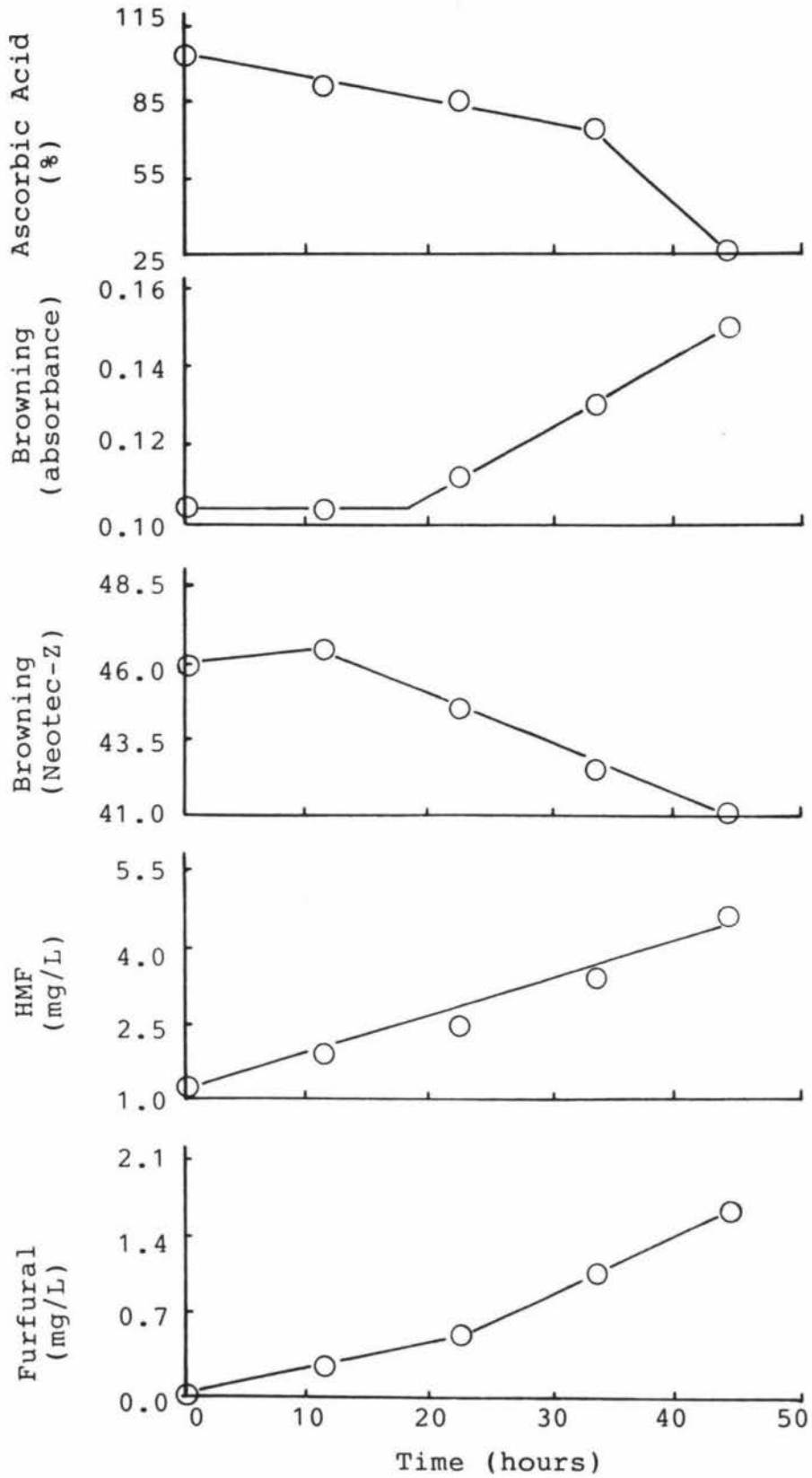

FIGURE 4.1: Quality changes in lemon juice during storage (55°C).

TABLE 4.2: Correlation coefficients among the quality parameters of lemon juice.

	Time	Ascorbic Acid	Browning (Absorbance)	Browning (Neotec) Y	X	Z	HMF
Ascorbic Acid	-0.915						
Browning (absorb-ance)	0.924	-0.960					
Browning Neotec Y	-0.728*	0.579*	-0.747*				
X	-0.652*	0.458*	-0.643*	0.989			
Z	-0.951	0.917	-0.978	0.850*	0.769*		
HMF	0.995	-0.947	0.954	-0.711*	-0.623*	-0.962	
Furfural	0.975	-0.952	0.985	-0.768*	-0.677*	-0.989	0.989

* Not significant at p = 0.05

Ascorbic acid content decreased with storage time at 55°C. After 44 hours storage, a 73.43% destruction of ascorbic acid had occurred. Up to 33 hours storage, ascorbic acid loss seemed to follow a linear trend, after which there was an observed rapid loss of ascorbic acid (Figure 4.1). Kanner *et al.* (1982) had observed accelerated ascorbic acid loss during long storage of orange juice concentrate (58 °Brix) at 37°C (150 days storage). This observation was attributed to the many breakdown products developed from juice constituents during the storage period, which seem to affect and accelerate the degradation of ascorbic acid. At a high temperature such as 55°C, reactions leading to the formation of these breakdown products are highly probable.

A significant correlation coefficient between ascorbic acid retention and time of $r = -0.915$ ($r^2 = 0.837$) was obtained. This means that 83.7% of the variations in values of y (ascorbic acid) can be explained in terms of values of x (time), and that 1 - .837, or 16.3% of the variations in y are not associated with x, but with other factors, or with error. However, a statistically significant correlation may not necessarily be adequate for accurate prediction purposes.

Among the CIE readings of Y, X, and Z using the Neotec Du-Colorimeter, only the Z value gave a significant correlation coefficient with the other parameters.

The browning reaction as measured by the Neotec-Z values and the absorbance readings (420 nm) was seen to proceed slowly initially (lag period) and later relatively rapidly (postlag period). No change in browning was observed for the first 11 hours. The initial effect observed by Karel and Nickerson (1964) and Clegg and Morton (1965) was referred to as the time during which colourless intermediates of the browning reaction formed. Change in the browning

index was observed after 10% of the ascorbic acid had disappeared. This agrees with the report of Passy and Mannheim (1979) who observed browning in citrus juices only after 10-15% of the ascorbic acid had been degraded.

The correlation coefficient for ascorbic acid and the browning measurement (absorbance at 420 nm) was r = -0.960, while for ascorbic acid and the Neotec-Z values it was r = 0.917. Although it cannot be concluded (based on the correlation coefficient alone) that browning at a low pH is a result of ascorbic acid degradation, the significant correlation coefficients obtained tend to support this conclusion.

Furfural and HMF, which are both products of ascorbic acid degradation, increased with storage time and a decrease in ascorbic acid. Considering that ascorbic acid loss and the browning reaction are measures of quality deterioration in lemon juice, the significant correlation coefficients obtained between these paramaters and furfural, and HMF confirms the reports of several authors (Dinsmore and Nagy, 1972; Nagy and Randall, 1973; Maraulja *et al.*, 1973; Meydav and Berk, 1978) that furfural and HMF may be used as chemical indices of quality loss in citrus juice. Furfural had slightly higher correlation coefficients with ascorbic acid retention and browning than HMF formation.

4.1.4 Conclusion

From the data gathered, it was observed that all the parameters measured had a time function. The significant correlation coefficients obtained among the different parameters confirms their suitability as indicators or measures of quality deterioration during storage of lemon juice. Based on the correlation coefficients, furfural appears to be a better index of quality deterioration than HMF.

However, in circumstances like this where the parameters are all highly correlated with one another, the choice of a method (between the two browning methods) or an index (between furfural and HMF) may be made based on other factors such as simplicity, labour and time requirements, and reliability.

4.2 Quality Changes in Lemon Juice During Accelerated Temperature Storage (55°C): Sensory Parameters

4.2.1 Introduction

In the estimation of shelf-life, the basic problem is to correlate measurable objective tests with sensory analyses (Labuza and Kamman, 1983). Since sensory testing may be a costly and time consuming procedure, some simple objective tests may be the only way in which a change in shelf-life can be measured.

This experiment was thus undertaken to determine the relationship between the instrumental measurement of browning and the sensory evaluation scores for the brown colour in lemon juice, and to determine flavour changes during accelerated storage at 55°C.

4.2.2 Experimental

A different batch of lemon juice samples were exposed to the same storage conditions as in experiment 4.1. Pasteurized single-strength lemon juice in stoppered flasks were stored at 55°C. Sampling for analysis was done every 11 hours up to 44 hours.

The degree of browning in the samples was determined using the spectrophotometric method. Based on the results of experiment 4.1, both instrumental methods of browning measurement correlated significantly with the other quality parameters. It was decided to use the spectrophotometric method (absorbance at 420 nm) rather than use the Neotec Du-Colorimeter for this experiment, because the former was more simple to conduct. Aside from this, browning in the samples used for the sensory trial session had been measured using absorbance in 420 nm, as mentioned in Section 3.9.3.

For the sensory evaluation of the brown colour in lemon juice, the magnitude estimation method was employed while for the flavour evaluation, the descriptive scoring method was used. Sensory evaluation was conducted under the conditions given in Section 3.9.3 and following the procedure given in the same section.

4.2.3 Results and discussion

The results of the sensory evaluation and the spectrophotometric browning measurements are given in Table 4.3. The sensory scores are averages of values obtained from 12 panelists. Analysis of variance was done to determine if significant differences existed among the samples (Appendix 4). The correlation coefficients among the different parameters are given in Table 4.4.

Significant differences in fresh lemon flavour and off-flavour were detected after 44 hours storage. At this point, the mean score for the presence of the fresh lemon flavour corresponded to a 'slightly noticeable' level. There was a loss of the "sharp" and "clean" flavour which the panelists considered characteristic of fresh lemon juice. The off-flavour score after 44 hours storage corresponded to a 'recognizable' level of the presence of a stored or stale flavour.

TABLE 4.3: Changes in flavour and colour of lemon juice during storage at 55°C.[1]

Time (hours)	Lemon Flavour	Off-Flavour	Brown Colour	Browning (abs. at 420nm)
0	3.33 [a]	4.75 [a]	1.75 [a]	0.118
11	3.17 [a]	4.83 [a]	1.82 [a]	0.129
22	3.08 [a]	4.25 [a]	2.17 [a]	0.142
33	2.75 [a]	4.08 [a]	3.36 [b]	0.174
44	2.08 [b]	3.25 [b]	5.16 [c]	0.208

[1] Means followed by a common letter are not significantly different at p = 0.05.

TABLE 4.4: Correlation coefficients among the quality parameters of lemon juice.[1]

	Time	Lemon Flavour	Off-flavour	Browning (sen. eval.)
Lemon Flavour	-0.931			
Off-Flavour	-0.933	0.963		
Browning (sen. eval.)	0.916	-0.994	-0.967	
Browning (absorbance)	0.970	-0.985	-0.964	0.985

[1] All correlation coefficients are significant at p = 0.05.

The flavour scores seemed to decrease with storage time (which meant a deterioration in flavour) and with an increase in browning. Both flavour scores gave high correlation coefficients with browning as measured by instrumental and sensory methods. This may indicate that flavour deterioration occurs following the same trend as browning, although the two reactions may not be related to each other.

A significant difference in the brown colour was detected after 33 hours. This coincided with the increase in the rate of the browning reaction as measured using absorbance at 420 nm, after an observed initial lag period (Figure 4.2). A correlation coefficient of $r = 0.985$ was obtained between the two browning measurements. This suggests that the absorbance readings give a good measurement of the brown colour as actually perceived by the panelists. Intensity responses such as the ones made by the panelists for colour evaluation have been reported by Trant *et al*. (1981) to be positively correlated ($p < 0.001$) with corresponding instrumental analysis. In their study, perceived intensities of the orange colour of orange juice were observed to be highly correlated with Hunter *a* values.

Assuming that the same reactions occurred in experiments 4.1 and 4.2, a significant change in colour of the lemon juice was perceived by the panelists after approximately 28.5% of the ascorbic acid had disappeared. Development of off-flavour was detected after 73% ascorbic acid loss. However, no firm conclusions can be drawn from these results since different lemon juice samples were used for the two experiments.

The time of storage at which a significant change in colour is detected may not be the same as the time of storage when the colour becomes unacceptable for a panelist (i.e. a significant change may be detected but the sample may still be regarded as acceptable based on colour). Thus,

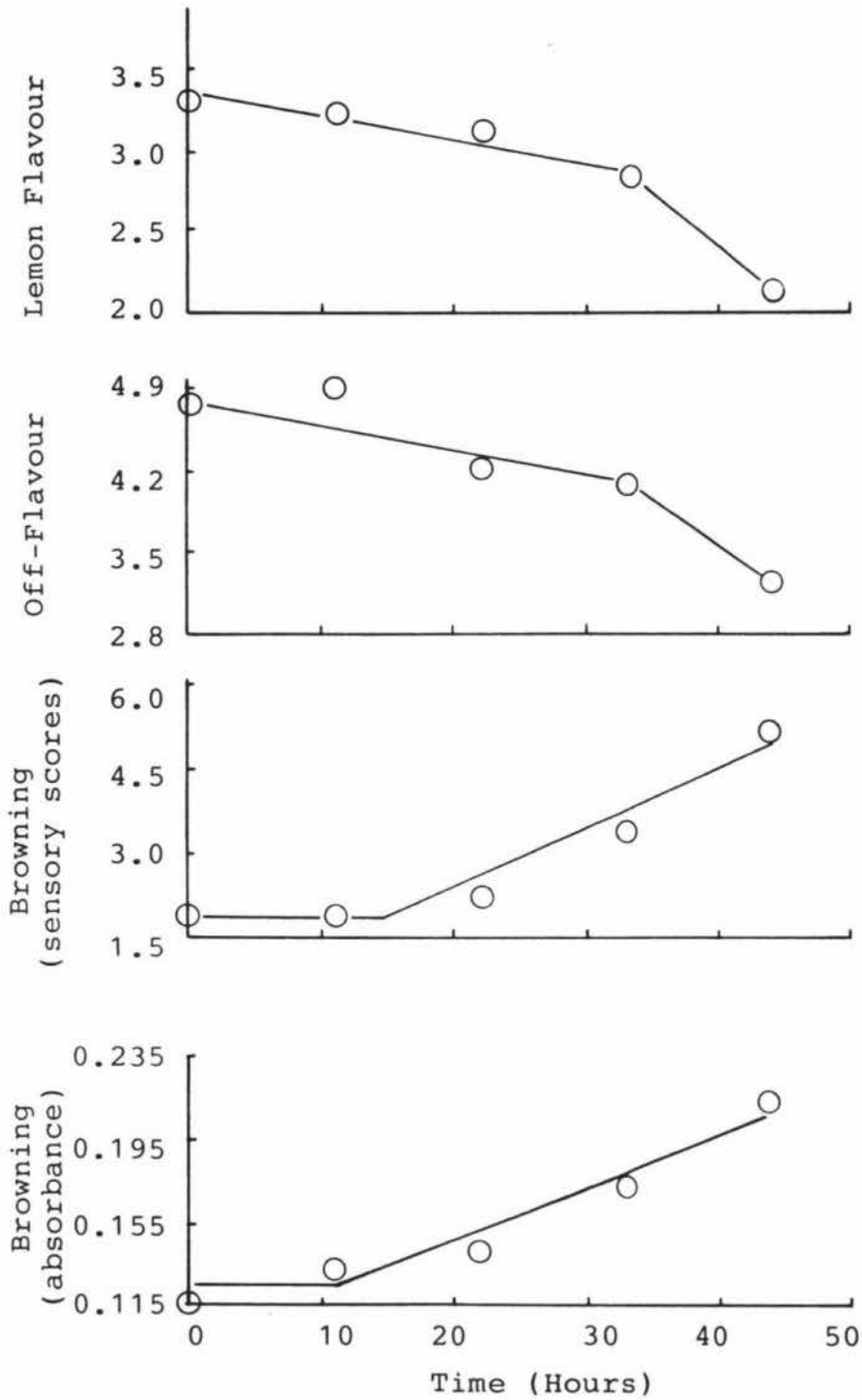

FIGURE 4.2: Quality changes in lemon juice during storage at 55°C.

at this stage it can not be said whether it is the flavour or colour (or a combination of both) which first becomes unacceptable to a panelist.

4.2.4 Conclusion

A good correlation between the objective and sensory measurements of browning was obtained. The loss of the fresh lemon flavour and the presence of a stale off-flavour were significantly detected after 44 hours storage at 55°C. Both flavour scores correlated well with the browning measurements.

Based on these results, a need was seen to further look into the relationship of these sensory evaluation tests with the other objective tests (ascorbic acid retention, furfural and HMF formation).

4.3 Quality Changes in Lemon Juice During Accelerated Storage (55°C) - Second Trial

4.3.1 Introduction

Experiments 4.1 and 4.2 were combined and repeated to determine the relationship between the sensory and objective measurements of quality change in lemon juice during accelerated temperature storage.

4.3.2 Experimental

Pasteurized single-strength lemon juice was stored at 55°C. In comparison to the previous experiments, the juice was contained in stoppered flasks without headspace to control

the amount of oxygen available. Sampling was done every 11 hours up to 44 hours. The samples were analysed for the different quality parameters of lemon juice using sensory evaluation, chemical and instrumental methods. The pH, total soluble solids and titratable acidity were also measured.

4.3.3 Results and discussion

The results, which are averages of six replicates, are given in Table 4.5. The lemon juice had an ascorbic acid content of 46.12 mg/100 mL initially.

No significant changes in titratable acidity and total soluble solids were observed. The pH was observed to decrease with storage time. This may be due to the presence of residual pectinesterase which catalyzes the hydrolytic removal of the methoxyl groups from the pectin molecule to form pectinic acid resulting in more free carboxyl groups and hence increasing its acidic properties.

Ascorbic acid content decreased with storage time but at a rate slower than that of experiment 4.1. After 44 hours storage, ascorbic acid breakdown was only 10.24% as compared to 73.43% for experiment 4.1. This big difference may be due to the absence of a headspace, thus making less oxygen available for aerobic degradation of ascorbic acid. It is known that aerobic breakdown of ascorbic acid occurs at a faster rate than anaerobic breakdown (Nagy, 1980). Since a large headspace was present in the samples of experiments 4.1 and 4.2, it is possible that sufficient oxygen was available for a complete aerobic breakdown of ascorbic acid for the whole storage period.

TABLE 4.5: Quality changes in lemon juice during storage at 55°C.

Time (Hours)	pH*	TSS (°Bx)*	Tit. Acidity (mg/100mL c.a.)*	Brix:Acid*	Ascorbic Acid (%)	HMF (mg/L)	Furfural (mg/L)
0	2.56	8.0	5.31	1.51	100.00	1.72	0.10
11	2.57	8.0	5.33	1.50	96.01	1.97	0.35
22	2.55	8.0	5.34	1.50	94.25	2.92	0.93
33	2.53	8.0	5.36	1.49	92.76	3.92	1.39
44	2.51	8.0	5.33	1.50	89.76	4.26	1.89

Browning (abs. at 420nm)	Browning (Neotec) Y	X	Z	Browning (sen. eval)+		Fresh Lemon Flavour*	Off-Flavour*
0.148	45.4	43.0	47.0	1.704	a	3.083	4.417
0.153	45.5	43.1	46.5	1.943	ab	3.333	4.917
0.168	45.4	42.6	45.3	2.764	bc	2.917	4.750
0.170	45.6	42.6	44.9	3.211	c	2.667	4.583
0.175	44.0	41.4	43.1	4.371	d	3.000	4.500

* No significant difference among the samples (p = 0.05)

\+ Means followed by a common letter are not significantly different at p = 0.05

For the juice samples in this experiment, aerobic degradation could have been the predominant reaction taking place during the first 11 hours when a bigger ascorbic acid loss was observed. Storage studies (Kefford *et al.*, 1959; Nagy and Smoot, 1977) on the loss of vitamin C potency in canned, single-strength juice have shown an initial period of rapid loss of ascorbic acid (about 1-2 weeks at 29-46°C) that was attributed to oxidation by residual oxygen. After the oxygen in the juice was depleted, ascorbic acid could have degraded anaerobically at a rate lower than by the aerobic process.

The differences in the lemon juices used (such as in pH, titratable acidity and total soluble solids) could also have contributed to the difference in the reaction rates between the two experiments.

With the low rate of ascorbic acid degradation, formation of furfural and HMF would be expected to decrease. However, the increase in the total concentration of furfural and HMF over 44 hours was not significantly different from that of experiment 4.1. The increase in furfural and HMF for experiment 4.1 was 1.66 mg/L and 3.2 mg/L respectively, and 1.79 and 2.54 mg/L for this experiment. The reason for the insignificant difference cannot be explained at this point.

Furfural concentration was actually higher and HMF lower, although not significantly, in this experiment. This could be accounted for by the pathway proposed by Bauernfiend and Pinkert (1970) (Figure 2.2), which shows that anaerobic degradation (such as would have predominated in this experiment) favours the production of furfural.

An initial lag period for browning was also observed as in experiments 4.1 and 4.2 (Figure 4.3). Initial absorbance readings were probably higher for the samples of this experiment due to the juice being more "yellow" (higher carotenoid content).

Browning as measured by the two instrumental methods occurred at a slower rate than in experiment 4.1. This can be explained on the basis of the theory that browning in citrus juices is due to ascorbic acid breakdown. However, the difference in change in browning for the two experiments does not seem to be as big or as significant as the difference in ascorbic acid breakdown.

Browning as evaluated by panelists, was found to be significantly different after 22 hours storage as compared to 33 hours for experiment 4.2. This corresponds to a bigger change in absorbance readings over 22 hours in the samples of this experiment, than in the samples of experiment 4.2 for the same storage period.

The correlation coefficients of the colour scores with the other objective parameters are given in Table 4.6. Browning as perceived by the panelists correlated well with the objective measures of quality.

No significant differences were detected for the presence of the fresh lemon flavour and any off-flavours. After 44 hours storage, the fresh lemon flavour was maintained at a 'recognizable' level and no off-flavours were detected. This may be due to the low reactivity of whatever causes flavour deterioration as indicated by the low rate of ascorbic acid breakdown and browning. It is also possible that flavour deterioration that can be attributed to oxidative reactions had been inhibited due to the low availability of oxygen.

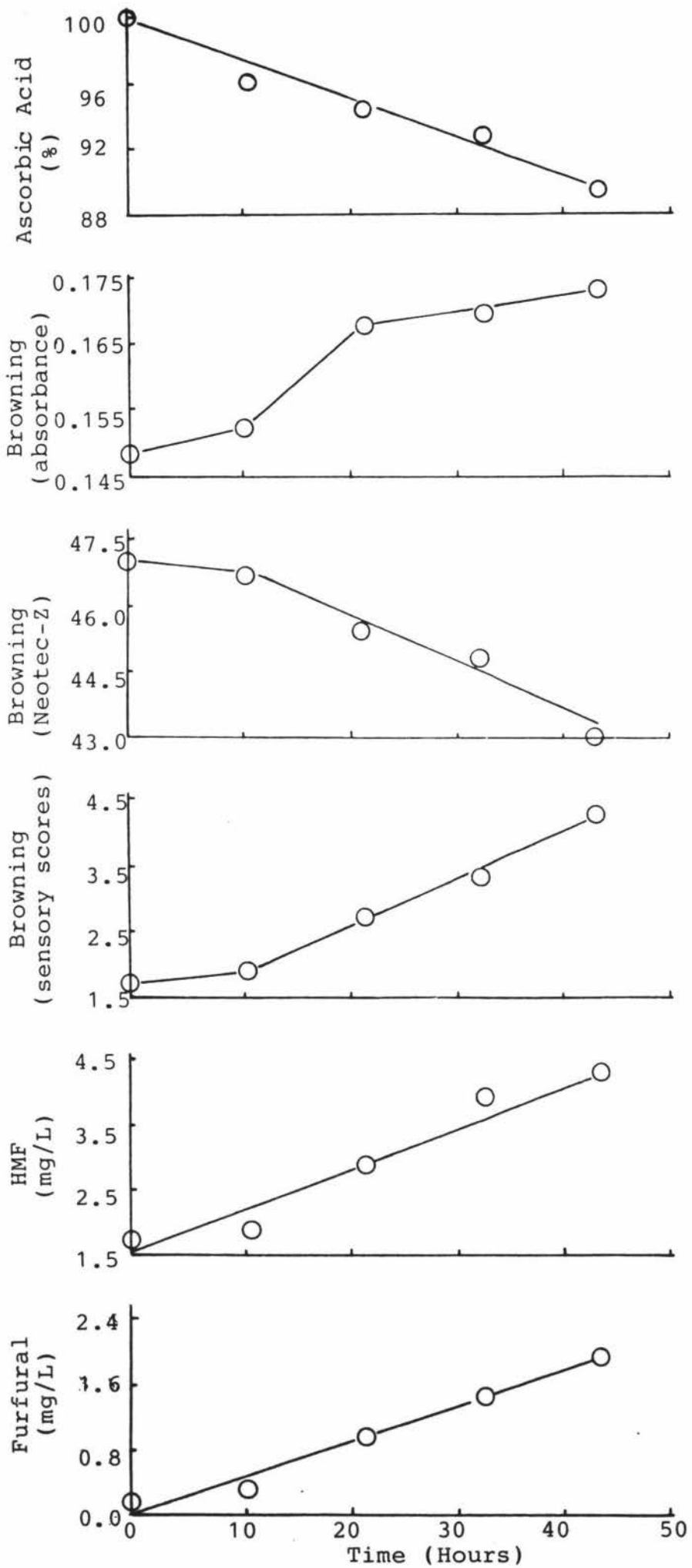

FIGURE 4.3: Quality changes in lemon juice during storage at 55°C.

TABLE 4.6: Correlation coefficients among the different quality parameters measured in lemon juice stored at 55°C.

	Time	Ascorbic Acid	HMF	Furfural	Browning (absorbance)	Browning (Neotec) Y	X	Z	Browning (sen. eval.)	Lemon Flavour
Ascorbic Acid	-0.985									
HMF	0.981	-0.935								
Furfural	0.994	-0.964	0.990							
Browning (absorbance)	0.964	-0.945	0.959	0.964						
Browning (Neotec) Y	-0.642*	0.642*	-0.574*	-0.669*	-0.536*					
X	-0.864*	0.840*	-0.832*	-0.892	-0.807*	0.925				
Z	-0.974	0.958	-0.946	-0.982	-0.937	0.785*	0.953			
Browning (sen. eval.)	0.976	-0.949	0.959	0.988	0.934	-0.775	-0.951	-0.998		
Lemon Flavour*	-0.542	0.422	-0.681	-0.582	-0.647	-0.052	0.304	0.455	-0.492	
Off-Flavour *	-0.132	-0.023	-0.263	-0.231	-0.103	0.384	0.396	0.234	-0.280	0.429

* Values are not significant at p = 0.05

A close correlation between furfural content and flavour changes has been observed in citrus juices (Nagy *et al.*, 1972; Maraulja *et al.*, 1973; Nagy and Randall, 1973) although furfural does not have any flavour properties even at high concentrations. Since no significant changes in flavour were detected in this experiment, very low correlation coefficients were obtained between flavour and the other quality parameters. Perhaps if the lemon juice samples had been stored for a longer period and quality deterioration was allowed to proceed further, then furfural formation could have served as an index of flavour deterioration.

4.3.4 Conclusion

The rates of quality changes were observed to be slower in this experiment than in experiments 4.1 and 4.2. This appears to be due to the absence of headspace in the storage flasks making anaerobic degradation of ascorbic acid predominant and thus also retarding the rate of browning and HMF formation.

Significant correlation coefficients were obtained among the objective measures of quality change in lemon juice and the browning sensory scores. Similar results were obtained for the first trial in experiments 4.1 and 4.2.

4.4 Quality Changes in Calamansi Juice During Accelerated Temperature Storage (55°C)

4.4.1 Introduction

The chemical characteristics of calamansi juice (ascorbic acid = 52.8 mg/100 mL; titratable acidity = 7.02 mg/100 mL citric acid; pH = 2.22; total soluble solids = 8.0 °Brix; as reported by Nisperos *et al.*, 1982) closely resembles those of lemon juice. Calamansi juice is also light coloured. It would therefore be expected that the quality deterioration in calamansi juice would be similar to that of lemon juice.

An accelerated temperature storage was thus undertaken to observe quality changes in calamansi juice during storage and to find out if these changes or reactions paralled those for lemon juice.

4.4.2 Experimental

Calamansi juice that had been extracted, pasteurized and canned in the Philippines, was used (refer to Section 3.1.2 for specific conditions and procedure). The juice from different cans was mixed together and transferred to stoppered Erlenmeyer flasks without headspace and then stored at 55°C. Sampling was done every 11 hours up to 44 hours. Due to the limited amount of calamansi juice, only three replicates per sampling time were analyzed. The calamansi juice samples were analyzed for the same parameters as lemon juice in the previous experiments, with no sensory evaluation being conducted.

4.4.3 Results and discussion

The results are given in Table 4.7. The calamansi juice at zero time had a pH of 2.63, titratable acidity of 7.49 mg/100 mL citric acid, total soluble solids of 11.97 °Brix and 44.93 mg/100 mL ascorbic acid.

TABLE 4.7: Quality changes in calamansi juice during storage at 55°C.

Time (Hours)	Ascorbic Acid (%)	Browning (abs. at 420 nm)	Browning (Neotec) Y	X	Z	HMF (mg/L)	FURF (mg/L)
0	100.00	0.295	40.5	37.3	23.1	0.89	0.48
11	90.85	0.371	42.2	37.1	20.5	0.91	0.83
22	89.65	0.598	39.8	36.1	24.2	0.86	1.89
33	86.67	0.710	39.1	36.2	23.0	1.29	2.49
44	68.10	0.530	39.3	36.1	23.6	1.22	2.86

The colour of the juice was darker orange than was expected. This was probably due to the calamansi fruits being ripe when used. The ascorbic acid content was low for freshly squeezed calamansi juice indicating that ascorbic acid breakdown had taken place during processing and storage.

Ascorbic acid was degraded to 68.10% after 44 hours. The curve for ascorbic acid retention against time resembles the curves obtained in the previous experiments (Figure 4.4). There is an initial high rate of ascorbic acid destruction which may also be attributed to aerobic degradation of ascorbic acid predominating up to 11 hours. A rapid loss of ascorbic acid was observed after 33 hours, similar to that observed in experiment 4.1. The same reason discussed in Section 4.1.3 may apply in this case.

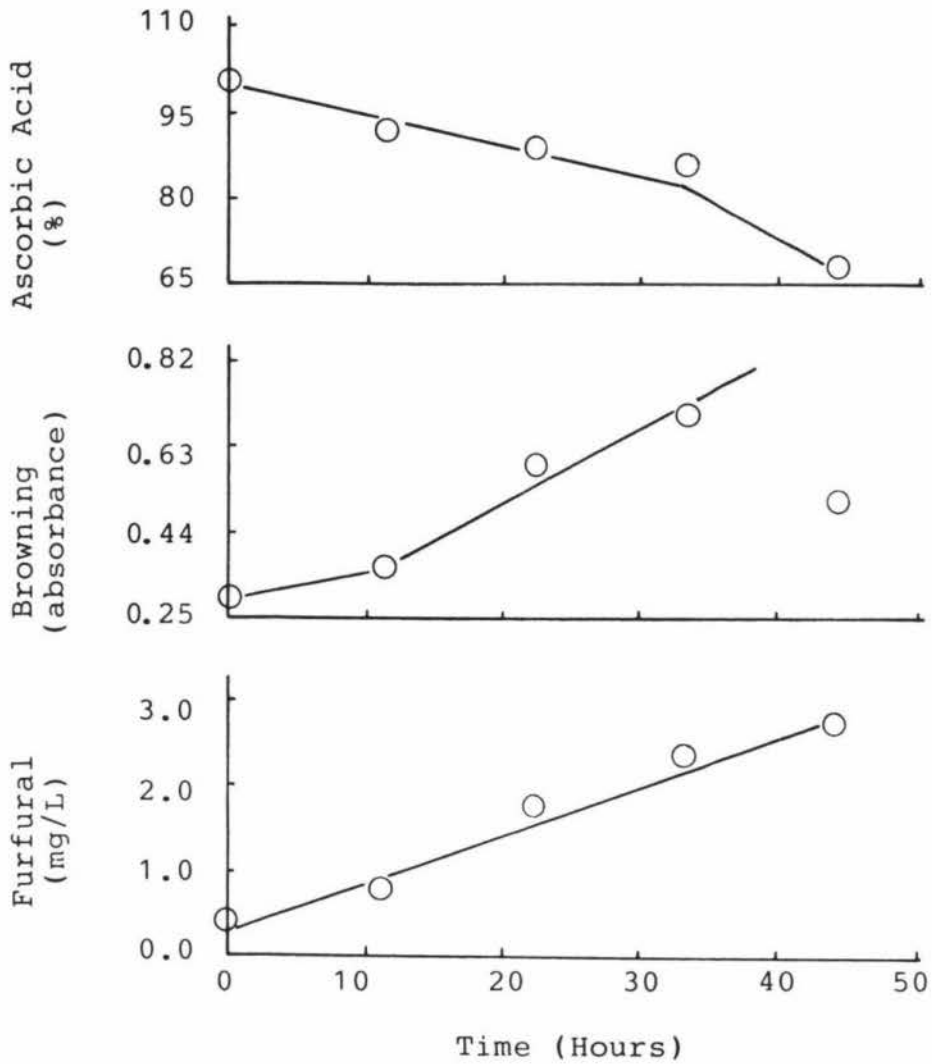

FIGURE 4.4: Quality changes in calamansi juice during storage at 55°C.

For the browning index (absorbance at 420 nm) and the Neotec-Z readings, the typical trend was followed up to 33 hours. After 44 hours storage the values dropped unexpectedly. These results are in error because the samples were cloudy, even after filtration. Further filtrations were tried with no success. Other tests to look into the problem could not be done due to limited material. The values at 44 hours were thus disregarded for the correlation computations (Table 4.8). The Neotec values were erratic and did not even show a trend. Thus, analysis of the browning reaction was solely based on the absorbance readings at 420 nm.

TABLE 4.8: Correlation coefficients among the quality parameters of calamansi juice.

	Time	Ascorbic Acid	Browning[1] (absorbance)	HMF
Ascorbic Acid	-0.918			
Browning (absorbance)	0.983	-0.854		
HMF	0.806*	-0.671*	0.689*	
Furfural	0.985	-0.847	1.000	0.795*

[1] Value at 44 hours disregarded

* Not significant at p = 0.10

The initial amount of furfural in the juice (0.48 mg/L) was considerably higher than that obtained for lemon juice. This could be due to ascorbic acid breakdown and furfural formation during storage in the cans prior to the accelerated temperature study.

The results obtained for HMF were erratic. As was the case with the browning determinations, some samples did not give clear solutions after filtration. It seems that some substances present or formed in calamansi juice during storage at a high temperature, interfere with the reactions required to determine some of the quality parameters.

Lower correlation coefficients among the quality parameters were obtained compared to lemon juice.

4.4.4 Conclusion

From the limited data obtained, calamansi juice appears to undergo the same ascorbic acid degradation, furfural formation and browning reaction as in lemon juice, although they may occur at different rates.

Further investigations on the suitability of the parameters, particularly HMF and the browning measurements, as quality indicators for calamansi juice are necessary.

CHAPTER 5

THE EFFECT OF INITIAL DISSOLVED OXYGEN CONTENT ON THE RATE OF QUALITY DETERIORATION IN LEMON JUICE DURING STORAGE

5.1 Introduction

The difference in the rates of reactions in experiments 4.1 and 4.3 demonstrates the possible effects of available oxygen on the quality changes in lemon juice during storage.

In some studies which concluded that the amount of oxygen present significantly affects ascorbic acid degradation (Singh *et al*., 1976; Eison-Perchonok and Downes, 1982), dissolved oxygen was maintained at saturation levels, or oxygen was made continuously available throughout storage. This situation, however, does not normally occur in commercial operations. Citrus juices are deaerated and/or hot-filled, which reduces dissolved oxygen in the juice and oxygen in the headspace, prior to packaging in gas-impermeable containers. Hence, in commercial packs of citrus juice, oxygen availability is limited.

In view of these, the following experiment was conducted to determine the effect of different initial amounts of dissolved oxygen on quality changes in lemon juice during storage. The kinetics of the different reactions taking place during storage were also investigated.

For quality changes in biological systems such as foods, the reaction order has generally been shown to be either 0 or 1, depending on the reaction involved (Labuza, 1982). Studies have shown that this applies to quality degradation in citrus juices (Nagy, 1980).

Graphically, for a zero-order reaction, a plot of the amount of reactant (C) remaining versus time yields a straight line with the slope equal to the rate constant k in units of concentration per unit time.

When first-order data are plotted as amount of reactant left or C versus time, a curved line is derived. However, the data will follow a straight line when plotted on a semilog scale. In this case, the slope of the straight line is equal to -k/2.303. The units of k on such a plot are $time^{-1}$.

The reaction order and reaction constants can be determined using a linear regression model. The basic equation is a regression of C (the dependent variable y) against time (the independent variable x). For a zero-order reaction the equation is then:

$$C = C_o \pm kt \qquad (5\text{-}1)$$

and for a first-order reaction it is:

$$\ln C = \ln C_o \pm kt \qquad (5\text{-}2)$$

where: C = concentration at time t
C_o = concentration at t = 0
k = rate constant
t = time

To determine the best order as well as the goodness of fit of the data, the coefficient of determination, R^2, is normally computed. R^2 can be interpreted as the fraction of the variation in C that can be explained by means of the straight-line prediction equation. Thus, the nearer the R^2 is to 1.00 the better the fit.

Another method of analyzing for a rate constant, reported by Hill and Grieger-Block (1980) and Labuza and Kamman (1983), is to assume each data point to be a separate experiment. The rate constant is then determined by application of the proper rate equation using this value and the averaged zero-time value $(C_o)avg$. This method is called the long interval method (Hill and Grieger-Block, 1980) or the point-by-point analysis (Labuza and Kamman, 1983). For zero-order, the point-method rate constant is:

$$k = \frac{C - (C_o)avg}{t} \qquad (5\text{-}3)$$

where C is the value determined at time t. If there are n + 1 data points (including the initial value), the procedure yields n values of k. The "average" value of k is then taken to be the arithmetic average of these computed values.

5.2 Experimental

5.2.1 Experimental conditions and procedure

Pasteurized lemon juice was used for this study. To obtain three different levels of dissolved oxygen in the juice, the juice was divided into three equal volumes (approximately 5 litres) and treated in the following way:

The first batch (I) was bubbled with nitrogen gas for 15 min, the second batch (II) was vacuum deaerated (-70 kpa), and the third batch was mixed vigorously and then bubbled with air for 20 min. The juices were filled into flasks immediately after treatment and tightly covered with aluminium foil-lined rubber stoppers. The juice temperature

was kept at 36 ± 0.7°C during treatment and filling. No headspace was allowed to remain in the flasks. The initial amount of dissolved oxygen measured for each batch was: I - 0.41 mg/L, II - 1.44 mg/L, and III - 3.74 mg/L.

All samples were stored in a room whose temperature was controlled at 37°C. The actual temperature of the juice samples in the room was 36 ± 0.5°C. A change of storage temperature from 55°C, which was used for the preliminary experiments, to a lower temperature was found necessary to reduce the rates of the reactions occurring in the juice and thus make the interval between sampling times longer (every 7 days) and hence provide the time needed to analyze all the samples. Aside from this, at very high storage temperatures such as 55°C, it is possible that other reactions may take place which do not normally occur under typical commercial storage conditions (i.e. refrigerated storage at 4-10°C or ambient temperature storage).

Analyses for dissolved oxygen content and other quality parameters were conducted using the methods described in Sections 3.2 to 3.7. Sampling and analyses were done 3 days after storage and every 7 days thereafter up to 6 weeks. Duplicate samples were analyzed for each treatment.

5.2.2 Data analysis

The reaction order of the different quality parameters measured was determined graphically and by computation for both zero- and first-order reactions. A regression analysis and a point-by-point analysis were performed to determine the rate constants of the different reactions.

An analysis of covariance (Anacova) was done to determine if significant differences existed among the samples due to the three treatments. The Anacova permits the researcher to employ a covariate (a quantitative independent variable

such as storage time) along with the qualitative groupings (dissolved oxygen levels I, II and III) in order to study the responses (i.e. ascorbic acid retention, browning). The Anacova was performed using the Biomedical Computer Program (BMDP) 1V run on a PRIME 750 computer.

5.3 Results and Discussion

The changes that took place during the storage of lemon juice at 36°C are summarized in Table 5.1. The results of the computations for zero- and first-order reactions based on regression analyses are shown in Table 5.2.

5.3.1 Comparison of the regression and point-by-point analyses

The results of the point-by-point analysis were compared to those of the regression computations using the reaction order that gave the best fit (zero-order for the browning measurements and furfural formation, and first-order for ascorbic acid retention and HMF formation). These are given in Table 5.3.

Generally, the confidence intervals at 95% for the k values using the point-by-point method were bigger than for the k values using the regression method. This means that the point-method gives a much wider range of times for a given degree of quality change, and this is a disadvantage of the method.

Table 5.1: Quality changes in lemon juice stored at 36°C.

O_2 (mg/L)	Days	pH[a]	°Bx[a]	%TA[a]	B:A[a]	AA (%)	BI (abs)	Neotec-Z	HMF	Furfural
	0	2.58	9.44	5.40	1.75	100.00[b]	0.138	46.6	1.31	0.09
	3	2.57	9.38	5.38	1.74	93.24	-[c]	46.2	1.42	0.32
	7	2.59	9.26	5.43	1.70	74.48	0.134	45.2	1.48	0.87
	14	2.62	9.24	5.45	1.70	63.54	0.141	45.8	1.64	1.35
0.41	21	2.60	9.39	5.45	1.72	58.02	0.148	46.0	2.49	2.54
	28	2.54	9.38	5.42	1.73	50.27	0.172	42.8	3.10	3.21
	35	2.56	9.06	5.49	1.65	51.20	0.186	41.2	3.52	4.87
	42	2.37	9.06	5.44	1.66	50.54	0.189	40.3	4.51	5.31
	0	2.58	9.44	5.40	1.75	100.00	0.138	46.6	1.31	0.09
	3	2.58	9.26	5.40	1.71	91.85	-	46.9	1.40	0.32
	7	2.59	9.26	5.42	1.71	78.45	0.130	45.5	1.46	0.86
1.44	14	2.61	9.14	5.45	1.68	60.32	0.151	46.0	1.60	1.34
	21	2.61	9.31	5.42	1.72	60.86	0.141	44.4	1.99	2.60
	28	2.60	9.36	5.42	1.73	52.73	0.161	44.0	2.94	3.24
	35	2.54	9.06	5.47	1.66	51.38	0.177	42.3	3.64	4.74
	42	2.38	9.06	5.42	1.67	50.31	0.196	40.2	4.64	5.47
	0	2.58	9.44	5.40	1.75	100.00	0.138	46.6	1.31	0.09
	3	2.58	9.51	5.40	1.76	85.93	-	46.2	1.56	0.32
	7	2.56	9.26	5.43	1.70	72.99	0.148	45.5	1.72	0.89
3.74	14	2.62	9.29	5.43	1.71	56.78	0.150	-	1.78	1.43
	21	2.61	9.36	5.45	1.71	55.98	0.160	43.5	2.42	2.69
	28	2.57	9.34	5.48	1.70	45.97	0.181	42.0	3.42	3.53
	35	2.56	9.06	5.50	1.65	46.37	0.199	40.4	4.02	5.09
	42	2.36	9.06	5.48	1.65	47.08	0.200	39.1	5.09	5.77

a No significant differences detected among the samples at p = 0.05

b Initial ascorbic acid content = 47.6 mg/100 mL

c Blanks represent discarded results due to analytical error

Table 5.2: Results of the computations for zero- and first-order reactions based on regression analysis .

Parameter	Oxygen Level (mg/L)	Zero-Order			First-Order		
		k (unit day^{-1})	C_o	R^2 (%)	($x10^{-2}$ k day^{-1})	ln C_o	R^2 (%)
Ascorbic Acid	0.41	1.16	89.4	81.6	1.67	4.49	86.3
	1.44	1.15	89.8	82.8	1.65	4.50	87.3
	3.74	1.18	86.0	79.8	1.80	4.45	84.9
Browning	0.41	0.00191	0.14	94.2	1.16	-1.96	93.6
(Absorbance)	1.44	0.00180	0.14	83.9	1.07	-1.96	83.0
	3.74	0.00199	0.15	94.6	1.13	-1.89	94.3
Browning	0.41	0.226	46.4	91.9	0.52	3.84	92.3
(Neotec-Z)	1.44	0.196	46.1	95.9	0.45	3.83	95.3
	3.74	0.211	44.9	99.8	0.51	3.81	99.9
HMF	0.41	0.075	1.03	95.4	3.05	0.218	97.7
	1.44	0.077	0.93	91.4	3.09	0.177	96.5
	3.74	0.086	1.05	94.4	3.18	0.275	97.0
Furfural	0.41	0.130	-0.11	98.3	8.50	-1.35	84.0
	1.44	0.131	-0.12	98.7	8.53	-1.35	84.2
	3.74	0.140	-0.15	98.8	8.69	-1.34	84.2

Table 5.3: Comparison of the regression and point-by-point method for determining rate constants.

Parameter	O_2 (mg/L)	Rxn Order	k x 10^{-1}*	
			Regression	Point-by-point
Ascorbic Acid	0.41		0.167 ± 0.066	0.262 ± 0.080
	1.44	1	0.165 ± 0.063	0.258 ± 0.070
	3.74		0.180 ± 0.076	0.330 ± 0.114
Browning	0.41		0.0191 ± 0.0067	0.0177 ± 0.0052
(absorbance)	1.44	0	0.0180 ± 0.0112	0.0053 ± 0.0126
	3.74		0.0199 ± 0.0067	0.0194 ± 0.0038
Browning	0.41		2.26 ± 0.95	1.50 ± 1.11
(Neotec-Z)	1.44	0	1.96 ± 0.57	1.88 ± 0.34
	3.74		2.11 ± 0.06	2.15 ± 0.06
HMF	0.41		0.305 ± 0.046	0.256 ± 0.058
	1.44	1	0.309 ± 0.059	0.228 ± 0.062
	3.74		0.318 ± 0.047	0.353 ± 0.105
Furfural	0.41		1.30 ± 0.17	1.10 ± 0.19
	1.44	0	1.31 ± 0.15	1.10 ± 0.19
	3.74		1.40 ± 0.15	1.16 ± 0.21

* 95% confidence limits

One advantage of the point-method is that it gives more rate constant values which can then be used in evaluating temperature effects (Labuza and Kamman, 1983). However, the method gives more weight to values that have a large deviation from the average. This is demonstrated by the k values for browning (absorbance at 420 nm) at 1.44 mg/L dissolved oxygen level, browning (Neotec-Z) at 0.41 mg/L dissolved oxygen and HMF at the 3.74 mg/L dissolved oxygen level. The narrower the confidence interval, the nearer the k values of the two methods are to each other. It seems then that the point-by-point method is useful if small deviations are observed from the average.

Another disadvantage of using the point-method, is that one assigns a much greater weight to the first point, $(C_o)avg$ than to any succeeding one, since all the k values for the different time intervals are computed using this initial value. For this reason, Hill and Grieger-Block (1980) considered it reasonable to employ this method of computation only in cases where the initial concentration was known more accurately than any of the succeeding values. In cases where the standard deviation of C is equal to $(C_o)avg$, such as in this present experiment, then the use of the point-method of averaging is inappopriate.

Considering these reasons, the regression method was deemed more applicable to the data being analyzed. Thus, for the rest of the analysis, the results of the regression computations were used.

5.3.2 Dissolved oxygen

There was a rapid disappearance of dissolved oxygen after three days storage as shown in Table 5.4 and Figure 5.1.

Table 5.4: Changes in dissolved oxygen levels in lemon juice stored at 36°C.

Days	Dissolved Oxygen Levels (mg/L)		
0	0.41	1.44	3.74
3	0.12	0.22	0.15
7	0.12	0.15	0.12
14	0.14	0.15	0.12
21	0.14	0.15	0.14
28	0.14	0.14	0.14
35	0.12	0.12	0.12
42	0.12	0.14	0.14

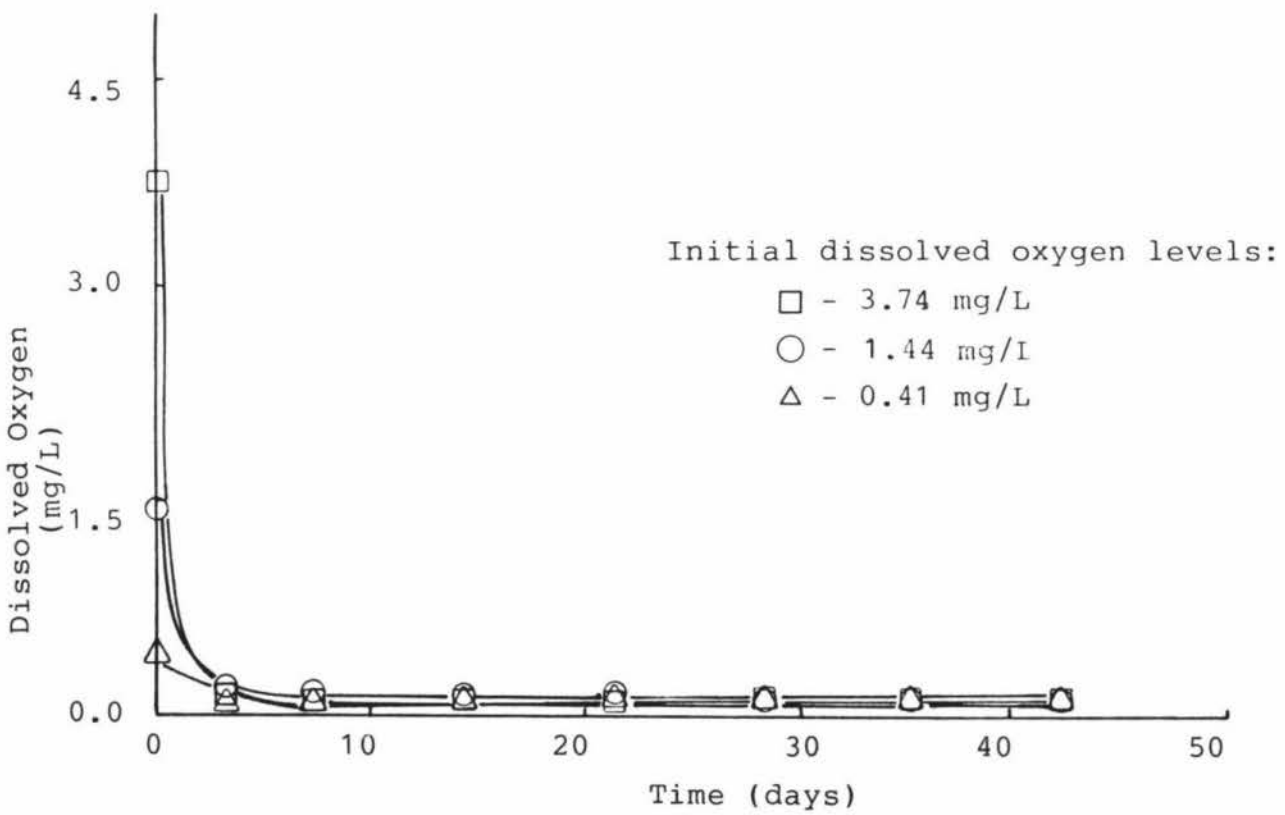

Figure 5.1: Changes in dissolved oxygen levels in lemon juice stored at 36°C.

After 7 days storage the dissolved oxygen content remained constant for all three treatments and did not disappear completely from the samples through the entire period of storage. A similar observation was made by Passy and Mannheim (1979) for grapefruit juice concentrate.

Within the first week, dissolved oxygen was consumed by ascorbic acid degradation and other oxidation reactions after which it seemed that an almost anaerobic condition existed in the juice and reactions proceeded anaerobically.

5.3.3 Acidity and total soluble solids

No significant changes were detected with pH, total soluble solids and titratable acidity for the three treatments. This is not unexpected. In a study on pH values and citric acid content of grapefruit juice samples stored at 50°C for 12 weeks, Smoot and Nagy (1980) concluded that the acid environment of the juices do not change during storage.

5.3.4 Ascorbic acid

Ascorbic acid disappeared with storage time and a first-order reaction was observed (Figure 5.2).

Numerous studies have shown ascorbic acid degradation to be first-order (Huelin, 1953; Waletzko and Labuza, 1976; Saguy *et al*., 1978a; Passy and Mannheim, 1979). However, Singh *et al*. (1976) and Lin and Agalloco (1979) have reported that the first-order rate of equation of ascorbic acid degradation is valid only if the oxygen is present in abundance in solutions (for aerobic degradation) or if it is totally excluded (for anaerobic degradation). In instances where oxygen is present in a limited amount, such as in this experiment, second-order kinetics is followed, i.e. the reaction depends on both the oxygen and ascorbic acid concentrations.

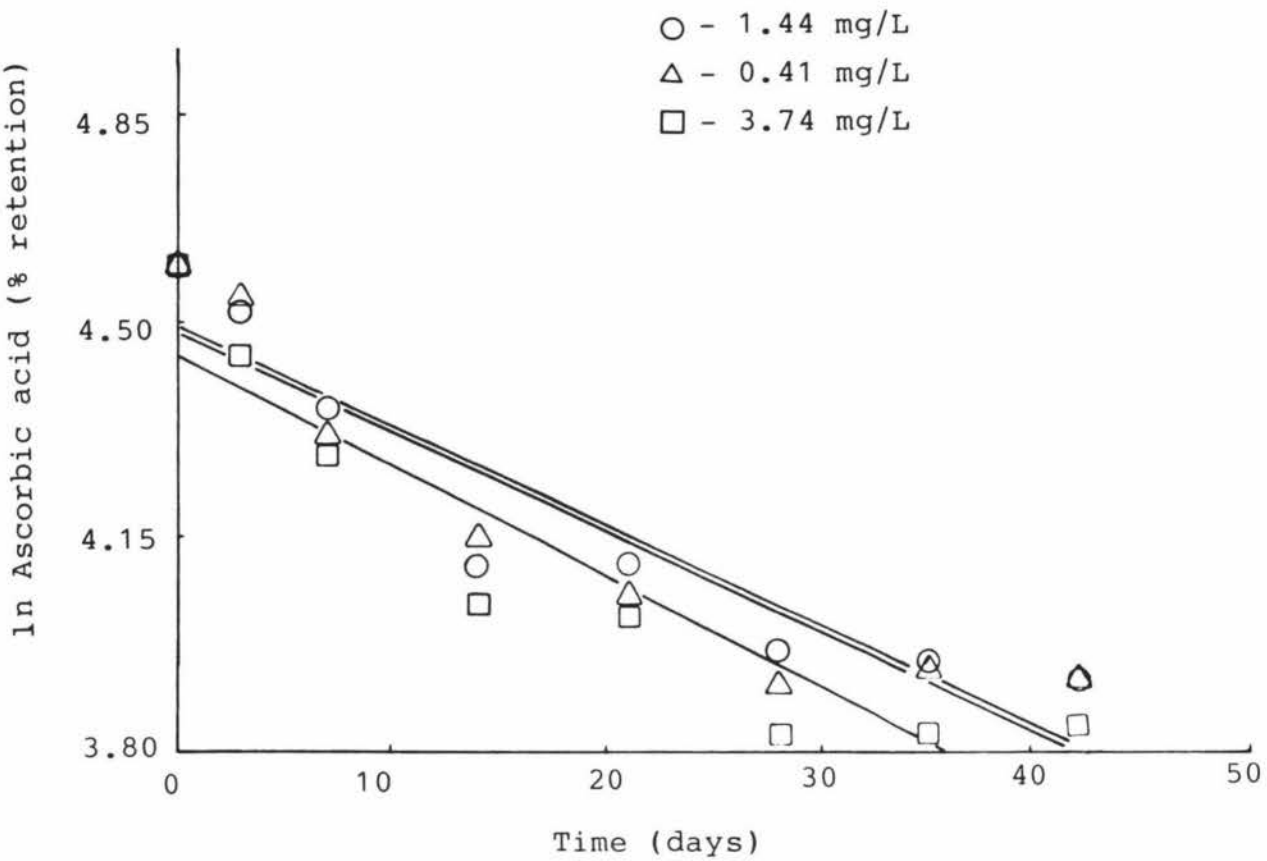

FIGURE 5.2: Ascorbic acid retention in lemon juice stored at 36°C, at three levels of initial dissolved oxygen (0.41, 1.44 and 3.74 mg/L).

In the study of Singh *et al.* (1976) on ascorbic acid oxidation in infant formula (55 mg/L ascorbic acid content) during storage at 7.2°C, a first-order reaction was found to apply to samples in which dissolved oxygen was maintained at levels above saturation (> 8.71 mg/L) throughout the storage period. For the samples with initial dissolved oxygen concentrations of 1.00, 4.86 and 8.71 mg/L (saturation at 7.2°C), a second-order reaction was observed.

Similar results were reported by Lin and Agalloco (1979). Ascorbic acid solutions of various concentrations were prepared by using distilled water, propylene glycol and sugar syrup held at 40°C. At a low initial oxygen concentration level of 3.2 mg/L, ascorbic acid degradation followed second-order kinetics while at a higher initial oxygen concentration of 8.5 mg/L, it was found to follow a first-order reaction regardless of the type of ascorbic acid solution. The difference in reaction order for the samples with an initial dissolved oxygen level of 8.71 mg/L (second-order, infant formula) and 8.5 mg/L (first-order, ascorbic acid solutions) in these two studies indicates that the mechanism of ascorbic acid degradation is not only dependent on the oxygen level but may also depend on the type of system in which ascorbic acid degrades, and on the storage temperature. Departures from a pure first-order plot were noted for canned, single-strength orange juice stored at high temperatures (37.8 - 46.1°C) by Nagy and Smoot (1977).

Since the juice samples in this present experiment had a limited dissolved oxygen content, the results were also tried for a second-order reaction. However, as expected, a poor fit was obtained. This is due to ascorbic acid degradation becoming independent of oxygen concentration after 7 days when oxygen remained constant at a very low level.

Aerobic degradation could have predominated up to 7 days after which ascorbic acid degraded anaerobically at a slower rate. It is suggested therefore, that since anaerobic degradation was the major mode of ascorbic acid breakdown, a first-order reaction was observed.

It is also possible that second-order kinetics may apply to systems such as infant formula (Singh *et al.*, 1976) and ascorbic acid solutions (Lin and Agalloco, 1979), but not to citrus juices due to differences in the reaction mechanism of ascorbic acid breakdown affected by other constituents and reaction products in the juice.

The computed rate constants for the different treatments are shown in Table 5.2.

The results of the Anacova (Appendix 6A) show no significant difference among the three treatments at a 5% probability level. This means that oxygen at the levels used does not affect ascorbic acid degradation significantly. Singh *et al* (1976) in their study of the effect of dissolved oxygen in infant formula, reported that no significant difference existed in rate constants for samples with initial dissolved oxygen concentrations between 1.00 and 4.86 mg/L stored under dark conditions at 7.2°C. Kefford *et al* . (1959) concluded that complete removal of oxygen from orange juice improves the retention of ascorbic acid and flavour during processing but has little effect on the retention of these quality factors during storage.

The zero-point intercept (C_o) should be at 4.60 (to correspond to the ln of the initial level of ascorbic acid in the samples). A noticeable deviation from this value for the three treatments was observed, particularly for the treatment with the highest dissolved oxygen level (3.74 mg/L). This may be due to an initial rapid degradation caused by the dissolved oxygen in the juice.

This deviation and the fact that the last three readings corresponding to 28-42 days did not vary greatly, explains why the R^2 values are not as high as the ones for the other quality parameters. The unexpected constant results cannot be accounted for since the other parameters attributed to ascorbic acid degradation such as browning and furfural formation continued to increase up to 42 days.

5.3.5 Browning

Browning as measured by the absorbance at 420 nm and the Neotec-Z readings gave similar results.

In all treatments there was a lag period, where the browning measurement remained almost constant, and only after a certain period was an increase in browning observed. The lag period was found to be approximately 14 days (Figures 5.3 and 5.4).

At the end of the lag period an increase in browning was observed which can be described as a zero-order reaction, according to the following equation:

$$B = B_{lag} + k\,(t - t_{lag}) \qquad (5\text{-}4)$$

where: B_{lag} = browning at the lag period
t = time in days
k = rate constant in B units/day

The browning reaction is probably predominantly anaerobic, but it is almost impossible to obtain a "free oxygen" environment to confirm this definitely (Passy and Mannheim, 1979).

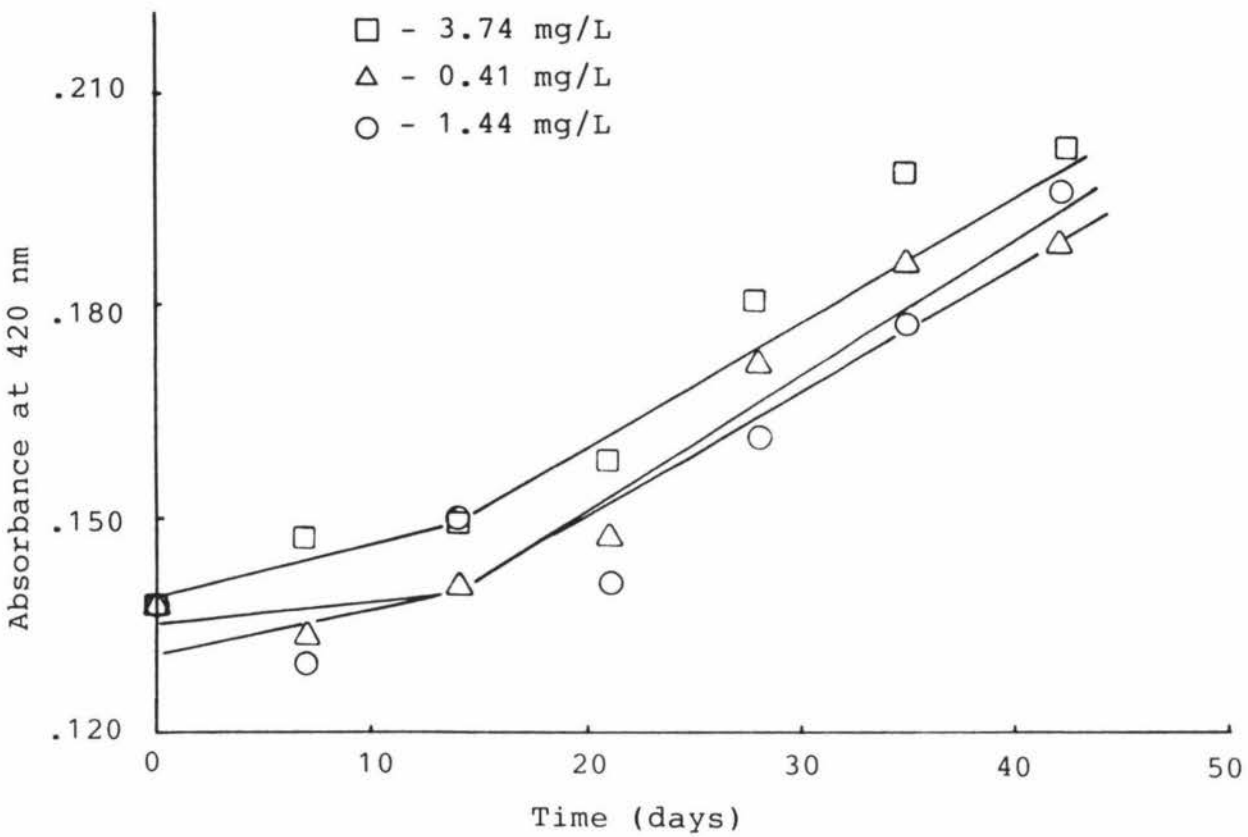

FIGURE 5.3: Browning in lemon juice stored at 36°C, at three levels of initial dissolved oxygen (0.41, 1.44 and 3.74 mg/L).

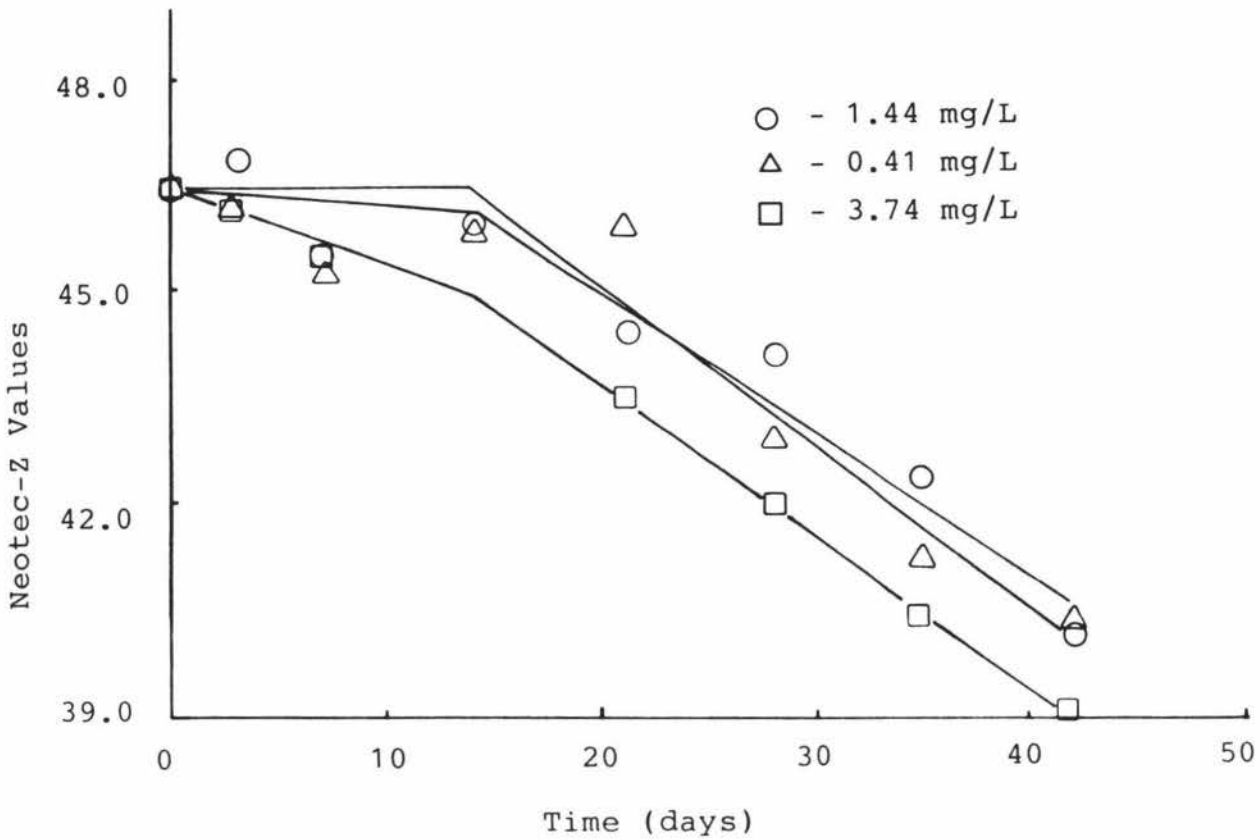

FIGURE 5.4: Browning in lemon juice stored at 36°C, at three levels of initial dissolved oxygen (0.41, 1.44 and 3.74 mg/L).

Computations show that the browning reaction fits both zero and first-order as can be seen in the R^2 values which are very near each other (Table 5.2). The zero-order R^2 is slightly higher. Studies (Saguy *et al.*, 1978b; Mannheim and Passy, 1979) have reported browning to be a zero-order reaction. It is possible that if the browning reaction was allowed to proceed past 42 days and ascorbic acid degradation was greater than 50%, then a more distinct difference between zero and first-order could be observed (Labuza and Kamman, 1983).

The Anacova for both browning measurements (Appendices 6B and 6C) show that significant differences exist among the samples in terms of mean values. The slopes of the three regression equations are parallel to each other indicating the same reaction response as a function of storage time. Browning in the samples with 0.41 mg/L and 1.44 mg/L initial dissolved oxygen was not significantly different. However, browning in the juice with 3.74 mg/L dissolved oxygen was significantly greater than in the samples of the two other treatments.

Since browning in lemon juice is assumed to be a result of ascorbic acid degradation, it was expected that the initial dissolved oxygen level would not significantly affect the browning reaction as it did for ascorbic acid breakdown. A possible explanation for the actual result is that although ascorbic acid degradation is the predominant source of browning, there may be other oxidative browning reactions taking place at a high temperature such as 36°C. Other compounds (i.e. sugars) present in lemon juice may breakdown producing carbonylic intermediates which react to form brown polymers (Hodge and Osman, 1976).

5.3.6 Furfural and hydroxymethylfurfural (HMF)

Furfural formation during storage was found to be zero-order while HMF formation was a first-order reaction (Figures 5.5 and 5.6). For both, good fit of the data to the regression line was observed.

The Anacova results (Appendices 6D and 6E) show that the different initial dissolved oxygen levels did not significantly affect furfural production. However, the highest dissolved oxygen level of 3.74 mg/L was found to result in a significantly higher HMF production.

This could be interpreted by the pathway proposed by Bauernfiend and Pinkert (1970) (Figure 2.2) which shows HMF as one of the products of aerobic degradation of ascorbic acid. Ascorbic acid degrades anaerobically to form furfural while the presence of oxygen leads to HMF formation as well. Hence, the presence of dissolved oxygen would affect HMF production.

Significant correlations were obtained between HMF and furfural and the quality parameters of ascorbic acid and browning. This confirms the suitability of furfural and HMF as chemical indices of quality deterioration in lemon juice during storage.

5.4 Conclusion

When lemon juice was stored at 36°C, ascorbic acid degradation and HMF formation were found to be first-order reactions while browning and furfural formation were zero-order reactions.

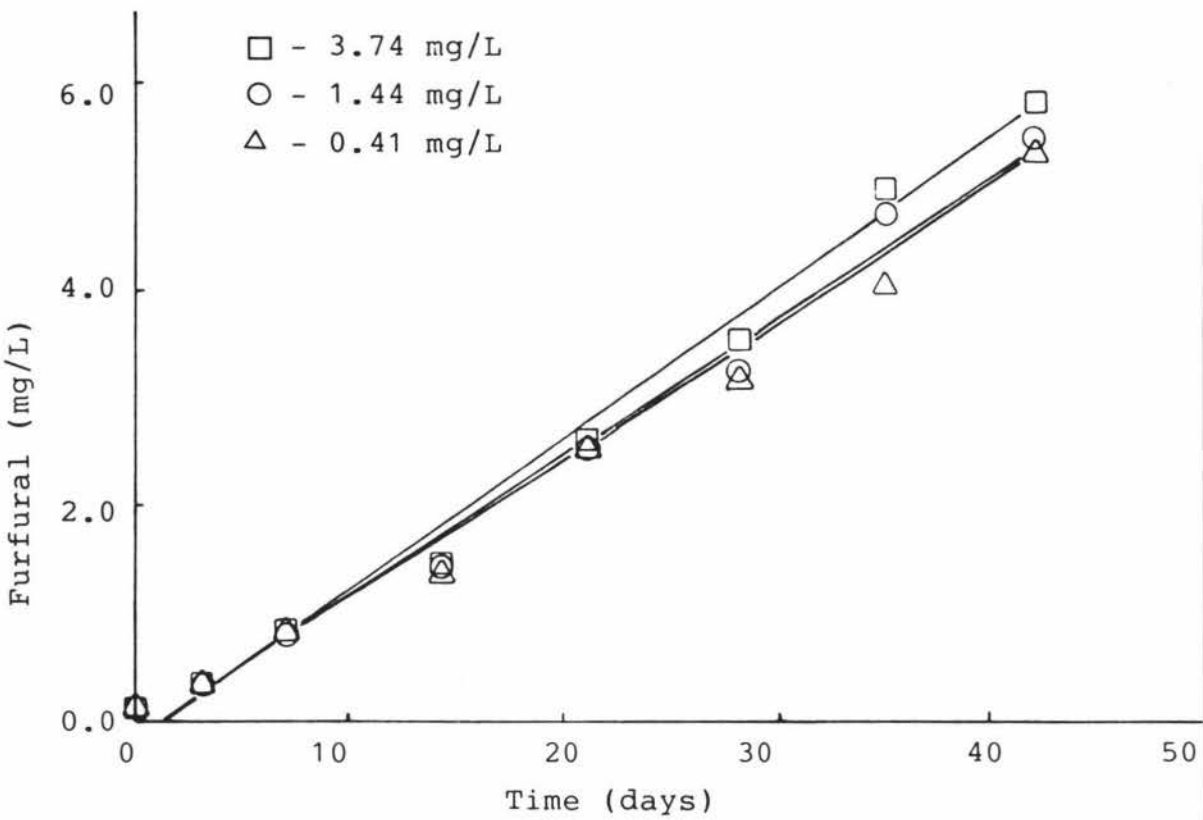

FIGURE 5.5: Furfural formation in lemon juice stored at 36°C, at three levels of initial dissolved oxygen (0.41, 1.44 and 3.74 mg/L).

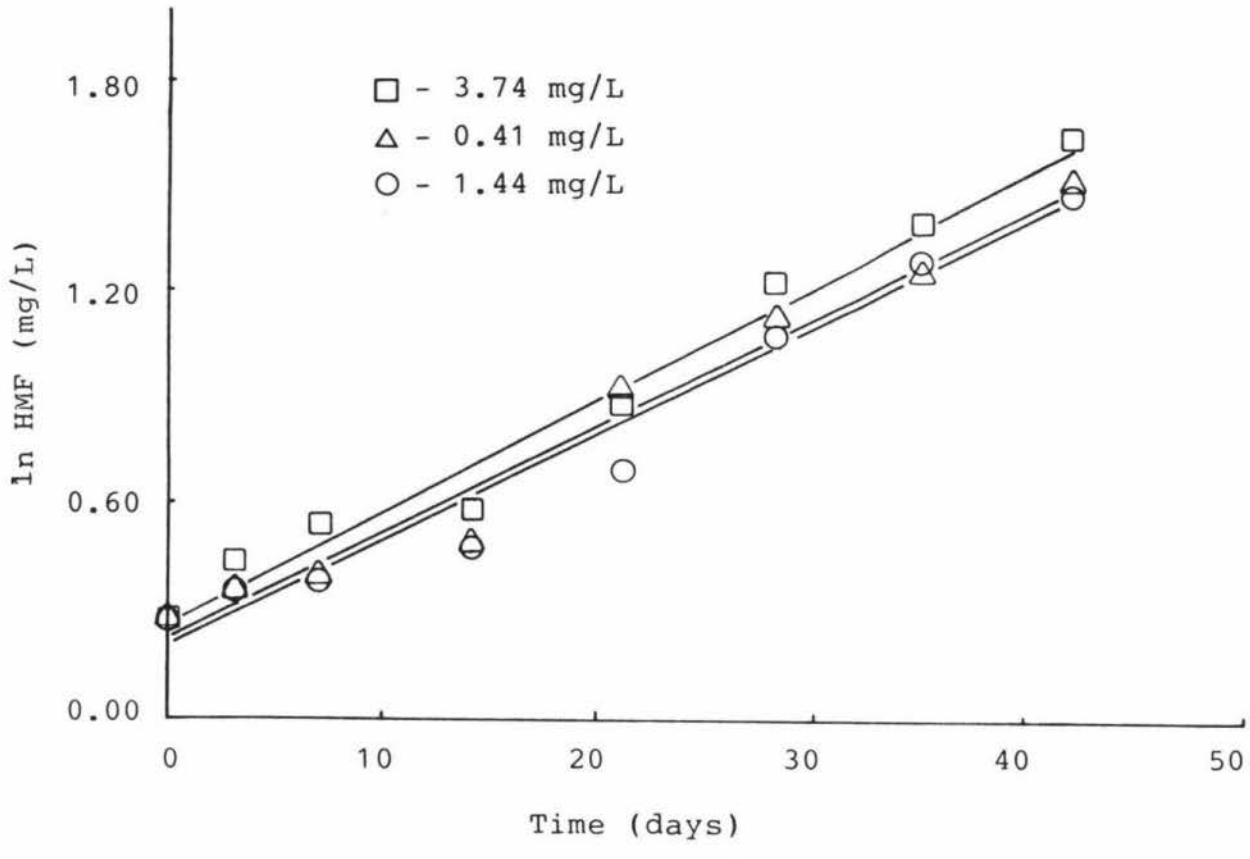

FIGURE 5.6: HMF formation in lemon juice stored at 36°C, at three levels of initial dissolved oxygen (0.41, 1.44 and 3.74 mg/L).

No significant effects on the rate of ascorbic acid degradation and furfural formation could be attributed to the different initial dissolved oxygen levels. For browning and HMF formation, no significant differences were observed between the juice samples with initial dissolved oxygen levels of 0.41 mg/L and 1.44 mg/L. However, a significant difference was found to exist between the samples with the highest dissolved oxygen level of 3.74 mg/L and the samples of the two other treatments. This shows that oxygen can be an important factor leading to quality deterioration when present at high levels.

Under commercial operations, the levels of dissolved oxygen attained would be sufficiently low to make oxygen a non-critical factor in the quality deterioration of lemon juice during storage. Studies on the effect of three deaeration methods (vacuum deaeration, hot-filling, and nitrogen sparging) on the quality of bottled orange juice and grapefruit juice (Mannheim and Passy, 1979) and on grapefruit juice (Passy and Mannheim, 1979) found no differences in quality parameters (ascorbic acid retention, browning, and sensory properties) and shelf-life due to the different deaeration treatments. It was further concluded that the commercial hot-filling procedure is sufficient for obtaining a reasonable removal of oxygen, provided a small headspace is used. The adequacy of vacuum deaeration was demonstrated in this experiment by the insignificant differences between the samples that were vacuum deaerated and those bubbled with nitrogen gas prior to packing.

Although this experiment did not consider the presence of headspace which is present in commercial packs of citrus juices, this is not an unrealistic approach since citrus juices are hot-filled commercially and the headspace would be water vapour. This would condense on cooling of the cans to leave a vacuum (typically -50 kpa) and a very low oxygen concentration.

When juice is cold-filled (0-5°C) as is the case with the aseptically-filled juices now on the market, higher levels of dissolved oxygen would be expected to be involved. In these circumstances the oxygen level could become an important factor in quality deterioration.

CHAPTER 6

THE EFFECTS OF STORAGE TEMPERATURE AND TOTAL SOLUBLE SOLIDS CONTENT ON THE KINETICS OF QUALITY DETERIORATION IN LEMON JUICE

6.1 Introduction

Commercially processed citrus juices are distributed in most countries at concentrations of 42 or 45 °Brix (Berry, 1979). Super concentrates at values higher than 45 °Brix have been prepared from orange, grapefruit, lemon and lime juices. Lemon juice is also processed to frozen concentrate for lemonade (Cole, 1954; Varsel, 1980).

Some advantages of these high Brix concentrates are the significant energy savings during storage and distribution because of their reduced volume and an increased microbial stability at higher temperatures (Crandall and Graumlich, 1982).

Most studies on the effect of the solids content of the juice on the kinetics of the quality changes during processing and storage have been done on orange and grapefruit juices. The composition of lemon juice is, however, distinctly different from other citrus juices excepting limes (see Appendix 7). Therefore, it follows that reaction responses in lemon juice to different soluble solids contents and storage temperatures could differ from other citrus juices.

This experiment was conducted to determine the effects of soluble solids content and temperature on the kinetics of quality changes in lemon juice during storage.

6.2 Experimental

6.2.1 Experimental conditions and procedure

Commercially pasteurized and frozen lemon juice from the Te Puke orchard of Schweppes (NZ) Ltd was used. The juice was thawed and concentrated to 55 °Brix under conditions specified in Section 3.1.3. The juice was cooled immediately after concentration and then stored at 1°C for 24 hours prior to dilution.

The concentrate was diluted with water to 9, 20, 30, 40 and 50 °Brix. Sodium benzoate was added to the juice at a level of 0.1% by weight, to inhibit mould and yeast growth. The presence of this permissible preservative has been reported to have no detrimental effect on the retention of ascorbic acid in orange juice (Evenden and Marsh, 1947).

The 9 °Brix and 20 °Brix juice samples were vacuum dearated (-70 kpa) prior to bottling. The 30, 40, and 50 °Brix juices were too viscous to be dearated. The 9 °Brix juice was filled into 300 mL clear glass bottles and covered with rubber stoppers lined with aluminium foil. The samples with higher juice concentrations were filled into 100 mL clear glass bottles and sealed with screw caps containing rubber gaskets. A larger amount of 9 °Brix juice was required for analysis, hence the difference in bottle sizes. This difference in bottle sizes was not expected to contribute to any difference in the rates of quality deterioration in the juice concentrates during storage.

Samples of each concentration were stored in constant temperature rooms giving juice temperatures of 10, 20 and 36°C. Samples at 36°C were analyzed every 7 days while samples at 20 and 10°C were analyzed every 14 days, up to 16 weeks. Reactions were expected to occur at a faster rate at 36°C hence, the more frequent sampling at this temperature.

All samples were diluted to 9 °Brix (single-strength) prior to analysis for the different quality parameters.

Duplicate samples were analyzed at each sampling time for ascorbic acid, browning and a chemical index of quality deterioration (furfural formation). Both browning measurements (absorbance at 420 nm and Neotec-Z values) gave significant correlations with the other quality parameters in lemon juice (see Sections 4.1 and 4.3). It was decided, however, to measure browning using absorbance at 420 nm because it is simpler and less time consuming to conduct considering that the samples for both methods have to undergo the same clarification and filtration steps.

Furfural formation was chosen as the chemical index rather than HMF for the same reasons. Furfural also had a slightly higher correlation coefficient with the other quality parameters compared to HMF (see Section 4.1), and some problems were encountered with HMF determination in calamansi juice (see Section 4.4). Aside from these, degradation of ascorbic acid was expected to be anaerobic and hence lead to furfural formation, which is known to be one of the principal end products of this reaction. Rapid depletion of residual oxygen was observed during the initial period of storage in experiment 5 and by Kefford *et al.* (1959) and Nagy and Smoot (1977). Thus, for the remaining storage period an almost anaerobic condition existed and ascorbic acid degraded anaerobically. The same conditions were expected to exist for this experiment.

Sensory evaluation of colour and flavour was conducted every 4 weeks. The same procedure as detailed in Section 3.9.3 was followed. Single-strength lemon juice was evaluated for colour using the magnitude estimation method.

Lemon juice stored at 1°C was used as a reference juice in the colour evaluation. A reference juice was thought necessary so that monthly results could be compared with

each other. The reference juice was analyzed for browning (using absorbance at 420 nm) before each sensory evaluation session, to ensure that it did not exhibit any colour change for the 4 months duration of the experiment.

The panelists were also asked to indicate the acceptability of the lemon juice based on colour (refer to scoresheet in Appendix 2B). The juice was considered unacceptable if more than half of the total number of panelists considered it was so.

For flavour evaluation, the lemon juice was mixed into a lemonade (see Section 3.9.3 for formulation details) and evaluated using a descriptive scoring method (see Appendix 2A for details of scoring form).

One set of samples of different Brix concentrations stored at a single constant storage temperature was evaluated at a time. The three sets of samples corresponding to the three storage temperatures were evaluated within a week. Order of evaluation of the different sets by the sensory panel was changed every month.

6.2.2 Data Analysis

The results were statistically analyzed using regression and correlation analyses, and analyses of variance and covariance.

Kinetic data were also computed from the results. The results were fitted to zero- and first-order reaction models and the model that gave the best fit was determined. The temperature dependence of the rates of the different reactions, was determined using the Arrhenius and linear models (the equation for each model is given in Section 6.3.2).

A two-way analysis of variance (Anova) was performed to determine if any significant differences in the rate constants of the different reactions were attributable to storage temperature and/or total soluble solids content. The Biomedical Computer Program (BMDP) 2V run on a PRIME 750 computer was used for the two-way Anova.

An interaction effect between the two factors, temperature and soluble solids content, could not be obtained using the two-way Anova since only one rate constant value was obtained for each treatment combination and the error effect could not be separated from the interaction effect. The interaction effect was determined for this case (a two-factor model without replication) by using a graphical approach and by employing the Tukey's test for additivity (Berenson *et al*., 1983) (see Appendix 8 for details of the test).

6.3 Results and Discussion

The results (summarized in Tables 6.1-6.4, 6.10-6.14) are averages of the duplicate samples analyzed each sampling time. Initial concentrations were actually 9.10, 19.12, 28.98, 38.50 and 50.16 °Brix, but for ease in the discussion they shall be referred to as 9, 20, 30, 40 and 50 °Brix, respectively. The initial ascorbic acid concentration was 52.86 mg/100 mL juice.

Separation in the lemon juice samples was observed during storage. The liquid became clearer as storage time became longer and brownish precipitates were formed. The clear liquid turned brown while the solids lost their light yellow colour, became muddy, and ultimately turned the same hue as the darkened juice. The same observations were made by Joslyn and Marsh (1935) in orange juice. Cloud loss

appeared to increase with increase in soluble solids content of the juice and storage temperature.

The explanation for this observations is that unfavourable storage temperatures and long storage times tend to distabilize the cloud in concentrated juice presumably by acid hydrolysis of the natural pectin (Swisher and Swisher, 1980; Chandler and Robertson, 1983).

6.3.1 Acidity and total soluble solids

No significant differences in pH, % titratable acidity and total soluble solids (TSS) as a function of storage time were found among the samples based on an analysis of variance. The occasional low TSS reading for the 9 °Brix samples may be due to slight fermentation taking place in the sample as suggested by a detected slightly fermented flavour.

The stability of these parameters were also observed in experiment 5 and by Smoot and Nagy (1980) in a study on grapefruit juice stored at 50°C for 12 weeks.

6.3.2 Ascorbic acid

6.3.2.1 Effects of temperature and total soluble solids content on ascorbic acid retention

Ascorbic acid retention decreased with an increase in temperature. At a constant temperature, ascorbic acid retention increased with soluble solids content (Table 6.4 and Figure 6.1).

This relationship of ascorbic acid retention to solids content is contrary to what other studies have reported. Saguy *et al*. (1978a) observed that ascorbic acid loss in grapefruit juice increased with soluble solids content, with a steep change upward at concentrations above 50 °Brix.

Table 6.1: pH of lemon juice during storage[1]

Temp. (°C)	Time (wk)	Total Soluble Solids (°Bx)				
		9	20	30	40	50
10	0	2.68	2.68	2.68	2.68	2.68
	2	2.57	2.58	2.58	2.54	2.54
	4	2.62	2.60	2.59	2.58	2.57
	6	2.57	2.58	2.58	2.58	2.58
	8	2.50	2.51	2.50	2.50	2.50
	10	2.66	2.62	2.60	2.61	2.60
	12	2.62	2.62	2.61	2.60	2.59
	14	2.66	2.63	2.61	2.60	2.60
	16	2.67	2.66	2.66	2.65	2.62
20	0	2.68	2.68	2.68	2.68	2.68
	2	2.56	2.58	2.56	2.57	2.57
	4	2.64	2.62	2.60	2.59	2.58
	6	2.60	2.59	2.55	2.56	2.56
	8	2.60	2.58	2.58	2.57	2.56
	10	2.62	2.62	2.60	2.61	2.60
	12	2.67	2.64	2.62	2.62	2.61
	14	2.61	2.59	2.58	2.57	2.56
	16	2.64	2.64	2.62	2.62	2.61
36	0	2.68	2.68	2.68	2.68	2.68
	1	2.64	2.64	2.65	2.66	2.66
	2	2.60	2.55	2.53	2.48	2.52
	3	2.64	2.60	2.58	2.58	2.58
	4	2.67	2.58	2.62	2.60	2.59
	5	2.59	2.59	2.58	2.58	2.57
	6	2.59	2.58	2.60	2.60	2.60
	7	2.58	2.61	2.61	2.60	2.60
	8	2.56	2.51	2.50	2.49	2.49
	9	2.64	2.64	2.63	2.63	2.62
	10	2.62	2.62	2.61	2.60	2.59
	11	2.65	2.65	2.64	2.64	2.64
	12	2.66	2.64	2.64	2.65	2.65
	13	2.66	2.63	2.64	2.65	2.65
	14	2.60	2.61	2.58	2.58	2.58
	15	2.67	2.63	2.66	2.64	2.64
	16	2.66	2.66	2.64	2.64	2.64

[1] No significant differences were detected among the samples at $p = 0.05$

Table 6.2: Titratable acidity (%) of lemon juice during storage[1].

Temp. (°C)	Time (wk)	Total Soluble Solids (°Bx) 9	20	30	40	50
	0	4.90	4.90	4.90	4.90	4.90
	2	4.86	5.05	4.94	5.06	5.11
	4	5.00	5.01	5.01	5.26	5.20
	6	5.36	5.60	5.35	5.40	5.42
	8	4.92	5.18	5.02	5.11	5.20
10	10	4.91	4.99	4.92	4.99	5.14
	12	4.80	5.08	4.89	4.78	5.16
	14	4.84	4.98	4.84	5.00	5.08
	16	4.86	5.02	5.14	5.10	5.18
	0	4.90	4.90	4.90	4.90	4.90
	2	5.02	4.95	4.74	4.84	4.92
	4	5.08	5.00	5.02	5.17	5.44
	6	5.12	5.12	5.40	5.52	5.44
20	8	5.07	4.90	5.11	5.20	5.18
	10	4.91	5.15	4.88	5.08	5.02
	12	4.84	5.10	4.97	5.00	5.04
	14	4.85	4.79	5.07	5.04	5.21
	16	4.86	5.00	4.90	4.93	5.01
	0	4.90	4.90	4.90	4.90	4.90
	1	4.93	4.54	4.66	4.77	4.88
	2	4.94	4.58	4.64	4.86	4.74
	3	4.58	4.90	4.93	4.94	5.08
	4	4.98	5.00	4.97	5.02	5.11
	5	4.60	5.24	4.98	5.07	5.38
	6	5.21	5.02	5.30	5.30	5.24
36	7	5.05	5.21	5.07	5.14	5.09
	8	5.01	5.73	5.04	5.35	5.26
	9	4.92	4.99	5.00	5.22	5.19
	10	4.90	5.09	4.94	5.09	5.13
	11	4.96	4.92	4.88	6.96	5.03
	12	4.45	5.06	4.90	5.11	5.05
	13	4.88	5.06	4.90	5.03	5.16
	14	4.82	4.97	5.17	5.24	5.17
	15	4.82	4.92	4.92	5.11	5.06
	16	4.88	4.99	5.14	5.28	5.30

[1] No significant differences were detected among the samples at $p = 0.05$

Table 6.3: Total soluble solids content (°Bx) of lemon juice during storage[1].

Temp. (°C)	Time (wk)	Total Soluble Solids (°Bx) 9	20	30	40	50
	0	9.10	19.12	28.98	38.50	50.16
	2	9.16	18.83	28.65	38.38	49.85
	4	9.48	18.61	28.04	38.62	49.82
	6	9.33	19.08	29.43	38.96	50.83
	8	9.26	18.80	28.81	38.64	50.22
10	10	9.32	18.94	28.86	38.82	50.30
	12	8.84	18.29	28.52	38.09	50.02
	14	8.95	18.38	28.42	38.34	49.96
	16	9.05	18.50	28.68	38.65	50.24
	0	9.10	19.12	28.98	38.50	50.16
	2	8.98	18.87	28.65	38.33	49.74
	4	9.00	18.77	28.56	38.71	50.26
	6	8.65	18.91	28.86	39.16	50.12
20	8	8.81	18.74	29.17	38.84	50.44
	10	9.06	19.08	28.72	38.87	50.25
	12	8.74	18.96	28.33	38.68	49.94
	14	8.65	18.85	28.65	38.26	50.10
	16	8.85	18.52	28.52	38.28	50.11
	0	9.10	19.12	28.98	38.50	50.16
	1	9.01	18.91	29.03	38.48	50.76
	2	8.97	18.89	29.00	38.55	49.10
	3	8.70	18.96	29.22	38.96	50.56
	4	9.10	18.83	28.46	37.68	50.19
	5	8.70	18.71	28.46	38.68	50.30
	6	8.98	18.96	28.58	38.54	50.58
36	7	9.40	19.14	29.00	39.32	50.84
	8	8.98	19.24	28.92	39.20	50.70
	9	8.84	18.49	28.58	38.68	50.22
	10	8.82	18.87	28.66	38.54	50.28
	11	8.68	18.52	28.84	40.39	52.61
	12	8.59	18.79	28.08	38.26	50.05
	13	8.96	19.00	28.46	38.24	50.30
	14	8.94	18.94	28.49	38.08	49.94
	15	8.91	18.89	28.30	37.75	49.94
	16	8.90	18.83	28.14	37.61	50.02

[1] No significant differences were detected among the samples with the same soluble solids content at p = 0.05

Table 6.4: Ascorbic acid retention (%) in lemon juice during storage[1,2].

Temp. (°C)	Time (wk)	Total Soluble Solids (°Bx) 9	20	30	40	50
	0	100.00[a]	100.00[b]	100.00[c]	100.00[d]	100.00[e]
	2	88.50	88.30	90.92	93.45	95.97
	4	85.24	85.47	88.61	93.87	94.29
	6	68.66	84.94	87.01	92.32	95.00
	8	72.50	79.40	83.79	88.97	94.85
10	10	66.36	78.76	85.92	90.94	96.06
	12	62.15	80.04	85.60	90.50	96.44
	14	52.45	78.96	85.77	90.09	97.01
	16	50.42	76.47	86.08	90.65	95.69
	0	100.00[a]	100.00[b]	100.00[c]	100.00[cd]	100.00[d]
	2	89.50	78.28	85.19	89.29	93.38
	4	83.22	76.35	84.18	88.01	93.34
	6	71.74	67.61	83.14	86.70	92.28
	8	52.44	68.84	79.68	81.72	88.50
20	10	47.71	69.73	81.35	82.00	87.76
	12	52.74	59.36	80.42	83.01	86.93
	14	49.58	59.91	83.09	81.93	85.77
	16	33.82	54.03	73.59	79.00	85.02
	0	100.00[a]	100.00[b]	100.00[c]	100.00[d]	100.00[d]
	1	88.31	76.73	80.70	87.82	92.36
	2	81.57	76.35	79.34	87.44	89.20
	3	76.11	66.27	78.45	81.88	86.45
	4	60.58	65.89	74.88	75.60	82.44
	5	45.86	61.75	75.75	76.79	78.55
	6	33.98	58.19	67.14	75.08	76.09
	7	23.44	58.04	66.63	71.55	73.21
	8	21.70	61.52	64.85	70.26	73.25
36	9	14.38	61.67	66.27	73.51	72.85
	10	12.62	56.89	66.40	72.27	70.71
	11	16.95	55.94	62.92	67.80	65.47
	12	10.50	58.02	61.08	66.40	65.98
	13	6.73	52.93	61.16	60.95	61.37
	14	5.62	54.26	61.54	64.38	62.84
	15	6.07	51.14	61.33	63.64	60.57
	16	7.02	48.58	55.16	58.34	54.94

[1] Initial ascorbic acid content = 52.86 mg/100 mL juice

[2] A common letter indicates that their adjusted means (see Appendix 6A for definition) are not significantly different at a 5% level of significance at a constant temperature (based on an Anacova with time as a covariate)

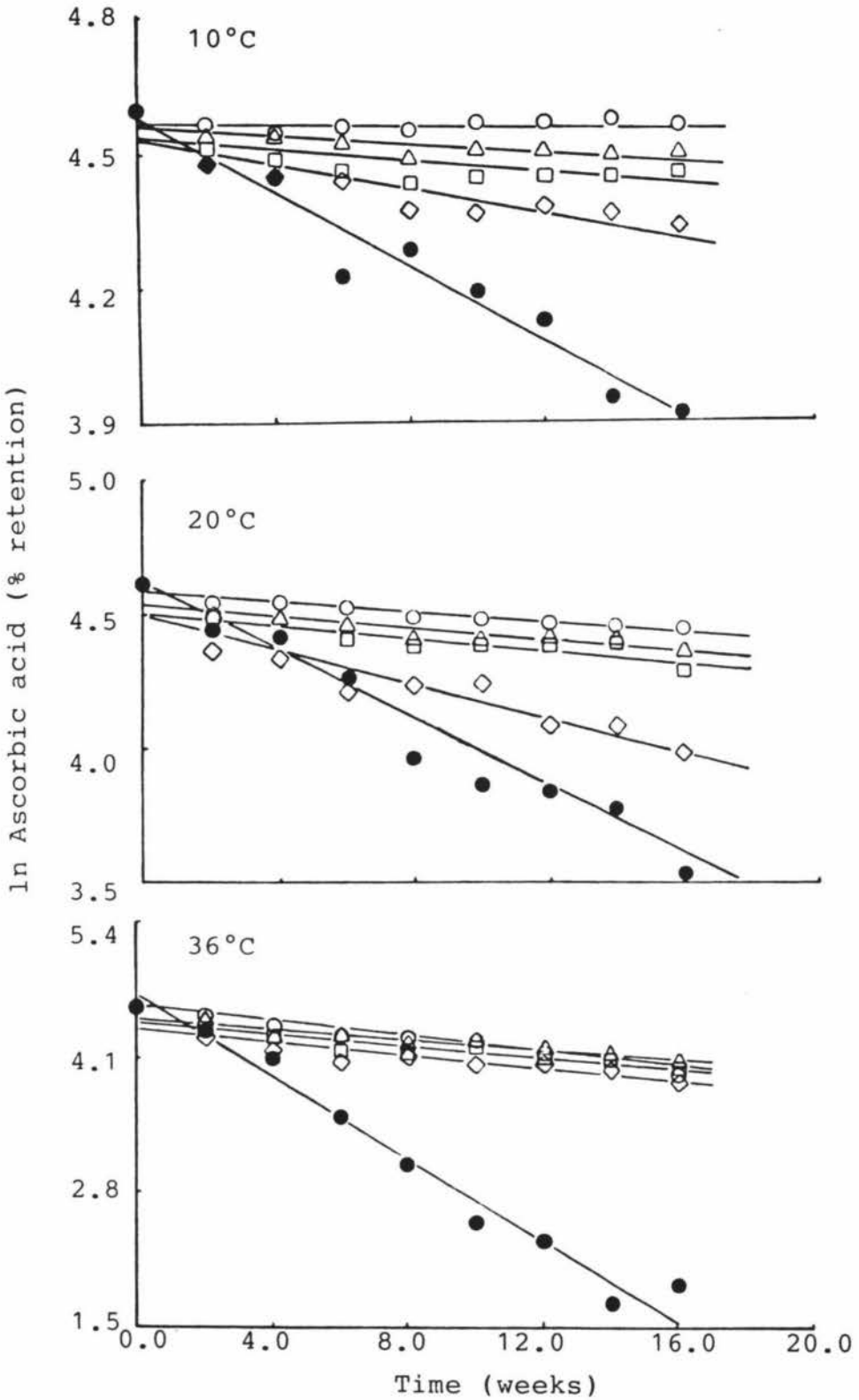

Figure 6.1: Ascorbic acid retention in lemon juices and concentrates (●- 9°Bx, ◇- 20°Bx, □-30°Bx, △-40°Bx, O - 50°Bx) during storage.

Similar results were obtained by Kanner *et al*. (1982) for orange concentrates.

The increased loss of ascorbic acid at higher soluble solids levels was attributed to the accelerating effect of higher concentrations of some of the components of the juice such as fructose (Curl, 1949; Huelin, 1953; Saguy *et al*., 1978a). Curl (1949) suggested that a reaction between the carbonyl groups of fructose (or a conversion product) and ascorbic acid was responsible for the accelerated loss in ascorbic acid in orange juice products.

The above studies were done on grapefruit and orange concentrate and not on lemon juice which has a different composition and, in particular, a higher acidity. It is possible that these factors have contributed to the difference in results.

Navarro *et al*. (1980) compared the stability of orange concentrates between 20 and 60 °Brix. They found higher Brix concentrates to be more stable with respect to ascorbic acid and the colour parameters tested.

The results of this present experiment indicate that at a higher soluble solids content there is an inhibition of ascorbic acid degradation. The reason for this is not clear, but it is possible that other components of lemon juice (e.g. sugars) at high concentration produce a stable or protective environment for ascorbic acid.

Joslyn (1957) reported that sugars exercise a protective effect on the enzymic and nonenzymic oxidation of ascorbic acid; hence, both glucose and fructose are inhibitory to browning. The study was on an ascorbic acid-amino acid-sugar system. Contradicting results were, however, reported by other authors (Curl, 1949; Huelin, 1953; Clegg, 1964) for model systems of citrus juices.

Lin and Agalloco (1979) observed that the degradation of ascorbic acid in an ascorbic acid solution was significantly retarded if sugar syrup was used in preparing the solution. The exact effect of sugar on the decreased ascorbic acid degradation is not known. They speculated that it may be due to the fact that some kinds of complex molecules are formed between the ascorbic acid and the liquid and that they provide better stability than do the original ascorbic acid molecules.

The observed decrease in ascorbic acid degradation with an increase in soluble solids content of the juice may also be attributed to a decrease in moisture content.

In dehydrated foods and model systems, it has been reported that the destruction rate of ascorbic acid increased with increasing moisture content and water activity (Karel and Nickerson, 1964; Lee and Labuza, 1975; Riemer and Karel, 1977; Singh *et al.* 1983). No values for the water activity (A_w) of citrus juice concentrates have been found in the literature. However, the A_w of single-strength juice would be expected to be near 1.0, and with an increase in soluble solids content, A_w would be expected to decrease due to a decrease in moisture content and an increase in solutes.

Lee and Labuza (1975) studied the destruction of ascorbic acid in model systems over a wide range of A_w (0.3 to 0.95). The increase in rate of ascorbic acid degradation with A_w or moisture content was attributed to its relation to the mobility of the reaction species in the aqueous phase. They observed that the rate constant k was inversely related to viscosity. Thus, it was postulated that the effect might be due to a decrease in viscosity and thus increased mobilization of the reaction species (i.e. ascorbic acid) (Labuza, 1975). This theory may well apply to the lemon juice samples of this experiment since viscosity of the juice was visually observed to significantly increase with

a decrease in moisture content (or increase in soluble solids content).

6.3.2.2 Kinetics of ascorbic acid degradation

Ascorbic acid retention was plotted against storage time (Figure 6.1) and both a zero-order and first-order model fitted to the data. Regression analysis indicated that ascorbic acid degradation followed the first-order model better than the zero-order model at all five soluble solids levels (Table 6.5).

The rate constant obtained for the single-strength juice at 36°C (0.198/wk) was higher than that in experiment 5 at the same temperature (0.126/wk). The difference may be due to differences in the lemon juice samples, the difference in the extent of ascorbic acid degradation and the fact that the lemon juice samples in this experiment had undergone more heat treatment (during pasteurization and concentration).

The k values for the 9 °Brix juice were significantly higher than those for the other four higher Brix concentrates at all three temperatures. This suggested that at a soluble solids level of 9 °Brix, ascorbic acid stability was very low. It is possible that at this single-strength level of lemon juice, the mechanism of ascorbic acid degradation differed from that occurring at 20 to 50 °Brix concentrates.

Very low R^2 values were obtained for the 30, 40 and especially the 50 °Brix samples at 10°C. After the decrease in ascorbic acid during the first 14 days, insignificant changes were observed during the remainder of the storage period, hence, the low R^2 values. Based on the table in Appendix 3, the R^2 values of 54.4% (at 30 °Brix) and 59.8% at (40 °Brix) ($R = 0.737$ and 0.773, respectively) are significant at the 5 percent level of significance. This

Table 6.5: Results of the computations for zero- and first-order reactions of ascorbic acid degradation based on regression analysis .

Soluble Solids Content (°Bx)	Temperature (°C)	Zero-order k (% AA wk^{-1})	Zero-order C_o (% AA)	Zero-order R^2 (%)	First-order k ($x10^{-2}$ wk^{-1})	First-order ln C_o (% AA)	First-order R^2 (%)
9	10	2.96	95.5	94.6	4.15	4.58	95.8
	20	3.91	95.8	91.4	6.19	4.61	91.1
	36	6.03	84.2	86.7	19.80	4.71	96.3
20	10	1.16	92.9	76.4	1.35	4.53	79.0
	20	2.26	88.5	82.4	3.11	4.49	87.3
	36	2.05	79.0	70.4	3.08	4.37	78.3
30	10	0.652	93.4	53.7	0.714	4.54	54.4
	20	1.01	91.5	60.8	1.18	4.51	62.8
	36	1.92	85.0	80.1	2.66	4.45	85.5
40	10	0.463	96.0	59.3	0.492	4.56	59.8
	20	1.01	93.8	76.1	1.14	4.54	78.3
	36	1.99	89.7	87.3	2.65	4.50	90.4
50	10	0.073	96.7	5.7*	0.073	4.57	5.4*
	20	0.834	97.0	89.7	0.914	4.58	90.9
	36	2.41	93.8	95.9	3.22	4.56	97.3

* Not significant at p = 0.05

means that 54.4% and 59.8% of the variations in ascorbic acid concentration can be accounted for by the fitted linear regression model. However, despite being significant at the 5% level of significance, it does not seem adequate for prediction and kinetic purposes.

The intercept (ln C_o) of the equation should be at 4.6 (ln of 100). As can be seen in Table 6.5, differences from this value were observed which may be due to the larger initial aerobic breakdown of ascorbic acid due to the presence of residual oxygen in the juice and the headspace. After 14 days, when oxygen had been consumed, anaerobic degradation of ascorbic acid would have predominated at a lower rate than the aerobic process.

The temperature dependence of the rate of ascorbic acid degradation was determined using the Arrhenius and linear models. The Arrhenius model is expressed as:

$$k = A \exp(-E_a/RT) \qquad (6\text{-}1)$$

where: k = rate constant, wk^{-1}
E_a = activation energy, kJ mol^{-1}
T = absolute temperature, K
R = gas constant, kJ mol^{-1} K^{-1}
A = frequency factor, wk^{-1}

while the linear form is:

$$k = k_o \exp(-bT) \qquad (6\text{-}2)$$

where: k_o = pre-exponential constant, wk^{-1}
b = constant, wk^{-1} K^{-1}
T = temperature, K

The activation energy (E_a) and the frequency factor (A) were determined by linear regression for each data set and are presented in Table 6.6.

Table 6.6: Computed Arrhenius coefficients and Q_{10} values.[1]

	Soluble Solids Content (°Bx)	ln A	E_a (kJ mol^{-1})	R^2 (%)	Q_{10} 10-20°C	Q_{10} 30-40°C
Ascorbic Acid	9	15.6 wk^{-1}	44.52	97.3	1.49	1.76
	20	4.95	21.33	64.1	2.30	1.31
Browning	9	13.1 abs.units/wk	42.61	96.8	1.44	1.71
	20	11.4	38.78	99.0	1.55	1.63
	30	15.3	48.20	99.7	1.85	1.84
	40	16.7	51.17	99.8	1.93	1.91
	50	21.2	61.75	99.8	2.22	2.19
Furfural	9	31.8 mg/L wk	83.55	92.4	7.39	2.88
	20	27.5	71.92	98.7	3.73	2.49
	30	29.4	76.52	98.3	4.23	2.64
	40	33.2	85.88	97.3	5.59	2.97
	50	33.6	86.68	98.6	4.94	3.00

[1] Arrhenius coefficients for ascorbic acid degradation in 30, 40 and 50 °Brix samples were not determined because the k values for these concentrates at 10°C were not considered adequate for kinetic purposes due to poor fit to the regression model as indicated by low R^2 values.

The model was only applied to 9 °Brix and 20 °Brix where high R^2 values for ascorbic acid retention as a function of storage time were obtained. While a high R^2 value for the Arrhenius plot was obtained for the 9 °Brix juice (97.3%) that for the 20 °Brix juice was considerably lower (64.1%). This latter value reflects the greater relative scatter of the points (Figures 6.2 & 6.3) and suggests that in 20 °Brix juice, it is likely that ascorbic acid degradation involves more than one reaction pathway, each having different E_a's.

The results of the regression analysis using the linear model are given in Table 6.7. High R^2 values were obtained for both the Arrhenius and linear model, indicating a good fit for all quality parameters over the temperature range of 10 to 36°C.

Since both models were applicable to the data of this experiment, a decision had to be made as to which of the two would be used for further discussion and analysis.

The use of the Arrhenius equation was considered by Saguy and Karel (1980) as the soundest approach to modelling temperature dependence. The Arrhenius model, unlike other possible expressions of temperature dependence, has a thermodynamic basis (Kwolek and Bookwalter, 1971; Saguy and Karel, 1980; Labuza and Kamman, 1983).

Kwolek and Bookwalter (1971) considered that the linear model does not present a suitable relation, since b is expected to increase rapidly at higher temperatures and slowly at lower temperatures, rather than at the same rate for all temperatures. This suggests that the linear model applies to a narrower range of temperatures.

Saguy and Karel (1980) recommended the use of the Arrhenius equation even if a linear approximation could be applied for a narrow range of temperature, as was the case in this experiment.

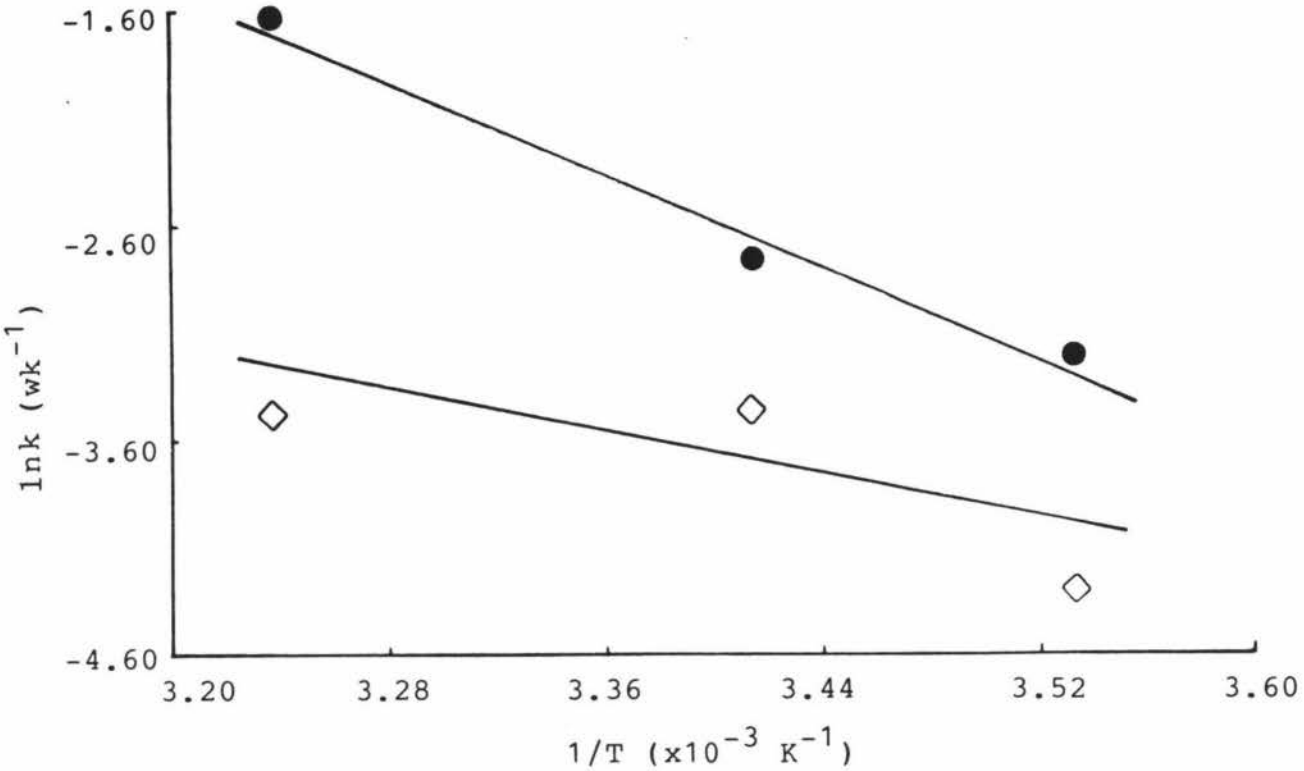

Figure 6.2: Arrhenius plots for ascorbic acid degradation in 9°Bx (●) and 20°Bx (◇) lemon juices.

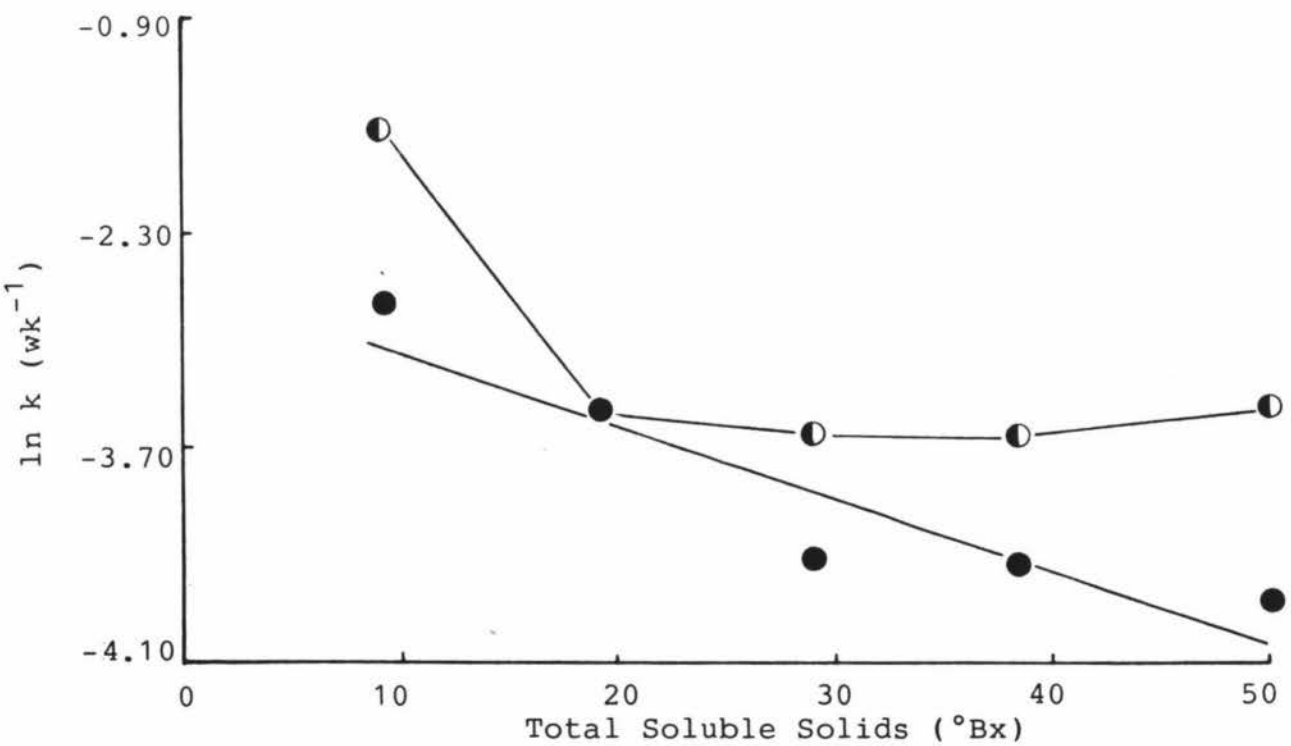

Figure 6.3: Changes in the rate of ascorbic acid degradation with increases in soluble solids level at 20°C (●) and 36°C (◐) (line of best fit at 20°C is based on linear regression analysis; points at 36°C have been joined by straight lines).

Table 6.7: Results of the regression analysis using the linear model.

	Soluble Solids Content (°Bx)	$\ln k_o$	b ($\times 10^{-1}$)	R^2 (%)
Ascorbic Acid	9	-20.6 wk^{-1}	0.613 $wk^{-1}K^{-1}$	98.0
	20	-12.2	0.287	61.8
Browning	9	-21.6 abs.units/wk	0.587 abs.units/wk K	97.6
	20	-20.2	0.533	99.5
	30	-23.9	0.662	99.9
	40	-24.9	0.702	99.9
	50	-29.0	0.847	99.9
Furfural	9	-35.9 mg/L wk	1.14 mg/L wk K	91.1
	20	-30.9	0.98	98.1
	30	-32.6	1.05	97.6
	40	-36.4	1.17	96.4
	50	-36.8	1.18	98.0

One advantage of the linear equation is that it is simple and thus easier for food processors and operators to understand than the Arrhenius equation. It seems then that for commercial purposes, where kinetic data is not a critical factor, the linear equation could be used for a specific temperature range.

However, since it was the objective of this experiment to determine the kinetics of the different reactions, it was considered best to use the Arrhenius model which is more appropriate from a theoretical viewpoint. Aside from this, kinetic data reported by other authors on reactions in citrus juices have been based on the Arrhenius model.

The "temperature quotient" or Q_{10} value was also computed. This describes the sensitivity of the reaction rates to a 10°C change in temperature. This is ordinarily defined as:

$$Q_{10} = \frac{k \text{ at } (T + 10°C)}{k \text{ at } T°C} \qquad (6\text{-}3)$$

The Q_{10} values were computed using this equation, for the temperature ranges 10-20°C and 30-40°C. The actual k values were used to compute the Q_{10} at 10-20°C. For the Q_{10} at 30-40°C, k at 30°C was interpolated and k at 40°C was extrapolated from the Arrhenius plot. The computed Q_{10} values are given in Table 6.6. A difference in values for the two temperature ranges was observed due to slight deviation of the actual k values from the Arrhenius plot. The higher the R^2 for the Arrhenius equations or the better the fit of the k values to the equation, the closer the Q_{10} values were, for a specific reaction and juice concentration.

An activation energy value of 44.52 kJ mol^{-1} and a Q_{10} of 1.49 (10-20°C) and 1.76 (30-40°C) were calculated for the 9 °Brix lemon juice. This E_a value was approximately double the value for the 20 °Brix juice (21.33 kJ mol^{-1}) further indicating the possibility that different mechanisms of

ascorbic acid degradation took place at the two soluble solids levels.

Evenden and Marsh (1947) reported an E_a of 49.0 kJ mol^{-1} for single-strength orange juice. Studies by Nagy and Smoot (1977) and Smoot and Nagy (1980) on single-strength grapefruit and orange juices reported that over the range of 4-28°C, regression analysis of the slope yielded an E_a value of 53.6 kJ mol^{-1} and a Q_{10} value of 2.2, whereas the region 28-50°C showed an E_a value of 102.5 kJ mol^{-1} and Q_{10} value of 3.7. These Q_{10} values agree with those reported for canned orange juice by Ross (1944), who showed that between 10 and 27°C, the rate of loss of ascorbic acid doubled for each 10°C rise, whereas from 27 to 37°C the rate quadrupled. Saguy *et al.* (1978a) reported a lower E_a value of 20.84 kJ mol^{-1} for grapefruit juice at 11.2 °Brix. Their value of 21.93 kJ mol^{-1} for 31.2 °Brix grapefruit juice is close to the E_a value of 21.33 kJ mol^{-1} for 20 °Brix lemon juice calculated in Table 6.6.

Differences in the reported and the observed E_a and Q_{10} values may be due to differences in the type of citrus juices used, the temperature range measured and the precision of the reaction rate constants.

The precision of the reaction rate constants is dependent on the analytical precision of the method used to measure the reactant concentration, and on the extent of reaction that has occurred (Benson, 1960). Benson (1960) presented a table which summarized what precision in analytical capability was required to measure k to a given degree of accuracy (Table 6.8).

Table 6.8: Errors in calculated rate constants caused by analytical errors.[1, 2]

Analytical Precision (%)	Percent error in reaction rate k at the following percent change in reactant species monitored:						
	1%	5%	10%	20%	30%	40%	50%
± 0.1	14	2.8	1.4	0.7	0.5	0.4	0.3
± 0.5	70	14	7	3.5	2.5	2	1.5
± 1.0	>100	28	14	7	5	4	3
± 2.0	>100	56	28	14	10	8	6

[1] From Benson (1960)

[2] Valid for a rate expression of the form $\frac{dC}{dt} = kC^n$ ($n = 0, \frac{1}{2}, 1 \ldots, 4$)

The analytical precision of the method for determining ascorbic acid was ± 0.2% (refer to Section 3.3.3). At 36°C for all soluble solids levels, the ascorbic acid degradation was around 40-50% (Table 6.4). Thus, from Benson's table, the error in calculated rate constants caused by analytical errors would be low, at around 0.6-0.8%. With a decrease in reactant conversion (increase in ascorbic acid retention) at the lower temperatures, precision of the rate constants decreased. Thus, for the k values at 10°C for 30, 40, and 50 °Brix samples where ascorbic acid degradation was only 5-10%, precision was decreased and uncertainty caused by analytical errors became 2.8-5.6%. A ± 5 to 10% error is considered acceptable for complex food systems (Labuza, 1984).

If higher precision of k values is desired, much higher analytical precision must be obtained and higher degrees of conversion used (Hill and Grieger-Block, 1980).

The precision of activation energy measurements is in turn dependent on the precision of the k values and on the size of the temperature interval chosen. According to Benson (1960), to measure E_a over a 10°C interval to within ± 0.5% would generally require temperature uncertainties of less than ± 0.03°C and rate constant uncertainties of ± 0.3%. The latter requirement would in turn necessitate an analytical precision of ± 0.1% over an extended range of concentration changes. These numbers indicate the difficulty in obtaining precise measurements of the activation energy and why it is difficult to be precise about the variation of E_a with temperature.

The precision in determining E_a within a 10°C interval at 27°C is generally about ± 3% for reactions in solution. This value given by Benson (1960) is applicable to simple chemical systems. For complex food systems, such as lemon juice, precision is expected to be lower.

Due to the limited number of results which could be used with confidence for prediction purposes, a single equation relating ascorbic acid degradation to temperature and soluble solids content could not be derived.

To determine if the effect of temperature and soluble solids level on the rate of ascorbic acid degradation was significant, a two-way Anova was performed. The results given in Table 6.9A show that if k values for all five soluble solids levels at the three storage temperatures were used for the computations, no significant difference in the rate could be attributed to either temperature or soluble solids level (as indicated by insignificant F-values). This did not seem correct since temperature was clearly observed to affect the rate of ascorbic acid degradation. Based on the assumption that the ascorbic acid breakdown mechanism was different in the 9 °Brix juice (as suggested by much higher k values and E_a value), a two-way Anova was conducted using the k values of the 20 to 50 °Brix juices.

Table 6.9A: Two-way Anova for the rates of ascorbic acid degradation in lemon juice and concentrates (9-50 °Brix).

Source	D.F.	SS	MS	F_c	F_t
Temperature	2	0.00664	0.00332	2.94	4.46
Soluble Solids	4	0.01692	0.00423	3.74	3.84
Error	8	0.00902	0.00113		
Total	14	0.03258			

Table 6.9B: Two-way Anova for the rates of ascorbic acid degradation in lemon juice concentrates (20-50 °Brix).

Source	D.F.	SS	MS	F_c	F_t
Temperature	2	0.0010183	0.0005091	18.25*	5.14
Soluble Solids	3	0.0002570	0.0000857	3.07	4.76
Error	6	0.0001675	0.0000279		
Total	11	0.0015527			

* Significant at p = 0.05

TUKEY TEST: $F_c = 1.18 < F_{.95;\ 1,\ 5} = 6.61$

The results, given in Table 6.9B, show that temperature had a significant effect on the rate of ascorbic acid degradation but not the soluble solids content.

The Tukey test was employed to determine if there was any interaction effect between temperature and soluble solids content. This procedure assumes that any interaction that may exist is a multiplicative function of the main effects of the two factors (Berenson *et al.*, 1983).

An insignificant F-value was obtained for the Tukey test which indicates that there was no interaction effect between temperature and soluble solids level.

It can thus be concluded that if ascorbic acid retention is used as the criterion of storage life of lemon juice, then temperature is the critical factor that has to be considered rather than soluble solids content. Ascorbic acid retention was highest in the juice samples stored at 10°C, and at this constant temperature, the rate of ascorbic acid degradation decreased with an increase in soluble solids content. Thus, for maximum retention of ascorbic acid in lemon juice it would be best to store it as a high Brix concentrate at low temperature (10°C). Over the 16-week storage period, maximum retention of ascorbic acid (95.69%) was attained in the 50 °Brix lemon concentrate stored at 10°C.

6.3.3 Browning

6.3.3.1 Effects of temperature and total soluble solids content on the degree of browning

The degree of browning of lemon juice as a function of total soluble solids content and time at three different storage temperatures is presented in Table 6.10. Not unexpectedly, for a given soluble solids content, there was more browning over a 16-week period at higher tempe-

Table 6.10: Browning (absorbance at 420 nm) in lemon juice during storage[1].

Temp. (°C)	Time (wk)	Total Soluble Solids (°Bx) 9	20	30	40	50
10	0	0.219[a]	0.219[b]	0.219[b]	0.219[c]	0.219[c]
	2	0.242	0.240	0.250	0.248	0.255
	4	0.244	0.241	0.251	0.262	0.262
	6	0.246	0.243	0.275	0.278	0.288
	8	0.280	0.271	0.284	0.293	0.298
	10	0.289	0.276	0.292	0.302	0.309
	12	0.300	0.298	0.300	0.318	0.325
	14	0.320	0.312	0.308	0.320	0.326
	16	0.335	0.318	0.314	0.330	0.325
20	0	0.219[a]	0.219[a]	0.219[b]	0.219[c]	0.219[d]
	2	0.250	0.260	0.274	0.278	0.291
	4	0.265	0.286	0.305	0.314	0.329
	6	0.292	0.302	0.311	0.332	0.346
	8	0.299	0.306	0.326	0.355	0.355
	10	0.336	0.336	0.364	0.390	0.410
	12	0.348	0.369	0.374	0.398	0.438
	14	0.354	0.370	0.415	0.420	0.452
	16	0.394	0.380	0.375	0.439	0.464
36	0	0.219[a]	0.219[a]	0.219[b]	0.219[c]	0.219[d]
	1	0.259	0.308	0.322	0.322	0.320
	2	0.290	0.306	0.332	0.359	0.380
	3	0.301	0.340	0.372	0.399	0.460
	4	0.378	0.386	0.416	0.452	0.510
	5	0.358	0.398	0.428	0.466	0.550
	6	0.425	0.405	0.472	0.512	0.620
	7	0.480	0.428	0.500	0.530	0.640
	8	0.468	0.460	0.522	0.598	0.730
	9	0.512	0.484	0.542	0.642	0.780
	10	0.545	0.508	0.588	0.660	0.840
	11	0.560	0.510	0.580	0.690	0.860
	12	0.610	0.610	0.630	0.770	0.940
	13	0.680	0.598	0.662	0.810	1.060
	14	0.680	0.630	0.728	0.840	1.060
	15	0.690	0.605	0.730	0.860	1.080
	16	0.700	0.640	0.760	0.920	1.260

[1] A common letter indicates that their adjusted means are not significantly different at a 5% level of significance at a constant temperature (based on an Anacova with time as a covariate)

ratures. Less clear-cut was the effect of soluble solids content on the degree of browning at constant temperature.

At 10°C, there was a small difference between the degree of browning for various soluble solids contents after 16 weeks storage, as compared to the browning at other temperatures. The 9 °Brix samples were, however, significantly browner than the other samples with higher soluble solids content.

At 20°C, the higher the soluble solids content, the greater the degree of browning, which can clearly be seen from the 10th to the 14th week. By the 16th week, the 9 °Brix juice exhibited more browning than the 20 °Brix juice. However, at 36°C the effect of soluble solids was reasonably consistent, a higher degree of browning being generally associated with a higher solids content for a given storage time.

In citrus juice, nonenzymic browning has been attributed to ascorbic acid degradation (Clegg, 1964). It was expected, therefore, that since ascorbic acid degradation was observed to decrease with an increase in soluble solids content, browning would follow the same trend. Such a trend was not observed in this experiment.

The findings of Saguy *et al*. (1978b) and Kanner *et al*. (1982) on grapefruit juice and orange juice supported the ascorbic acid theory of Clegg (1964), an increase in browning being accompanied by a decrease in ascorbic acid.

Browning at 9 °Brix juice concentration was significantly correlated ($r = -0.945$) (see Appendix 11 for correlation coefficients) with ascorbic acid loss. Thus, at this juice concentration, it is possible to conclude that ascorbic acid breakdown was the principal source of browning. The same conclusion was made by Clegg (1964) for single-strength lemon juice.

Since browning at higher juice concentrations increased with soluble solids level while ascorbic acid loss decreased, then there must be a different reaction or reactions predominating which results in browning at these high Brix concentrations.

Two other theories have been put forward to explain non-enzymic browning (Stadtman, 1948; Clegg, 1964). One is the Maillard reaction, involving the formation of sugar-amine condensation products, which, after undergoing Amadori rearrangement and a variety of secondary reactions, give rise to dark coloured 'melanoidin' compounds.

The other theory (called the active aldehyde theory) postulates that browning involves the decomposition of sugars and sugar acids to furfuraldehydes, or similar compounds, characterised by having an active carbonyl group. These products then condense with nitrogenous compounds and/or polymerise to form brown resinous materials.

Browning due to the Maillard reaction would be expected to increase with soluble solids levels because of increase in concentration of sugars and amines. However, some authors have indicated that the sugar-amino acid reactions of the Maillard type are of minor importance in citrus juices because of the high acidities involved. Clegg (1964) pointed out that the Amadori rearrangement requires a near neutral, or slightly alkaline medium for optimum efficiency of reaction; therefore it was thought unlikely that this mechanism would be the major contributor to the browning of a highly acidic product such as lemon juice. Acid pH values were reported to inhibit the Maillard reaction (Mauron, 1981). It has been observed that an alkaline medium was a prerequisite for significant browning in sugar-amino acid model systems (Schroeder *et al.*,1955; Willits *et al.*, 1958; Underwood *et al* ., 1959). The Maillard reaction could be one of the reactions occurring in the lemon

concentrates, but it is most probably not the predominant browning reaction.

Clegg (1964) stated that the "active aldehyde theory" appeared more likely to apply to a highly acidic product such as lemon juice with ascorbic acid being specifically involved. This pathway has furfuraldehydes and similar compounds as end products.

In the study of stored dehydrated orange juice by Tatum *et al*. (1967), 18 compounds were obtained that were not present in the control sample. Of these 18 storage decomposition products, most had been shown to be nonenzymic browning products for hexoses or ascorbic acid, based on model system studies (Shaw *et al*., 1977). Among the 18 compounds, 8 can originate from acid-catalysed degradation of D-fructose (Shaw *et al*., 1967) and another 8 from acid-catalysed degradation of ascorbic acid (Tatum *et al*., 1969). Three of the 8 products, one of which was furfural, were common for both D-fructose and ascorbic acid degradation.

Off-flavour development in single-strength orange juice during storage was studied by Tatum *et al*. (1975), who identified 10 storage products. Five of these compounds (including furfural and HMF), had been identified previously as storage decomposition products from instant orange juice (frozen concentrated orange juice dehydrated to a powder containing 1-3% moisture) (Tatum *et al*., 1967). These 5 compounds had been shown in model studies to be formed by degradation of fructose and/or ascorbic acid (Shaw *et al*., 1977). Reducing sugars (fructose and glucose) breakdown by simple acid-catalysed enolisation and dehydration to carbonyl compounds (Hodge and Osman, 1976; Shaw *et al*., 1977). Work with model systems of lemon juice by Clegg and Morton (1965) confirmed that the α,β-unsaturated carbonyls formed by ascorbic acid breakdown, were potent browning agents.

The above studies suggest that acid-catalysed breakdown of reducing sugars and ascorbic acid are both possible causes of browning in citrus juices. Carbonyl development and consequently browning in lemon juice model systems containing glucose, were attributed mainly to ascorbic acid breakdown, with glucose making a separate delayed contribution to α,β-unsaturated carbonyl development (Clegg and Morton, 1965). Thus, for the single-strength lemon juice in this present experiment, ascorbic acid degradation could have been the predominant source of browning. However, at higher soluble solids concentrations, sugar breakdown could have been the main source of furfural formation and browning. This is supported by the observation that ascorbic acid degradation decreased with an increase in soluble solids level in the juice, while browning and furfural formation increased with soluble solids concentration.

Citrus juices contain both nonreducing (sucrose) and reducing (fructose and glucose) sugars. In lemon juice, the reducing sugars dominate and may account for about 90% of the total sugars (Bartholomew and Sinclair, 1951). Sucrose may contribute to browning after acid-catalysed hydrolysis to the more reactive reducing sugars fructose and glucose (Curl and Talburt, 1961; Karel and Labuza, 1968).

Some studies have indicated that the amino acids and sugars are of more than just minor importance in the darkening of citrus juices (Varsel, 1980).

Curl (1949), in a study conducted with a synthetic orange juice, reported that the loss of ascorbic acid occurred in the presence of citric acid and potassium citrate buffer alone, but that the losses were increased by the addition of the sugars fructose, sucrose and glucose in that order. He found that darkening of the synthetic juice occurred

principally when both amino acids and sugars were present; and the effect was even more pronounced in the presence of ascorbic acid.

Kato and Sakurai(1964) studied the effect of ascorbic acid, organic acids, amino acids, and inorganic ions on browning in a model system. They determined that 3-deoxyglucosone and 5-hydroxymethylfurfural were formed by the action of organic acids on fructose (formed by inversion of sucrose). They concluded that amino acids played a role in the development of browning.

Clegg (1964) reported that the presence of amino-acids in lemon juice model systems increased the intensity of browning. The reduction of amino acid levels by 50% had no significant effect on the degree of browning of the model systems; this was in agreement with their earlier finding that only when the nitrogen content was lower than 0.3 µg of N/mL was the browning of lemon juice retarded. Some of the active groups resulting from oxidation of ascorbic acid, and possibly other reactive compounds, were polymerising and not involving amino acids as was shown by the browning of nitrogen-free model systems.

Thus, the amino acids appear not to be a limiting factor in the browning of lemon juice, based on the ascorbic acid theory. The principal role of amino-acids in the non-enzymic browning of fruit products appeared to be to increase the browning potential after the oxidation of the ascorbic acid to reactive carbonyl compounds rather than in the initial reaction leading to the formation of reactive compounds (Clegg, 1964).

In this present experiment, at high juice concentrations, it is possible that the amino acids were reacting with carbonyls and similar compounds, which were products of other reactions, such as acid-catalysed sugar breakdown.

Burton *et al.* (1963a) have suggested that furfural and hydroxymethylfurfural are relatively inert in the browning of sugar-amino acid model systems but may play a more important part in the browning of fruit juices (Burton *et al.*, 1963b). Kanner *et al.* (1981, 1982) reported that reaction between furfural and other compounds occurs at a high rate with increasing juice concentration. These reactions also lead to the development of strong colour and brown pigments (Burton *et al.*, 1962; Clegg and Morton, 1965; Rizzi, 1974). Thus, these reactions with furfural may also be contributing to the browning in lemon juice concentrates.

In a further study (Clegg, 1966), citric acid appeared to contribute to the build-up of browning but not in the capacity of accelerating the breakdown of ascorbic acid leading to greater pigment formation. The study showed that a specific brown pigment could be detected only when citric acid and ascorbic acid were included. The inference was that citric acid played an integral part in the browning of acidic products which contained ascorbic acid. At high juice concentrations, citric acid would be present at high levels and this could also have contributed to the increased browning observed in this experiment.

To confirm this theory, the citric acid content of the lemon juice samples of this present experiment were determined using the high pressure liquid chromatographic method discussed in Section 3.8. Analysis was conducted every month, and the results are given in Appendix 9. The analysis of variance of the results indicated that citric acid levels significantly decreased with an increase in storage time. No significant differences could be attributed to the differences in storage temperature or soluble solids level.

The loss of citric acid as a function of time, supports the theory that it is involved in the browning of lemon juice. However, despite the significant decrease in citric acid content, the loss in citric acid over the 16-week storage period was only 9.9 mg/mL (15.6%). The observation that loss of citric acid was not a function of temperature and soluble solids content suggests that although it contributes to browning in lemon juice, its contribution is minor and is not the cause of the observed differences in browning intensities among the different juice concentrates at the three storage temperatures.

When an accelerated temperature storage experiment was conducted with single-strength lemon juice at 55°C, no significant losses in citric acid content were observed over the 6-day storage period (results are presented in Appendix 10). This suggests that citric acid's role in the browning of lemon juice may be dependent on such factors as temperature, storage time and juice concentrations.

Malic, tartaric, and oxalic acids have been reported to cause colour formation when heated with ascorbic acid at 100°C (Lamden and Harris, 1950). Malic acid is one of the acids present in lemon juice in considerable amounts (refer to composition table in Appendix 7) (Bartholomew and Sinclair, 1951; Swisher and Swisher, 1980). Thus, several reactions are possibly resulting in the browning of lemon juice concentrates.

Many of the studies on browning have been conducted on model systems in an attempt to isolate reacting compounds. Lemon juice, however, is a complex system and it is possible that browning is not a simple reaction, but a group of reactions with one of them predominating in certain citrus juices and under certain juice conditions (i.e. solids content, oxygen content, metal ions).

6.3.3.2 Kinetics of the browning reaction

Browning measurements were plotted against time (Figure 6.4). No initial lag period was observed, although the 9 and 20 °Brix juices at 10°C showed insignificant change in browning over the 2-6 weeks period. Most studies have reported an initial lag period (Karel and Nickerson, 1964; Saguy *et al.*, 1978b) and a lag period was also observed in experiments 4 and 5.

The rate constant, obtained in experiment 5, for browning in single-strength lemon juice stored at 36°C was 0.0133-0.0139 absorbance units/week. The value obtained for 9 °Brix juice under the same storage temperature (36°C) in this experiment was considerably higher at 0.0316 absorbance units/week. This may explain the absence of the lag period. The reaction proceeded at a fast rate and the period where colourless intermediates are formed could have occurred within the first few days. The two-week interval between consecutive analyses of browning in the samples stored at 10 and 20°C, may have been sufficiently long for significant changes in browning to occur and thus any lag period would not have been detected.

A zero-order and first-order model were fitted to the browning data (Table 6.11). Regression analysis indicated that a good fit for the browning data was obtained for both zero- and first-order models. Little difference in R^2 values between the two models was observed in the samples where the browning reaction occurred at a slow rate, such as in samples at 10°C. The change in the browning measurements over the 16-week storage period at 10°C for all five soluble solids levels was less than 53%. With an increase in the extent of browning, the difference between the two models became distinct, with the zero-order model giving a better fit (higher R^2). This was demonstrated in the samples at 20 and 36°C, where change in browning measurements over the 16-week storage period was greater than 71%.

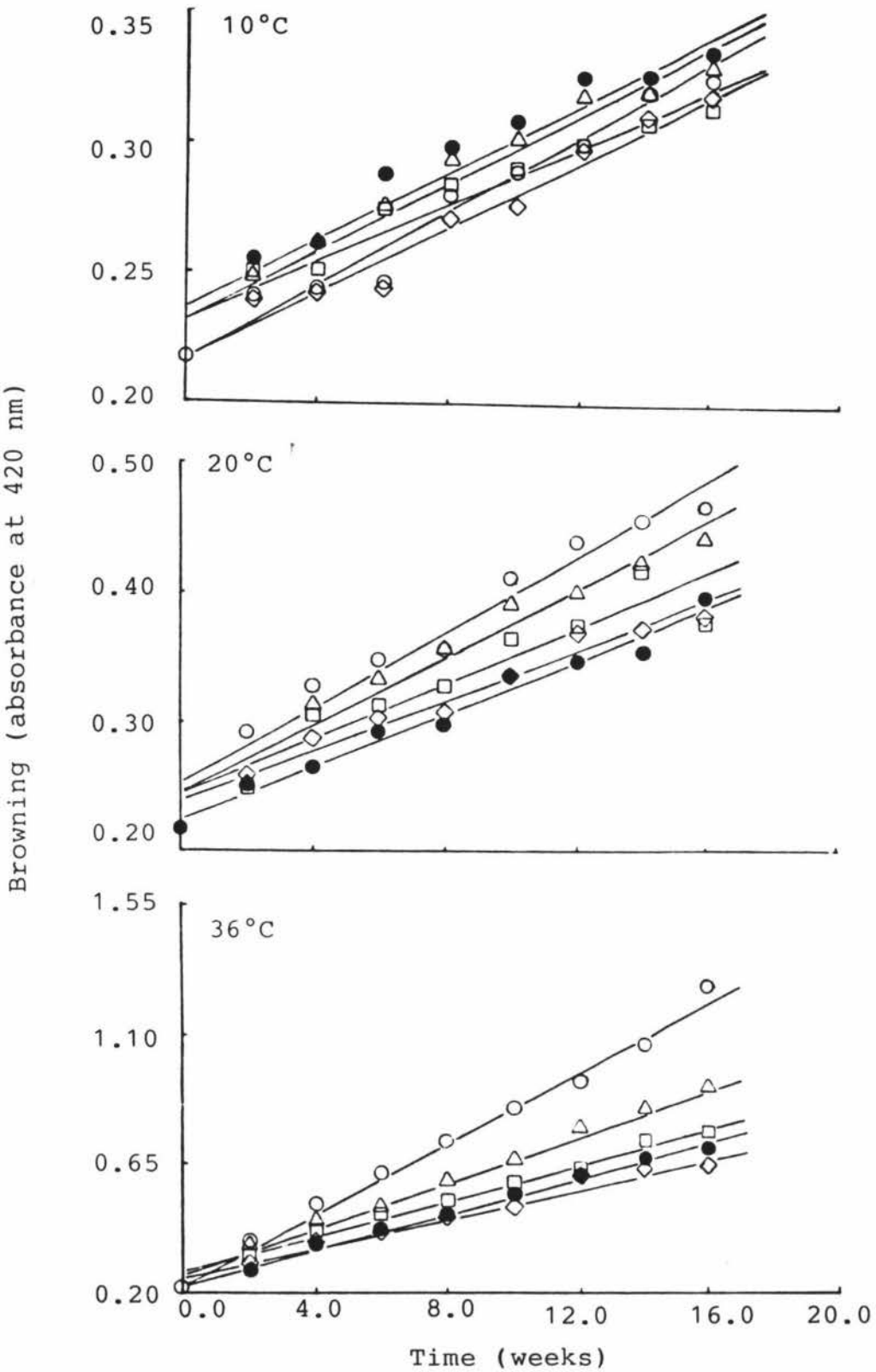

Figure 6.4: Browning in lemon juices and concentrates (● - 9°Bx, ◇ - 20°Bx, □ - 30°Bx, △ - 40°Bx, ○ - 50°Bx) during storage.

Table 6.11: Results of the computations for zero- and first-order reactions of browning based on regression analyis

		Zero-order			First-order		
Solids Content (°Bx)	Temperature (°C)	k ($\times 10^{-2}$ unit wk^{-1})	C_o (abs. units)	R^2 (%)	k ($\times 10^{-2}$ wk^{-1})	ln C_o (abs. units)	R^2 (%)
9	10	0.71	0.218	97.0	2.59	-1.51	97.0
	20	1.02	0.225	98.2	3.40	-1.47	97.3
	36	3.16	0.227	98.5	7.15	-1.37	95.5
20	10	0.632	0.218	96.6	2.36	-1.51	96.7
	20	0.978	0.236	96.0	3.23	-1.43	93.4
	36	2.49	0.261	96.9	5.79	-1.28	92.8
30	10	0.557	0.232	94.4	2.07	-1.46	91.9
	20	1.03	0.247	88.7	3.30	-1.39	86.2
	36	3.09	0.271	98.5	6.46	-1.22	92.8
40	10	0.663	0.232	96.1	2.40	-1.45	93.6
	20	1.28	0.247	96.1	3.88	-1.38	91.2
	36	4.09	0.264	99.2	7.60	-1.20	93.7
50	10	0.653	0.237	91.3	2.35	-1.43	88.5
	20	1.45	0.251	95.3	4.22	-1.36	90.3
	36	5.87	0.254	98.9	9.18	-1.15	93.3

It has been reported by Labuza and Kamman (1983) that a reaction conversion of 50% may be necessary to distinguish any difference between zero- and first-order plots of the data.

The intercept (C_o) of the regression equations should be 0.219 absorbance units, which corresponds to the initial absorbance reading of the juice samples. As can be seen in Table 6.11, deviations from this value were observed which may be attributed to a higher initial rate of browning due to the presence of residual oxygen in the juice and the headspace, particularly in the samples at 36°C and those with higher soluble solids levels. It has been shown in experiment 5 that the presence of oxygen accelerates the browning reaction. After this initial period (~ 14 days), where aerobic reactions could have predominated, an anaerobic condition could have prevailed throughout the rest of the storage period.

The precision of the rate constants of browning was expected to be lower than for ascorbic acid degradation since the analytical precision of the method for determining browning was 3.5% (refer to Section 3.6.1.3) as opposed to 0.2% for ascorbic acid determination. The change in the degree of browning was generally higher than 50%. From the table presented by Benson (1960) (Table 6.8), the errors in the calculated rate constants of browning caused by analytical errors would be around 10% or less for greater than 50% change in browning measurement.

Using the rate constant values for the browning reaction following the zero-order model, the relationship between rate of browning and temperature was observed to follow the Arrhenius model (Figure 6.5). The E_a and Q_{10} values are given in Table 6.6.

The Q_{10} values for the lemon juice concentrates (20-50 °Brix) for the two temperature ranges did not differ considerably from each other. This is due to the good fit of the k values to the Arrhenius plots (as shown by the high R^2 values).

The k,E_a and Q_{10} values for the 20 to 50 °Brix juices increased with increase in soluble solids content (Figures 6.6 and 6.7)

The E_a value for the 9 °Brix juice was 41.65 kJ mol^{-1} and ranged from 37.93 to 60.42 kJ mol^{-1} for 20 to 50 °Brix lemon juice, respectively.

The Q_{10} for 9 °Brix juice was 1.71 and ranged from 1.63 to 2.19 for the 20 to 50 °Brix lemon juice, respectively. The values are comparable to those reported by Saguy *et al.* (1978b) for grapefruit juice during thermal and concentration processes (see Table 6.12), despite the higher temperature range over which their values were obtained.

Table 6.12: E_a and Q_{10} values for grapefruit juice during thermal and concentration processes[1].

	°Bx	E_a (kJ mol^{-1})	Q_{10} (70-80°C)
Lag Period			
	11.2	34.1	1.40
	31.2	41.3	1.51
	47.1	64.9	1.90
Post Lag Period			
	11.2	63.4	1.88
	31.2	63.3	1.88
	47.1	71.8	2.04

[1] From Saguy *et al.* (1978b)

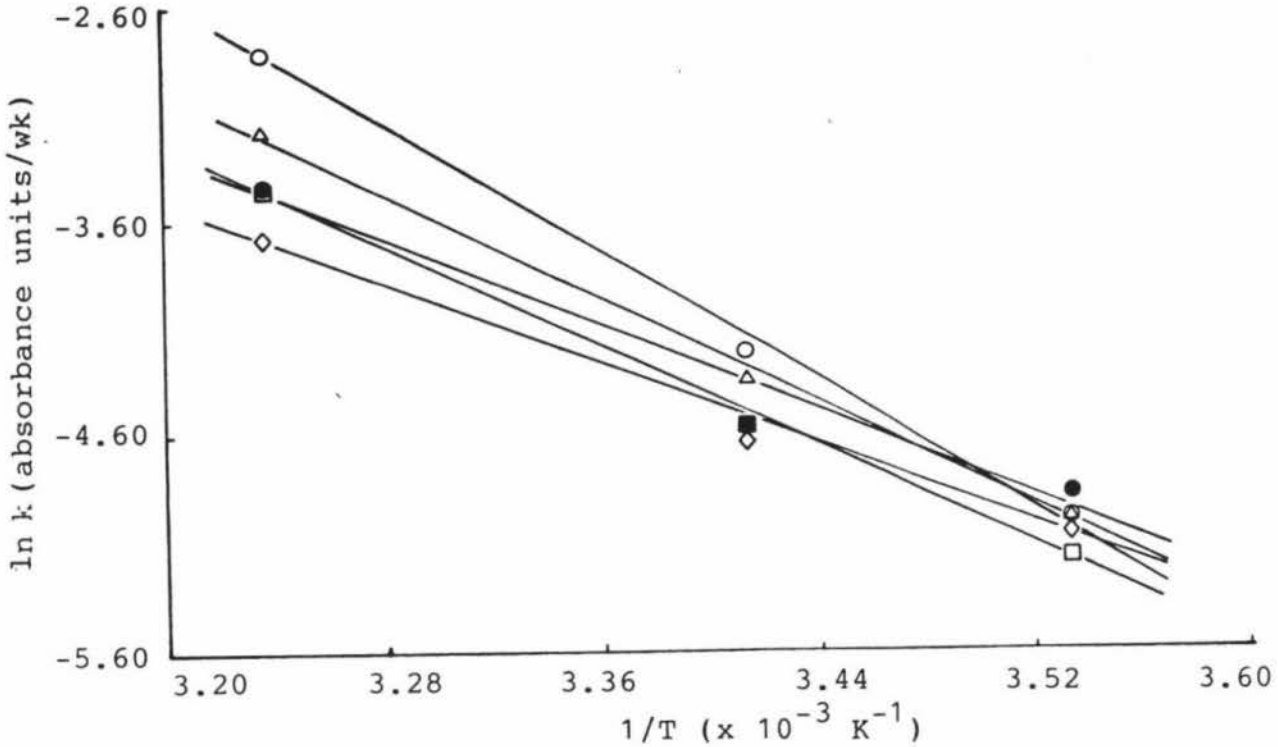

Figure 6.5: Arrhenius plots for the browning reaction in lemon juice and concentrates (● - 9°Bx, ◇ - 20°Bx, □ - 30°Bx, △ - 40°Bx, ○ - 50°Bx).

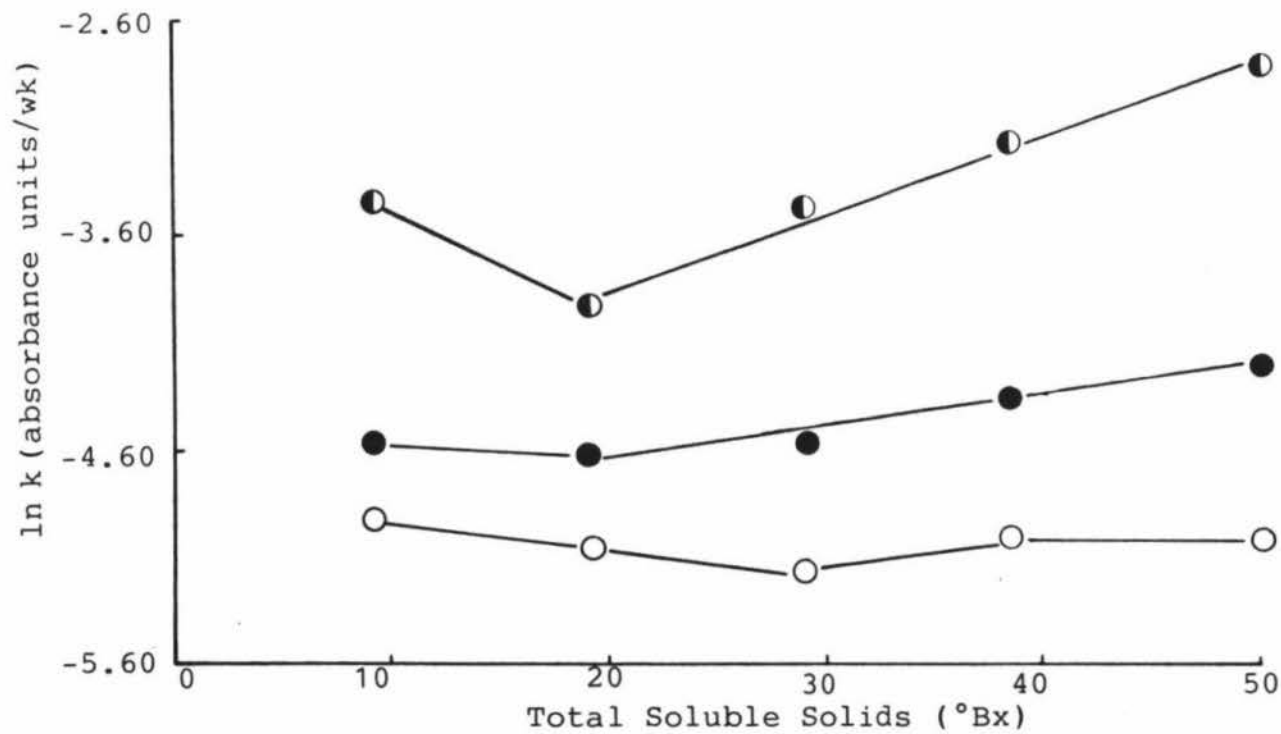

Figure 6.6: Changes in the rate of browning with increases in soluble solids level at 10°C (◐), 20°C (○) and 36°C (●) (lines of best fit based on linear regression analysis for the 20-50°Bx juices at 20 and 36°C; points at 10°C have been joined by straight lines).

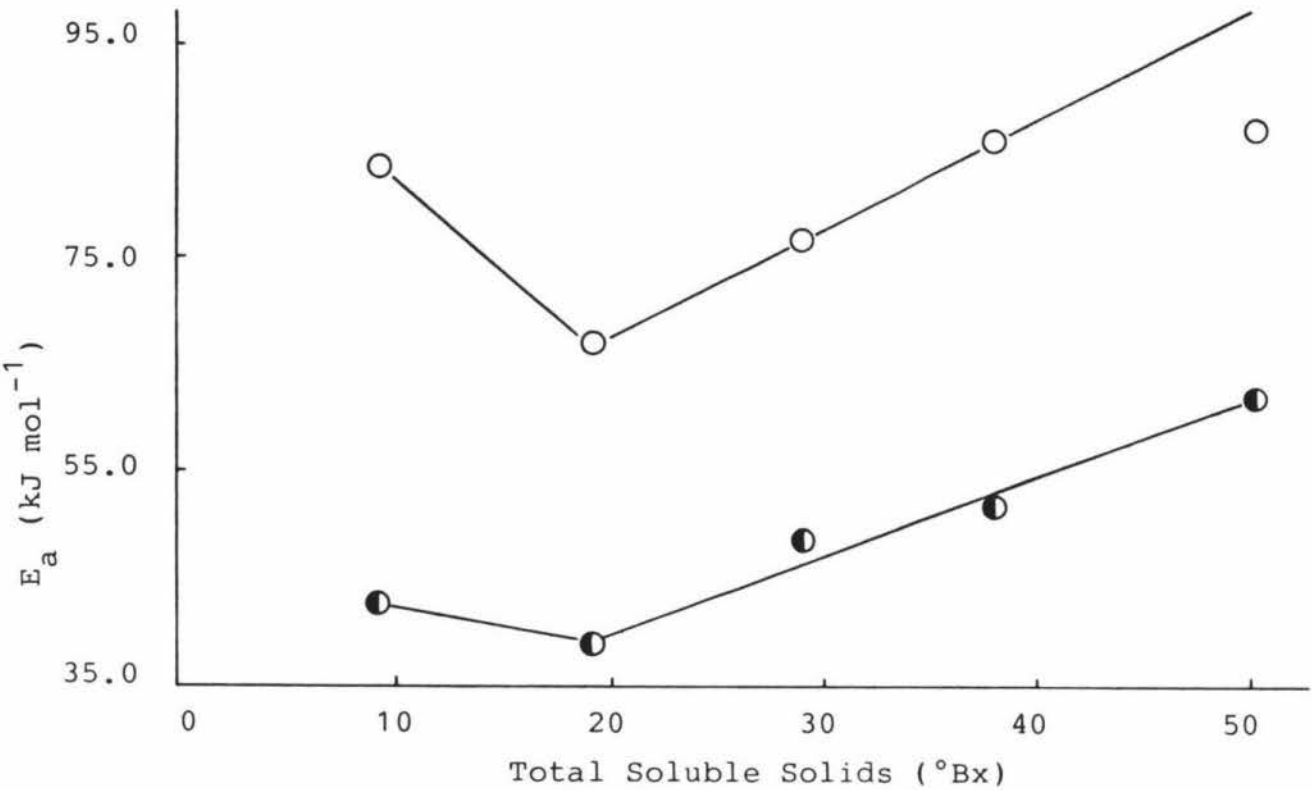

Figure 6.7: Activation energy (E_a) values for browning (◐) and furfural formation (○) in lemon juice and concentrates.

The rate constants and E_a value for the 9 °Brix juice were higher than those of the 20 °Brix samples, probably because a different browning reaction was predominating in each of the juice samples. Ascorbic acid degradation could have been the principal source of browning in 9 °Brix juice and this reaction was significantly lower in 20 °Brix juice. The browning reaction predominating in the 20 °Brix juice was not apparently as fast as the one taking place in the 9 °Brix juice samples. However, at higher soluble solids levels, this reaction was accelerated.

The two-way Anova of browning (Table 6.13A) shows that significant differences in rates are attributable to the storage temperature but not to the total soluble solids content at a 5% level of significance. Based on the assumption that the browning reaction in the 9 °Brix juice differed from that which had occurred in the higher Brix concentrates, a two-way Anova for the concentrates (20-50 °Brix) was also performed. As shown in Table 6.13B, the results are similar to those obtained for the 9 to 50 °Brix samples.

The insignificant effect of soluble solids concentration could be due to the small differences in k values among the juices of different soluble solids content at 10 and 20°C.

The Tukey test gave a highly significant F-value which indicates an interaction between temperature and soluble solids content. The interaction can be considered to give a synergistic effect. A synergistic effect occurs when the two factors interact in a joint, multiplicative manner that is greater than the mere presence of the two effects taken together (Berenson *et al.*, 1983).

The effect of this interaction on the rate of browning is also shown in the Arrhenius plots (Figure 6.5). If there was no interaction, then the regression lines corresponding to the different soluble solids levels would

Table 6.13A: Two-way Anova for the reaction rates of browning in lemon juice and concentrates (9-50 °Brix)

Source	D.F.	SS	MS	F_c	F_t
Temperature	2	0.0027583	0.0013792	27.42*	4.46
Soluble Solids	4	0.0003134	0.0000784	1.56	3.84
Error	8	0.0004027	0.0000503		
Total	14	0.0034744			

* Significant at p = 0.05

TUKEY TEST: $F_c = 867.01 > F_{.95;\ 1,\ 7} = 5.59$

Table 6.13B: Two-way Anova for the reaction rates of browning in lemon juice and concentrates (20-50 °Brix)

Source	D.F.	SS	MS	F_c	F_t
Temperature	2	0.0024298	0.0012149	19.44*	5.14
Soluble Solids	3	0.0002961	0.0000987	1.58	4.76
Error	6	0.0003752	0.0000625		
Total	11	0.0031012			

* Significant at p = 0.05

TUKEY TEST: $F_c = 643.70 > F_{.95;\ 1,\ 5} = 6.61$

be expected to be parallel. The graph shows that the total soluble solids concentrations had little effect on the rate of browning at 10°C but had a comparatively large effect at 36°C. The significant increase in E_a (which is proportional to the slope of the Arrhenius plot) of the lemon juice concentrates with an increase in soluble solids content, also indicates that a possible interaction between temperature and soluble solids content exists.

6.3.3.3 Predictive model for browning

An attempt was made to derive a predictive model describing the effect of these two factors (temperature and soluble solids content) on the rate of browning.

A plot of E_a against the soluble solids concentration (Figure 6.7) showed that there was a linear relationship between the E_a of browning and the soluble solids content for the lemon juice concentrates of 20 to 50 °Brix at 10 to 36°C.

$$E_a = 25.9 + 0.703\ (°Bx) \qquad (6\text{-}4)$$

$$(R^2 = 97.2\%)$$

Based on a regression analysis, a linear relationship between lnA and E_a for the 20 to 50 °Brix juices at 10 to 36°C was also indicated.

$$\ln A = -5.21 + 0.427\ (E_a) \qquad (6\text{-}5)$$

$$(R^2 = 100.0\%)$$

The temperature dependence of the rate of browning had been shown earlier to follow the Arrhenius model (Equation 6-1). The ln A and E_a values in the Arrhenius equation were substituted with equations 6-5 and 6-4, respectively, and an equation was derived which followed the model equation:

$$\hat{Y} = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 \qquad (6\text{-}6)$$

A multiple regression analysis based on this equation gave the following predictive model:

$$\ln k = 5.80 + 0.302\ (°Bx) - 3117(1/T) - 84.6(°Bx/T) \qquad (6\text{-}7)$$

where: ln k = ln of the rate constant of browning
°Bx = soluble solids concentration, °Brix
T = temperature, K

The detailed computer output for the predictive model equation is given in Appendix 13.

The third predictor variable (°Bx/T) demonstrates the multiplicative effect of temperature and total soluble solids content on the rate of the browning reaction.

A high R^2 value of 99.4% was obtained indicating a good fit of the data to the regression line. This also suggests that there is a good agreement between predicted and actual values of the rate of browning for the lemon juice concentrates of 20 to 50 °Brix soluble solids content at 10 to 36°C.

The Anova table for the regression equation (see Appendix 13) shows that significant F-values at the 5% level of significance were obtained for the three predictor values. This means that each of the predictor variables contributed significantly to the overall model.

A plot of this predictive model is shown in Figure 6.8.

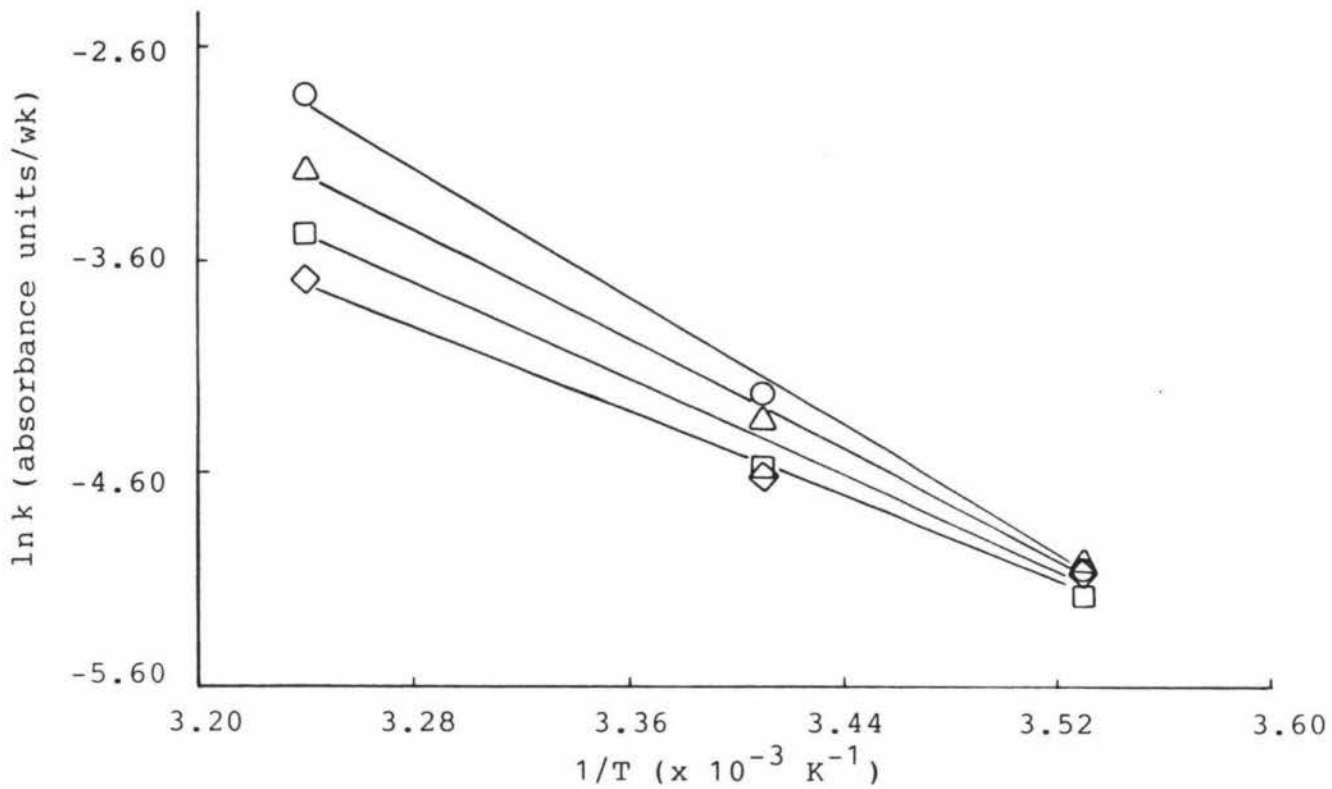

Figure 6.8: Plot of the predictive model (Equation 6-7) for browning, showing the actual k values (◇ - 20°Bx, □ - 30°Bx, △ - 40°Bx, O - 50°Bx) and the predicted regression lines for the lemon juice concentrates at 10 to 36°C.

6.3.4 Furfural

Furfural content increased with an increase in storage temperature for all juice concentrations (Table 6.14 and Figure 6.9). At a constant temperature of 36°C, furfural accumulated at an increasing rate with increasing soluble solids content. Furfural was formed in the 50 °Brix samples at 20°C at a slower rate than in the 30 and 40 °Brix samples. At 10°C, the formation of furfural in the 40 and 50 °Brix juice concentrates was lower than in the 20 and 30 °Brix juice concentrates.

Furfural formation was plotted against storage time (Figure 6.9) and both a zero-order and first-order model fitted to the data. Regression analysis indicated that furfural formation followed the zero-order model better than the first-order model at all five soluble solids levels (Table 6.15).

The precision of the rate constants for furfural formation would be similar to that for browning since the analytical precision of the method for determining furfural concentration was 3.4% (see Section 3.4.3) which was very near that for browning (3.5%). Changes in furfural concentration over the 16-week storage period were all greater than 50%. Based on Benson's table (Table 6.8), errors in calculated rate constants would be less than 10%.

Several research workers have shown that furfural is one of the main degradation products of ascorbic acid or dehydroascorbic acid (Huelin, 1953; Kurata and Sakurai, 1967; Tatum *et al.*, 1969). The results of this experiment, which correlated poorly with ascorbic acid retention at high juice concentrations (see Appendix 12), suggest that at these high soluble solids levels (20-50 °Brix) furfural was being formed by a different reaction sequence than via ascorbic acid breakdown.

Table 6.14: Furfural formation (mg/L) in lemon juice during storage[1].

Temp. (°C)	Time (wk)	Total Soluble Solids (°Bx) 9	20	30	40	50
	0	0.13[a]	0.13[b]	0.13[b]	0.13[b]	0.13[b]
	2	0.16	0.17	0.19	0.32	0.20
	4	0.17	0.22	0.22	0.23	0.23
	6	0.25	0.27	0.36	0.35	0.32
10	8	0.26	0.41	0.44	0.45	0.40
	10	0.31	0.49	0.54	0.51	0.46
	12	0.33	0.59	0.61	0.59	0.54
	14	0.39	0.65	0.66	0.62	0.55
	16	0.39	0.76	0.75	0.63	0.68
	0	0.13[a]	0.13[b]	0.13[c]	0.13[c]	0.13[c]
	2	0.24	0.40	0.47	0.50	0.50
	4	0.59	0.84	0.93	1.03	0.98
	6	0.87	1.13	1.33	1.44	1.30
20	8	1.01	1.18	1.39	1.46	1.33
	10	1.50	1.72	2.21	2.20	1.92
	12	1.67	1.93	2.29	2.31	2.10
	14	1.95	2.19	2.31	2.58	2.60
	16	2.05	2.68	2.99	3.02	2.80
	0	0.13[a]	0.13[b]	0.13[c]	0.13[cd]	0.13[d]
	1	0.38	0.84	1.14	1.23	1.21
	2	1.21	1.74	1.98	2.15	2.11
	3	2.06	2.55	3.00	3.33	3.30
	4	3.14	3.59	4.41	4.24	4.22
	5	3.73	4.07	4.95	5.02	4.66
	6	4.08	4.34	5.01	5.47	5.51
	7	5.05	6.20	6.80	6.76	6.68
36	8	5.23	7.21	7.55	7.82	8.08
	9	5.43	7.29	8.08	8.60	8.50
	10	5.32	7.36	8.30	8.70	8.57
	11	5.69	7.39	8.96	9.35	9.48
	12	5.47	7.39	8.94	9.51	10.00
	13	5.36	7.58	8.96	9.81	10.46
	14	6.15	8.50	9.55	10.72	10.79
	15	6.60	8.96	10.79	11.90	12.16
	16	6.18	9.02	10.56	11.64	13.21

[1] A common letter indicates that their adjusted means are not significantly different at a 5% level of significance at a constant temperature (based on an Anacova with time as a covariate)

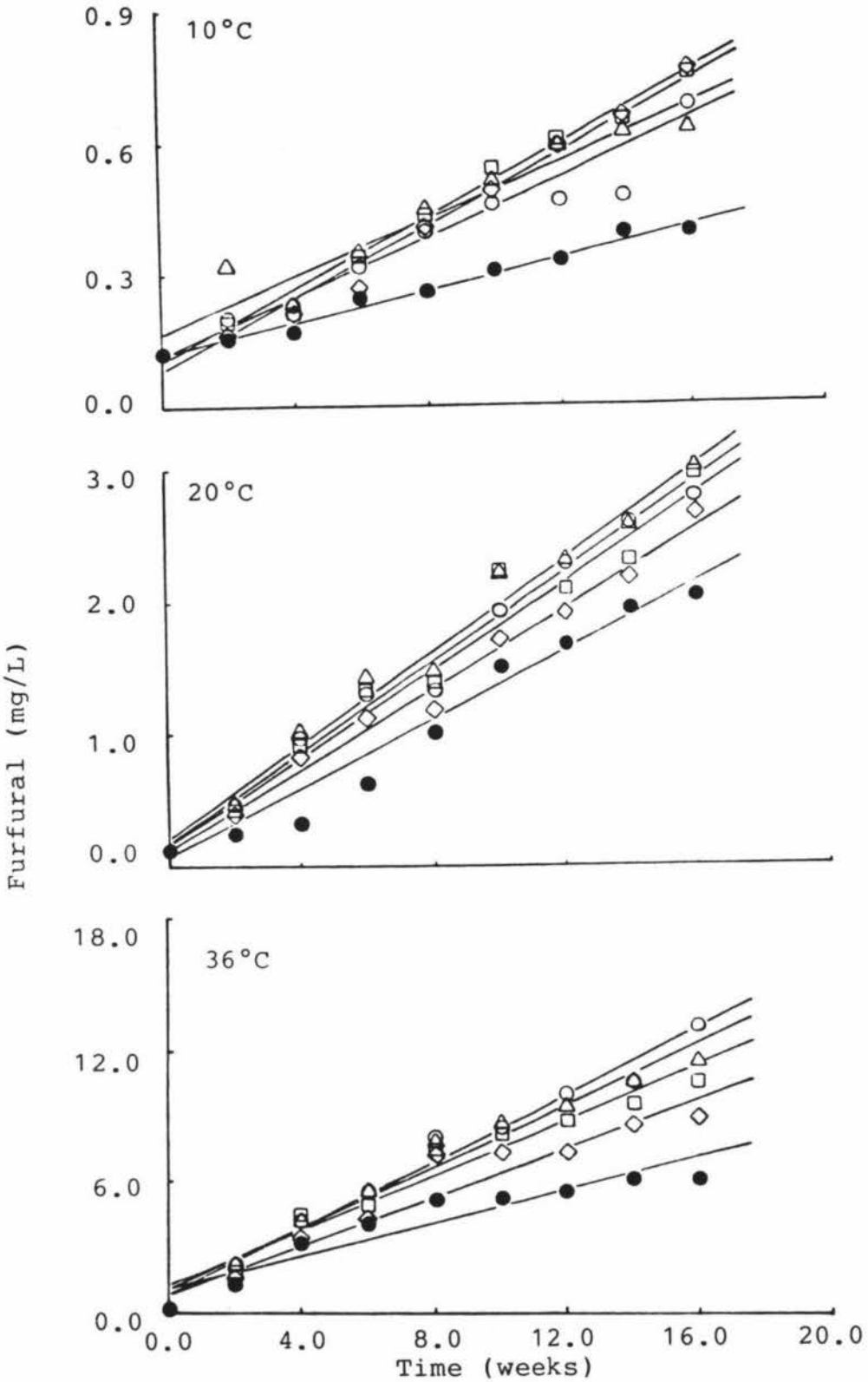

Figure 6.9: Furfural formation in lemon juices and concentrates (●- 9°Bx, ◇- 20°Bx, □- 30°Bx, △ - 40°Bx, O - 50°Bx) during storage.

Table 6.15: Results of computations for zero- and first-order reactions of furfural formation based on regression analysis .

Solids Content (°Bx)	Temperature (°C)	Zero-order k ($x10^{-1}$ mg L^{-1} wk^{-1})	Zero-order C_o (mg/L)	Zero-order R^2 (%)	First-order k ($x\ 10^{-1}$ wk^{-1})	First-order ln C_o (mg/L)	First-order R^2 (%)
9	10	0.176	0.125	97.6	0.717	-1.97	95.4
	20	1.30	0.072	98.7	1.66	-1.53	88.2
	36	3.83	1.13	86.3	1.70	-0.242	61.5
20	10	0.410	0.082	98.3	1.14	-1.96	97.7
	20	1.53	0.133	98.8	1.61	-1.27	84.0
	36	5.54	1.11	92.5	1.75	-0.001	64.3
30	10	0.404	0.110	98.8	1.10	-1.87	94.6
	20	1.71	0.190	96.8	1.64	-1.16	80.7
	36	6.48	1.24	95.0	1.76	0.142	63.1
40	10	0.315	0.174	92.7	0.880	-1.66	82.8
	20	1.76	0.222	98.2	1.63	-1.11	79.2
	36	7.18	1.10	97.3	1.79	0.169	64.3
50	10	0.334	0.123	98.7	0.977	-1.84	95.3
	20	1.65	0.195	98.7	1.59	-1.15	80.4
	36	7.71	0.834	98.5	1.85	0.134	66.5

With 9 °Brix juice where high ascorbic acid breakdown was observed, the furfural formed could be largely attributed to ascorbic acid degradation. A significant correlation coefficient of r = -0.971 (see Appendix 11) between furfural formation and ascorbic acid retention was obtained at this soluble solids level.

A correlation coefficient of r = 0.85 was obtained between browning and furfural content considering all juice samples at all concentrations of total soluble solids. It is possible that browning and furfural are results of the same reactions predominating at high juice concentrations (20-50 °Brix). The "active aldehyde theory" of nonenzymic browning suggests that the decomposition of sugars and sugar acids to furfuraldehydes, are involved (Clegg, 1964; Shaw *et al.*, 1977).

Furans and especially furfural and α,β-unsaturated aldehyde are known to be reactive compounds (Dunlop and Peter, 1953). Reactions of α-amino acids with furfural yielded pyrroles by a nucleophilic attack of the amines at the electrophilic position of the furan nucleus and alde-amines by an attack of the amine on the aldehyde group (Rizzi, 1974). These reactions also lead to the development of strong colours and browning pigments (Burton *et al.*, 1962; Clegg and Morton, 1965; Rizzi, 1974).

The decrease in the rate of furfural formation in the 50 °Brix juice samples at 20°C and in 40 and 50 °Brix juices at 10°C, suggests that furfural may be reacting with other compounds. Kanner *et al* . (1981, 1982) reported that furfural accumulation decreased with an increase in soluble solids content. This was explained by their observations that indicated that the reaction between furfural and other compounds occurred at a higher rate with increasing juice concentration.

At 36°C, the accumulation of furfural was higher than its decomposition, and hence the continued increase in the rate of furfural formation with increase in soluble solids content. At 10 and 20°C however, the rate of furfural decomposition became greater than its formation in lemon juice of high soluble solids content (30-50 °Brix) (Figure 6.10).

The results of the studies of Kanner *et al.* (1981, 1982) and of this experiment imply that at high juice concentrations and long storage periods, furfural and similar compounds may play a more significant role in the browning of lemon juice than was suggested by Clegg and Morton (1965).

In single-strength lemon juice, furfural could serve as an index of quality deterioration. This is supported by the high correlation coefficients obtained between furfural formation and ascorbic acid retention ($r = -0.971$) and between furfural formation and browning ($r = 0.945$) in 9 °Brix juice. Because furfural is formed and decomposed simultaneously in lemon juice concentrates, it would be more difficult to use it as an index for predicting quality changes in juices of high soluble solids content. This suggestion is in agreement with that made by Kanner *et al.* (1981).

At a constant soluble solids content, the temperature dependence of furfural formation followed the Arrhenius model (Equation 6-1). The E_a and Q_{10} values are given in Table 6.6. The Arrhenius plots are shown in Figure 6.11.

The fit of the k values to the Arrhenius plots were not as good as that for browning, as indicated by the lower R^2 values. This is probably due to the simultaneous formation and decomposition of furfural in the juice samples, which are temperature dependent and thus affect the k values.

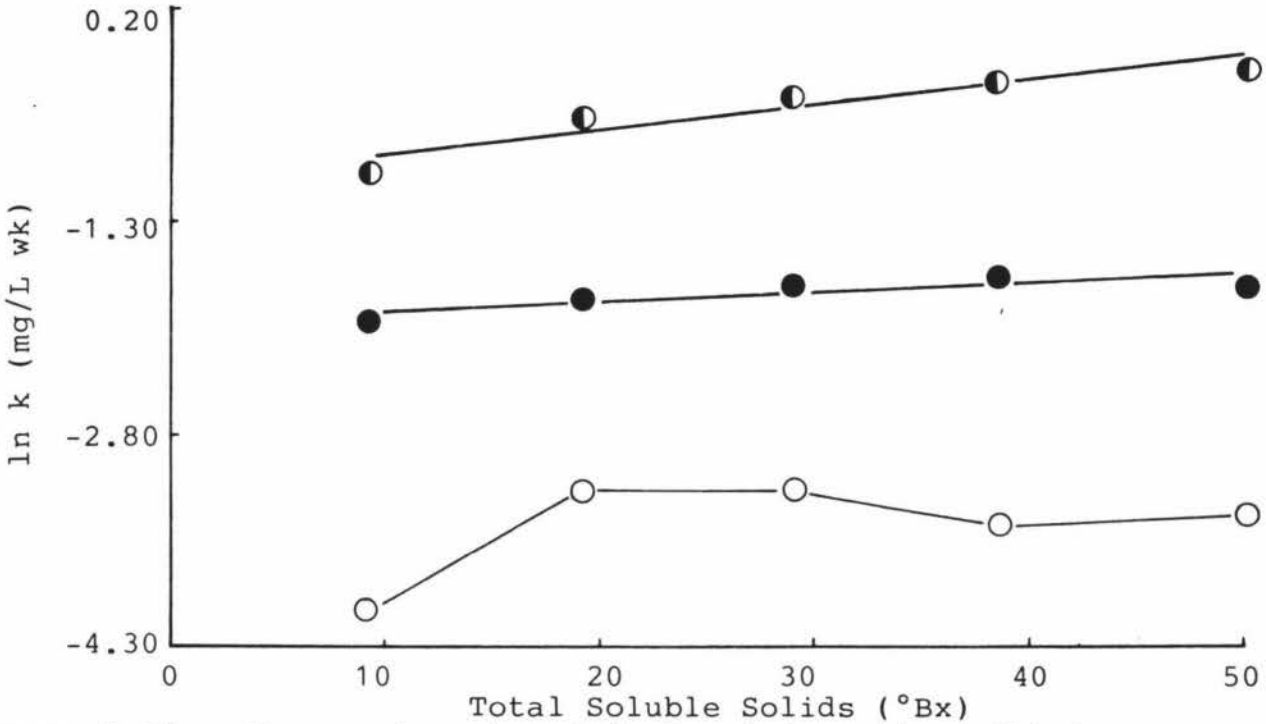

Figure 6.10: Changes in rate of furfural formation with increases in soluble solids level at 10°C (○), 20°C (●) and 36°C (◐) (lines of best fit at 20 and 36°C are based on linear regression analysis; points at 10°C have been joined by straight lines).

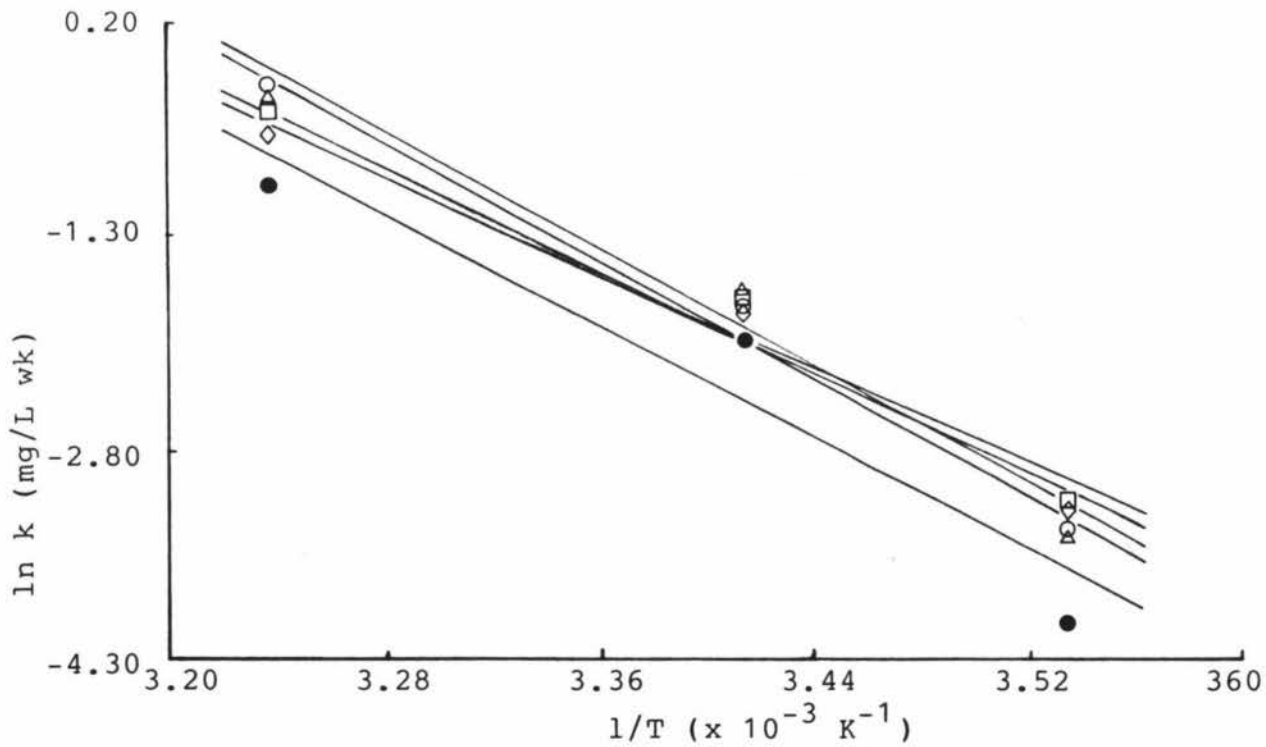

Figure 6.11: Arrhenius plots for furfural formation in lemon juice and concentrates (● - 9°Bx, ◇ - 20°Bx, □ - 30°Bx, △ - 40°Bx, 0-50°Bx).

The E_a and Q_{10} values increased linearly with an increase in soluble solids content for the 20 to 40 °Brix lemon juice concentrates (Figure 6.7). The E_a value at 9 °Brix appears to be unexpectedly high, further suggesting the possibility of a different reaction occurring at this low, single-strength level of soluble solids. The E_a and Q_{10} (30-40°C) values for the 50 °Brix concentrate are very near those of the 40 °Brix juice. The Q_{10} value for the temperature range 10-20°C of the 50 °Brix concentrate is lower than that of the 40 °Brix sample. These observations are also attributable to an increase in furfural decomposition at such high soluble solids level as 50 °Brix.

A two-way Anova was performed and the results are given in Tables 6.16A and B. They show that the rate of furfural formation was significantly affected by storage temperature but not by the soluble solids concentration. Similar results were obtained for ascorbic acid degradation and browning.

A significant F-value was obtained for the Tukey test, indicating an interaction between temperature and total soluble solids content. This interaction between the two factors had a synergistic effect on the rate of furfural formation. The Arrhenius plots (Figure 6.11) do not show this effect very clearly, but the increase of the E_a and Q_{10} values with an increase in soluble solids level for 20-40 °Brix concentrates demonstrates that the sensitivity of the reactions to a change in temperature increases with an increase in soluble solids content.

These observations, which are similar to those made for the browning reaction, further support the theory that furfural formation and browning in the lemon juice concentrates are due to the same reaction.

Table 6.16A: Two-way Anova for the rates of furfural formation in lemon juice and concentrates (9-50 °Brix).

Source	D.F.	SS	MS	F_c	F_t
Temperature	2	0.93739	0.46869	69.13*	4.46
Soluble Solids	4	0.04101	0.01025	1.51	3.84
Error	8	0.05427	0.00678		
Total	14	1.03267			

* Significant at p = 0.05

TUKEY TEST: $F_c = 187.28 > F_{.95;\ 1,\ 7} = 5.59$

Table 6.16B: Two-way Anova for the rates of furfural formation in lemon juice concentrates (20-50 °Brix).

Source	D.F.	SS	MS	F_c	F_t
Temperature	2	0.90410	0.45205	154.81*	5.14
Soluble Solids	3	0.00928	0.00309	1.06	4.76
Error	6	0.01750	0.00292		
Total	11	0.93088			

* Significant at p = 0.05

TUKEY TEST: $F_c = 179.63 > F_{.95;\ 1,\ 5} = 6.61$

Furfural is not recommended as an index of quality deterioration at high Brix concentrates (20-50 °Brix) due to its simultaneous formation and decomposition at these soluble solids levels. Hence, a predictive equation for furfural formation was not derived.

6.3.5 Sensory Evaluation

The sensory results are given in Tables 6.17, 6.18 and 6.21.

6.3.5.1 Flavour

The sensory scores for the lemon juice samples at 10°C corresponded with a 'recognizable' to a 'pronounced' fresh lemon flavour (see Appendices 2A and 2B for scoresheets). No significant differences among the samples of different concentrations for all the sampling times at 10°C were observed. This demonstrates that at this temperature, the lemon flavour is retained for all juice concentrations over the four-month storage period. The 9 °Brix sample seemed to have lost some characteristic lemon flavour, although not to a significant extent.

No off-flavours were detected in the samples with 20 to 50 °Brix soluble solids content at 10°C. A recognizable stored, burnt and slightly fermented off-flavour was detected in the 9 °Brix juice after four months storage.

Similar observations regarding lemon flavour were made with juice stored at 20 and 36°C. The fresh lemon flavour was retained in the juice with solids content of 20 to 50 °Brix over the four-month storage period. There was a loss of lemon flavour in the 9 °Brix samples. The presence of the fresh lemon flavour was down to a 'slightly

Table 6.17: Changes in the perceived levels of fresh lemon flavour in the lemonade samples.[1]

Temp. (°C)	Sol. Solids (°Bx)	Storage Period (months) 1	2	3	4
10	9	2.75	3.17	2.85	2.50
	20	3.25	3.08	3.00	3.50
	30	3.25	3.42	3.40	3.50
	40	3.25	3.17	3.00	3.30
	50	3.17	3.33	3.20	3.60
20	9	2.80	3.30	2.25*	2.30*
	20	3.20	2.90	2.90	2.90
	30	3.10	3.20	2.90	3.20
	40	3.00	3.30	3.10	3.10
	50	3.20	3.20	3.00	3.30
36	9	2.50	2.46*	2.30*	2.18*
	20	3.00	2.82	2.80	3.64
	30	3.17	3.00	2.90	3.46
	40	3.08	3.00	2.80	3.00
	50	3.08	2.82	2.90	3.36

[1] Scores without an asterisk (*) are not significantly different at $p = 0.05$

* Scores correspond to a 'slightly noticeable' level of fresh lemon flavour

Table 6.18: Off-flavour development in the lemonade samples.[1]

Temp. (°C)	Sol. Solids (°Bx)	Storage Period (months) 1	2	3	4
10	9	4.42	4.58	4.16*	3.30**
	20	4.50	4.67	4.80	4.80
	30	4.67	4.75	5.00	4.80
	40	5.00	4.58	4.70	5.00
	50	4.92	4.75	4.80	4.90
20	9	4.80	4.70	3.63*	2.89**
	20	4.70	4.40	4.90	4.10*
	30	4.80	4.70	4.60	4.90
	40	4.70	4.60	4.70	4.80
	50	4.90	4.70	4.70	4.70
36	9	3.83*	3.64*	3.50**	3.00**
	20	4.50	4.82	4.60	4.54
	30	4.50	4.54	4.90	4.82
	40	4.58	4.27	4.90	4.82
	50	4.75	4.46	4.60	4.27

[1] Scores without asterisk (*) are not significantly different at p = 0.05 and correspond to 'no detected' off-flavour

* Scores correspond to a 'slightly noticeable' level of off-flavour

** Scores correspond to a 'recognizable' level of off-flavour

noticeable' level after 3 months at 20°C and after 2 months at 36°C (Table 6.17).

These losses in lemon flavour coincided with the development of an off-flavour in the juices. The off-flavour was described by the panelists as being like a cooked citrus flavour, and/or having a stale aftertaste, and/or a slightly fermented flavour.

The lemon juice samples with soluble solids contents of 20 to 50 °Brix remained acceptable to the sensory panel at all temperatures in terms of flavour after four months storage. For the 9 °Brix samples, however, the juice became unacceptable after four months at 10°C, after three months at 20°C and after one month at 36°C.

A significant correlation was obtained between off-flavour development and ascorbic acid retention considering all five soluble solids levels (see Appendix 12). Off-flavour development was observed in the 9 °Brix juice, where ascorbic acid degradation was greatest. This suggests that ascorbic acid degradation parallels flavour deterioration. Tatum *et al*. (1969) showed a number of off-flavour contributing compounds and nonenzymic browning intermediates that could be formed from the degradation of ascorbic acid in acid medium, as well as fructose in acid or base media. The results of this present experiment indicate that ascorbic acid degradation is a more likely source of off-flavours in lemon juice rather than fructose degradation, although it may not be the only cause of off-flavour development. It was speculated in Section 6.3.3.1 that browning in the juices of high soluble solids content (20 to 50 °Brix) was primarily due to sugar breakdown. No off-flavours were detected, however, at these soluble solids levels, indicating that off-flavour development in lemon juice was probably not due to sugar degradation. It is also possible that at higher soluble solids concentrations, a protective effect is created on the

flavour components and/or an inhibitory effect on the reactants causing off-flavours.

It has been shown in Section 6.3.2.1 that ascorbic acid retention increased with increases in soluble solids content with optimum retention in the 50 °Brix juice stored at 10°C. Thus, based on the assumption that ascorbic acid breakdown is one of the sources of off-flavour development in lemon juice concentrates, it would then be expected that the desirable flavour properties of the product would be best retained in higher Brix concentrates.

The studies that have been made on the flavour characteristics of citrus juice were reviewed in Section 2.4 and summarized in Table 6.19.

Table 6.19: Summary of the results of studies that determined storage life of citrus juices based on flavour characteristics .

Juice Type	°Bx	T(°C)	Storage Life	Reference
Orange juice	single-strength	5 30	1½ years 2 weeks	Dinsmore & Nagy (1972)
Canned orange juice	single-strength	10 16 21 30	16 weeks 10 weeks 8 weeks 2 weeks	Nagy & Randall (1973)
Orange concentrate	55	15	2 months	Johnson & Toledo (1975)
Orange concentrate	58	5 12	17 months 10 months	Kanner *et al*. (1982)
Orange concentrate	20 30 40 50 60	0-2	5 months 5 months 5 months 9 months 9 months	Navarro *et al*. (1980)

The results of the studies summarized in Table 6.19 show that significant changes in flavour in orange juice take a longer time to occur in juice concentrates with higher soluble solids contents.

A poor correlation (see Appendix 12) between off-flavour development and browning, and between off-flavour development and furfural formation, was observed considering all juice concentrations. This was because for the samples of higher soluble solids concentrations (20-50 °Brix), no significant changes in flavour were detected, and the rate of furfural formation did not increase consistently with soluble solids content.

A close correlation between furfural content and flavour changes has been demonstrated with grapefruit juice (Nagy *et al.*, 1972; Maraulja *et al.*, 1973) and orange juice (Nagy and Randall, 1973) but furfural *per se* did not have any flavour properties, even at a high concentration of 2000 µg/L (Nagy *et al.*, 1972). It should be pointed out that this correlation was obtained using single-strength juices. It has been discussed in the previous section and reported by Kanner *et al.* (1982) that furfural formation is not a suitable index of quality deterioration in juice at higher soluble solids levels.

A good correlation between furfural formation and off-flavour development ($r = -0.873$) was obtained only with 9 °Brix juice. Apparently, formation of furfural at this single-strength level parallels the formation of components responsible for off-flavour development. As was discussed earlier, ascorbic acid degradation was suggested to be one of the sources of off-flavour development in citrus juices, and furfural is one of the major end products of ascorbic acid degradation. When the furfural exceeded a value of approximately 3.14 mg/L in lemon juice, the taste panel observed a significant difference in flavour.

These results support the conclusion that was made in the previous section, namely that furfural can serve as an index of quality deterioration in single-strength lemon juice but not for concentrates with higher soluble solids content.

The reported levels of furfural coinciding with changes in flavour are summarized in Table 6.20.

Table 6.20: Reported levels of furfural corresponding to significant changes in flavour.

Juice Type	Furfural Level (µg/L)	Reference
Canned grapefruit j.	175	Nagy *et al.* (1972)
Glass packed grapefruit j.	150	
Canned orange j.	50 - 70	Maraulja and Blair (1973)
Canned grapefruit j.	155 - 200	
Glass packed orange j.	55	Nagy & Randall (1973)

The furfural level for orange juice is considerably lower than that for grapefruit juice and the level obtained for lemon juice of 3.14 mg/L is considerably higher. Since furfural is only an index and not the cause of off-flavour development, the furfural level would not be expected to be the same for the different types of citrus juices which exhibit different rates of furfural formation.

There is a possibility that off-flavours may form at a different rate and in a different manner than furfural, depending on the conditions of the juice; i.e. oxygen content, pH, metal ions, soluble solids content and other factors (Randall and Nagy, 1972).

6.3.5.2 Colour

The results of the sensory evaluation of the brown colour in the lemon juice samples are given in Table 6.21.

The general observation was that the intensity of the brown colour in the juice samples increased with increase in storage time and soluble solids content at all storage temperatures. Figure 6.12 shows the extent of browning in the samples after 3 months storage.

A poor correlation was obtained between the colour scores and browning measurements (absorbance at 420 nm) ($r = 0.448$) for the samples at 10°C (see Appendix 12). The colour scores at 10°C did not consistently increase as a function of storage time as compared to the absorbance readings. This indicates that the sensory method is less sensitive in detecting small changes in colour than the instrumental method.

However, a significant correlation coefficient was obtained between the colour scores and browning measurements (absorbance at 420 nm) ($r = 0.865$) for all the samples stored at 20°C. This suggests a close correlation between sensory and spectrophotometric measurements of browning at this temperature where greater changes in colour were observed every month than in the samples stored at 10°C.

The magnitude estimation method involved the description of a perceived colour intensity in the juice samples. In a study by Trant *et al*. (1981) it was observed that intensity functions were positively correlated ($p < 0.001$) with corresponding instrumental analysis - Hunter *a* values for orange juice (colour in orange juice), Brookfield viscosity at 30 rpm for apricot nectar (oral viscosity of apricot nectar), and refractive index of lemonade (sweetness in lemonade).

Table 6.21: Changes in colour in the lemon juice samples during storage.[1]

Temp. (°C)	Sol. Solids (°Bx)	Storage Period (months) 1	2	3	4
10	9	1.61	4.94	5.30[u]	4.44[u]
	20	1.68	3.01	3.00	2.51
	30	1.84	3.36	3.50	2.12
	40	1.82	3.79	3.47	2.33
	50	2.01	3.68	3.32	2.83
20	9	1.64	3.29	4.68[u]	4.53[u]
	20	1.95	4.80[u]	4.20[u]	5.31[u]
	30	2.60	3.90[u]	4.91[u]	4.52[u]
	40	2.32	4.66[u]	5.07[u]	5.34[u]
	50	2.77	5.05[u]	5.88[u]	6.39[u]
36	9	3.28[u]	6.80[u]	9.97[u]	6.59[u]
	20	3.74[u]	5.86[u]	8.49[u]	6.30[u]
	30	3.84[u]	6.99[u]	11.17[u]	7.39[u]
	40	4.20[u]	9.09[u]	12.60[u]	9.80[u]
	50	4.89[u]	11.15[u]	15.02[u]	11.67[u]

[1] Score of the reference juice (9 °Brix lemon juice stored at 1°C) was 1.0

[u] Considered unacceptable by more than half the number of the panelists

Figure 6.12: Samples of lemon juices and concentrates (9-50 °Brix) after three months storage at 10, 20 and 36°C. Initial sample is the reference juice (9 °Brix) stored at 1°C.

A lower correlation coefficient (r = 0.768) was obtained at 36°C than at 20°C because all the colour scores decreased after the 4-month storage period (Figure 6.13). It is possible that sensitivity of the panelists to colour change reached a maximum, after which the samples were already very dark and the panelists were not as sensitive in detecting changes. It is further suggested that a linear relationship between sensory and instrumental measurements of browning exists only for a specific range and given extent of the browning reaction.

At 10°C, changes in browning in the 20 to 50 °Brix samples were small and the samples remained acceptable after four months storage. The 9 °Brix lemon juice became unacceptable after three months and corresponded to a browning intensity perceived to be 5.3 times the reference juice.

At 20°C, all samples except the 9 °Brix juice, were judged to be unacceptable after two months storage. At 36°C, samples of all five soluble solids levels were unacceptable after one month's storage. The juice samples with least browning at this sampling time (9 °Brix) was perceived to be 3.28 times browner than the reference juice.

As shown in Table 6.21, at 10°C, after two months storage, browning intensity was perceived to be higher than 3.28 times compared to the reference (9, 30-50 °Brix) but was still considered acceptable by the sensory panel.

After three months storage at the same temperature the browning intensity for 30 °Brix sample was scored as 3.5 times the reference and was considered as still having an acceptable colour.

These discrepancies may be due to the lack of sensitivity of the panelists to small changes in colour such as was observed for the lemon juice concentrates at 10°C. Another possible limitation is that acceptability is based on

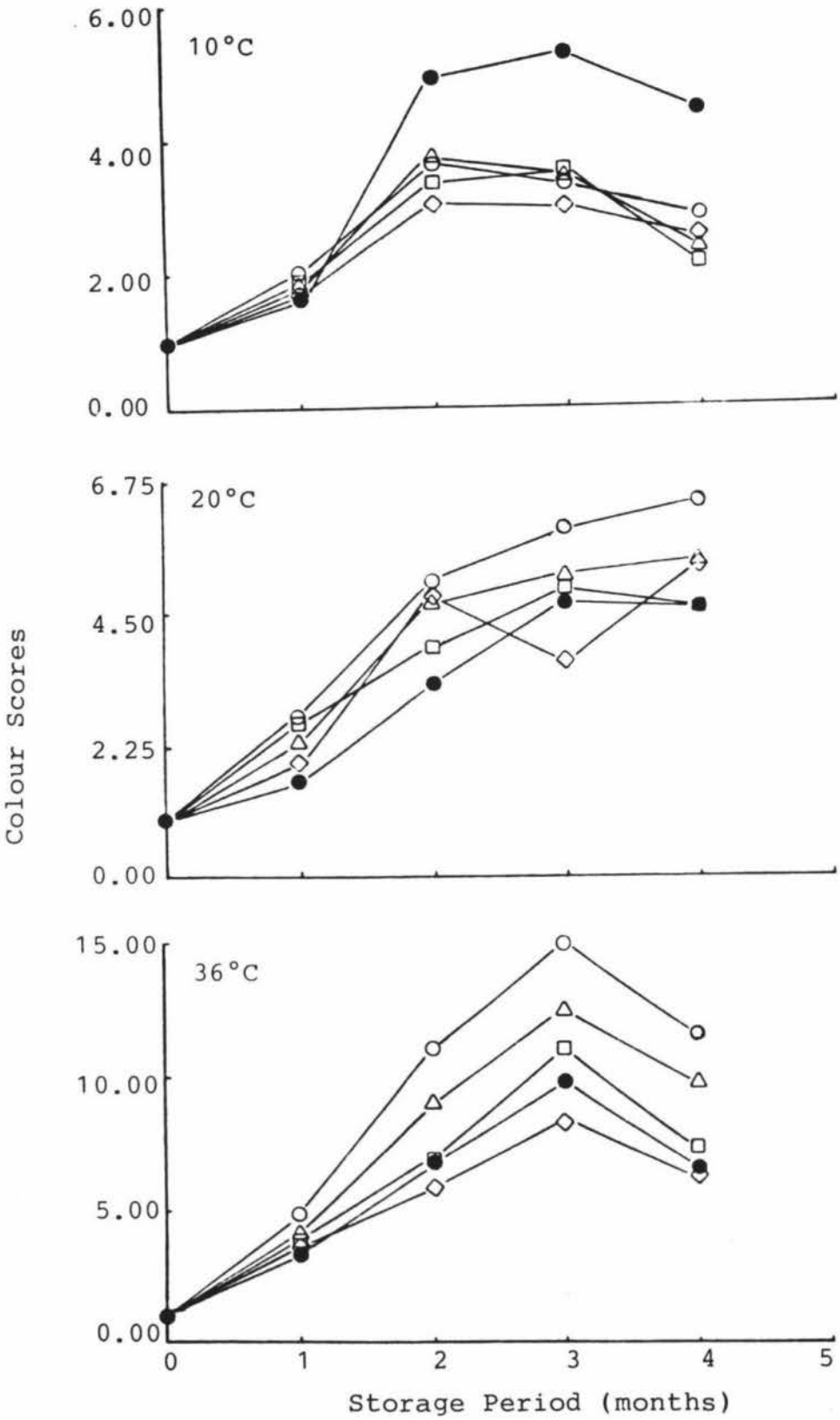

Figure 6.13: Changes in colour in lemon juices and concentrates (● - 9°Bx, ◇ - 20°Bx, □ - 30°Bx, △ - 40°Bx, O - 50°Bx) during storage.

hedonic responses while colour scores are perceived intensities. According to Trant *et al*. (1981), intensity and hedonic responses are distinct behaviours which exhibit different functions with stimulus concentration, usually linear for the former and cubic for the latter. In most sensory-instrumental comparisons, linear correlation can be applied validly only to perceived intensity but not to acceptance or hedonic responses.

All samples found unacceptable based on colour had scores higher than 3.5 except one (9 °Brix at 36°C - 3.28). Thus, it is considered reasonable to conclude that when browning intensity is perceived to be greater than 3.5 times the reference, then the lemon juice would be regarded as unacceptable.

The corresponding values in terms of browning (as absorbance at 420 nm) were also investigated (Table 6.10). The browning value corresponding to the least brown sample found unacceptable for all temperatures was 0.300 absorbance units (for 9 °Brix juice stored at 10°C). However, similar to the problem encountered with the sensory browning scores, some juice samples at 10°C were found to have browning measurements higher than 0.300 absorbance units but were still considered acceptable by the panelists. A 0.330 absorbance reading was the highest browning measurement which corresponded to an acceptable colour (for 40 °Brix at 10°C). A 0.330 absorbance reading appeared to be suitable as the level past which the brown colour in the lemon juice would be regarded unacceptable by the panelists. At this level only two juice samples, whose absorbance readings were actually lower than 0.330, were found to be unacceptable.

A correlation coefficient of $r = 0.90$ between the colour scores and furfural formation in the 9 °Brix juice was obtained. This further supports the conclusion earlier made (Section 6.3.4) that furfural could serve as an index of quality deterioration in single-strength lemon juice.

Significant changes in colour occur before changes in flavour. Sensory quality and storage life of lemon juice and concentrates, should therefore be based on browning rather than on flavour changes. Kanner *et al.* (1982) also concluded that nonenzymic browning rather than flavour changes was the main deteriorative phenomenon in orange juice concentrates (11-58 °Brix) stored between -18 and 36°C.

6.3.6 Storage life

6.3.6.1 Storage life determination

Storage life was determined based on the browning reaction (measured by absorbance at 420 nm) during storage of lemon juice. The end-point value for unacceptability was 0.330 absorbance units, which was determined in the previous section. This level corresponded to the browning intensity which was considered unacceptable by the panelists. Storage life was taken to be the time required to reach the 0.330 absorbance measurement using the zero-order equations for browning given in Table 6.11.

The storage lives of the samples with different soluble solids contents at the three storage temperatures are given in Table 6.22. The storage life values decreased with an increase in temperature and soluble solids content except for the 9 °Brix juice stored at 10°C which has a lower storage life than the 20 and 30°Brix juices. It was observed in Section 6.3.3.2 that the single-strength lemon juice had the highest rate of browning at 10°C, thus, the lower storage life.

There was an observed linear relationship between the storage life and soluble solids content for the lemon juice concentrates stored at 20 and 36°C (Figure 6.14). However, at 10°C the storage life of the concentrates did not

Table 6.22: Storage life in weeks of lemon juice and concentrates based on the browning reaction.

Temp. (°C)	Total Soluble Solids Content (°Bx)				
	9	20	30	40	50
10	15.8	17.7	17.6	14.8	14.1
20	10.3	9.6	8.1	6.5	5.4
36	3.2	2.8	1.9	1.6	1.3

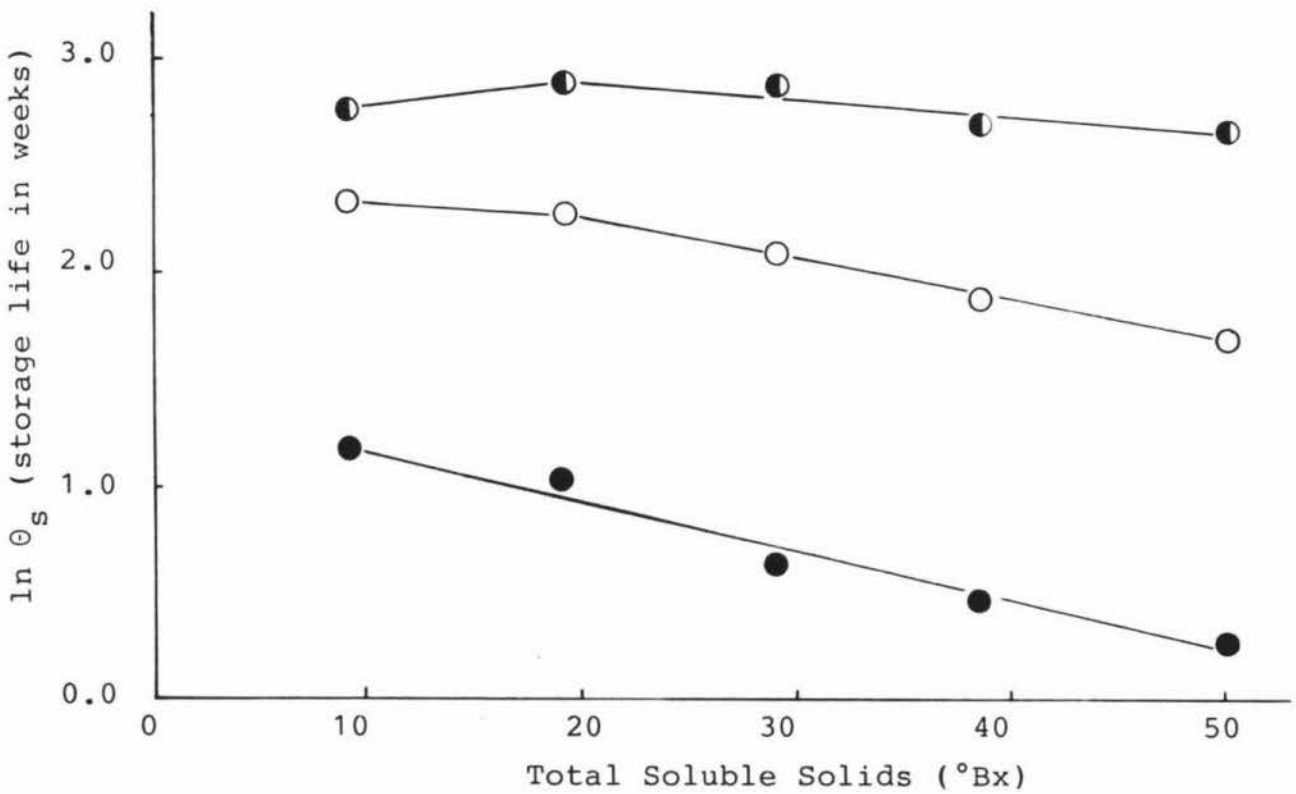

Figure 6.14: Changes in storage life with increases in soluble solids level at 10°C (◐), 20°C (○) and 36°C (●) (lines of best fit based on linear regression analysis for the 20-50°Bx juices at 10 and 20°C, and for the 9-50°Bx juices at 36°C).

decrease as consistently with increases in soluble solids concentration which is due to the insignificant differences among the k values for browning observed at this low temperature.

At 10°C, the storage lives for the 40 and 50 °Brix samples were 14.8 and 14.2 weeks, respectively. However, Table 6.21 shows that the juice colour of the samples at these soluble levels was still considered acceptable after 16 weeks storage at 10°C. This demonstrates the non-linearity between intensity measurements and hedonic responses. Except for these two values, the storage life value determined using the end-point value of 0.330 absorbance units, agreed with those based on the acceptability responses for colour given by the sensory panel as shown in the previous section.

The natural logarithm of storage life was plotted against the reciprocal of temperature (K) (Figure 6.15A). The slope of the plot is equivalent to E_a/R as in the Arrhenius model (Labuza and Kamman, 1983). This is based on the following equations:

$$k = \frac{\Delta C}{\Theta_s} \qquad (6\text{-}8)$$

where: ΔC = amount lost at time Θ_s
$= C_o - C_s$ for zero-order
$= \ln C_o/C_s$ for first-order

Θ_s = storage life

since $$\ln k = \ln \Delta C - \ln \Theta_s = \ln A - E_a/RT \qquad (6\text{-}9)$$

thus $$-\ln\Theta_s = \ln \frac{1}{\Theta_s} = -\ln\Delta C + \ln A - \frac{E_a}{RT} \qquad (6\text{-}10)$$

Figure 6.15B shows the linear relationship between the natural logarithm of storage life and temperature with the slope equivalent to a constant b.

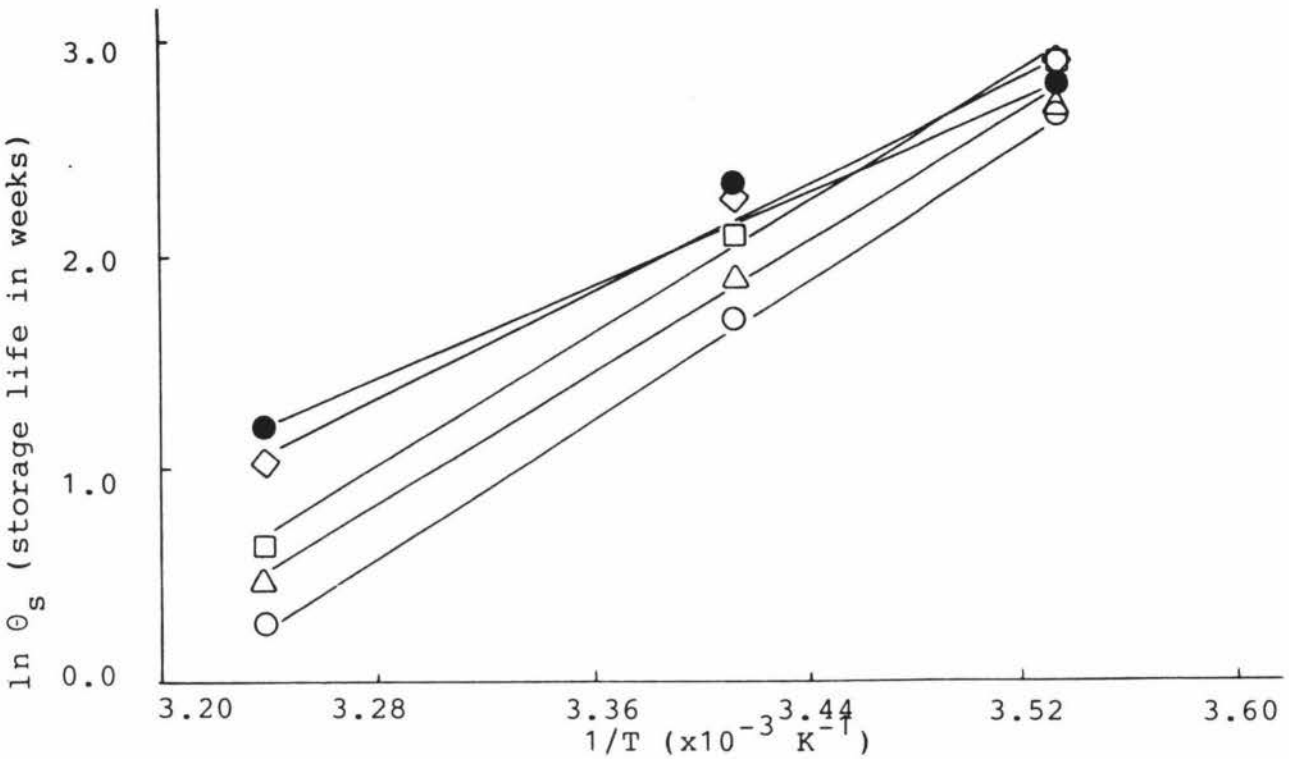

Figure 6.15A: Storage life plots of lemon juice and concentrates (● - 9°Bx, ◇- 20°Bx, □- 30°Bx, △ - 40°Bx, ○ - 50°Bx), showing the Arrhenius relationship between the natural logarithm of storage life and the reciprocal of absolute temperature.

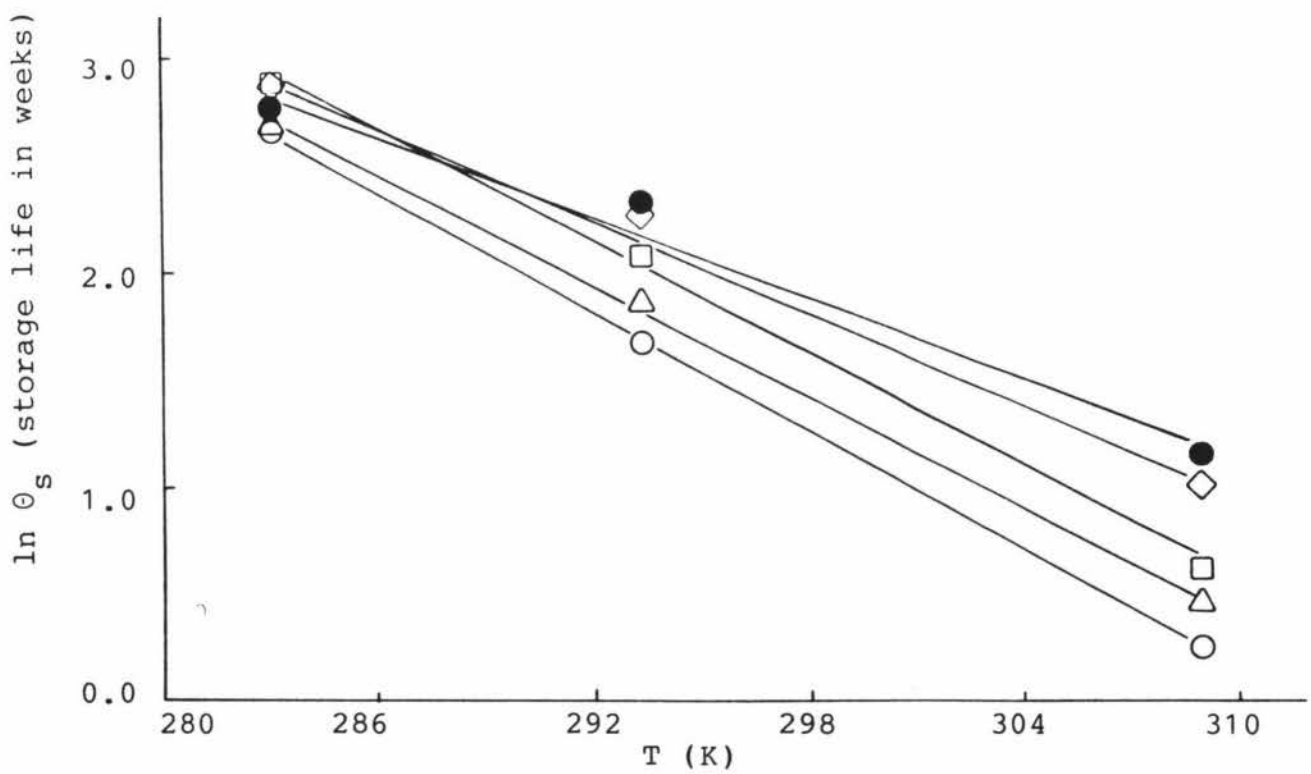

Figure 6.15B: Storage life plots of lemon juice and concentrates (● - 9°Bx, ◇- 20°Bx, □ - 30°Bx, △ - 40°Bx, ○ - 50°Bx), showing the linear relationship between the natural logarithm of storage life and temperature.

The Q_{10} can be calculated from the storage life plot as:

$$Q_{10} = \frac{\text{storage life at T°C}}{\text{storage life at (T+10°C)}} \qquad (6\text{-}11)$$

which assumes that the rate is inversely proportional to the storage life (Labuza, 1982).

The computed E_a and Q_{10} values are given in Table 6.23. The E_a and Q_{10} values increased with the increase in soluble solids content which is expected since the same was true for browning. The values for the 9 °Brix juice are comparable to those obtained for browning (absorbance at 420 nm) given in Table 6.6. For the juice concentrates of 20 to 50 °Brix, the storage life values were higher than the browning values. The difference may be due to the fact that the E_a and Q_{10} values for browning (absorbance at 420 nm) were based solely on the k values of the absorbance readings whereas the E_a's obtained from the storage life plots were dependent on the absorbance readings (0.330 absorbance units) which corresponded to the acceptability responses made by the panelists.

The end-point value, 0.330 absorbance units, was obtained based on the sensory evaluations which were conducted every four weeks. Thus, it may not be exactly the level corresponding to the time at which significant change in colour occurred (i.e. colour could have been considered unacceptable after two months, but the colour change could have occurred at any time between the fourth and eighth week).

Another possible cause of these discrepancies is that the E_a values determined from the storage life plots are less reliable than those for the browning measurements since the k values for the browning reaction were based on many data points (9-17 points) while the storage life (θ_s) was

Table 6.23: Computed activation energy (E_a) and Q_{10} values for lemon juice and concentrates.

Soluble Solids Content (°Bx)	ln Θ_o [1] (wk)	E_a (kJ mol^{-1})	R^2 (%)	Q_{10} 10-20°C	Q_{10} 30-40°C
9	-16.5	45.44	97.7	1.53	1.78
20	-19.2	52.06	99.3	1.84	1.93
30	-23.7	62.71	99.6	2.17	2.21
40	-23.8	62.50	99.8	2.28	2.21
50	-25.8	66.89	100.0	2.63	2.33

[1] Θ_o = storage life at T = 273°K (0°C)

based on just one point corresponding to 0.330 absorbance units. This end-point value was in turn based on the sensory evaluation of colour conducted every month which means, that there were only five data points (including the initial value) over the 16-week storage period.

The results of the two-way Anova given in Tables 6.24A and B, show that both temperature and soluble solids content significantly affects the storage life of lemon juice and concentrates. However, the effect of the soluble solids content is not as significant as that of temperature.

Thus, the critical factor to be considered for storage life optimization of lemon juice and concentrates is storage temperature. For maximum storage life, low temperature storage is essential (10°C). Low temperature storage is necessary for retention of ascorbic acid and to inhibit browning and any flavour changes (Smoot and Nagy, 1980; Kanner *et al.*, 1982; Crandall and Graumlich, 1982).

Total soluble solids content is not as important a factor as temperature although it does affect storage life. It is recommended that lemon juice be stored as high Brix concentrates at low temperature rather than as single-strength juice. Flavour changes and ascorbic acid loss were greatest at the 9 °Brix soluble solids level.

Over a 16-week period and at a temperature of 10°C, it would be best to store lemon juice as 50 °Brix concentrate. At this soluble solids level, there was maximum retention of ascorbic acid (95.69%), and colour and flavour remained acceptable throughout the whole storage period. The use of storage temperatures lower than 10°C (refrigerated storage) would be expected to result in even better storage stability of lemon juice concentrates.

Table 6.24A: Two-way Anova for storage life of lemon juice and concentrates (9-50 °Brix)

Source	D.F.	SS	MS	F_c	F_t
Temperature	2	484.356	242.178	250.70*	4.46
Soluble Solids	4	21.917	5.479	5.67*	3.84
Error	8	7.731	0.996		
Total	14	514.004			

* Significant at p = 0.05

Table 6.24B: Two-way Anova for storage life of lemon juice concentrates (20-50 °Brix)

Source	D.F.	SS	MS	F_c	F_t
Temperature	2	408.582	204.291	331.64*	5.14
Soluble Solids	3	17.809	5.936	9.64*	4.76
Error	6	3.698	0.616		
Total	11	430.089			

* Significant at p = 0.05

6.3.6.2 Predictive model for storage life

A predictive model was established for the storage life of lemon juice concentrates (20-50 °Brix) at 10 to 36°C. Since storage life was determined based on the browning reaction, a multiple regression analysis of the data was performed using the three predictor variables given in Equation 6-7. The following equation was obtained:

$$\ln \Theta_s = -16.6 - 0.192\ (°Bx) + 5576\ (1/T) + 51.6\ (°Bx/T) \qquad (6\text{-}12)$$

$(R^2 = 99.5\%)$

The results of the Anova of the equation given in Appendix 14, show that the three predictor variables significantly contribute to the overall predictive model.

A plot of this predictive model is shown in Figure 6.16.

A different browning reaction was assumed to occur in 9 °Brix juice (see Section 6.3.3). Thus, prediction of storage life at this soluble solids level would have to be based on its Arrhenius plot (Table 6.23) and would be:

$$\ln \Theta_s = -16.5 + 5091\ (1/T) \qquad (6\text{-}13)$$

$(R^2 = 97.7)$

Storage life of lemon juice and concentrates were determined using the predictive models given in Equations 6-12 and 6-13. The results are presented in Table 6.25. Storage life at 5°C was extrapolated from the equations to show the advantage of storing juices at a lower temperature.

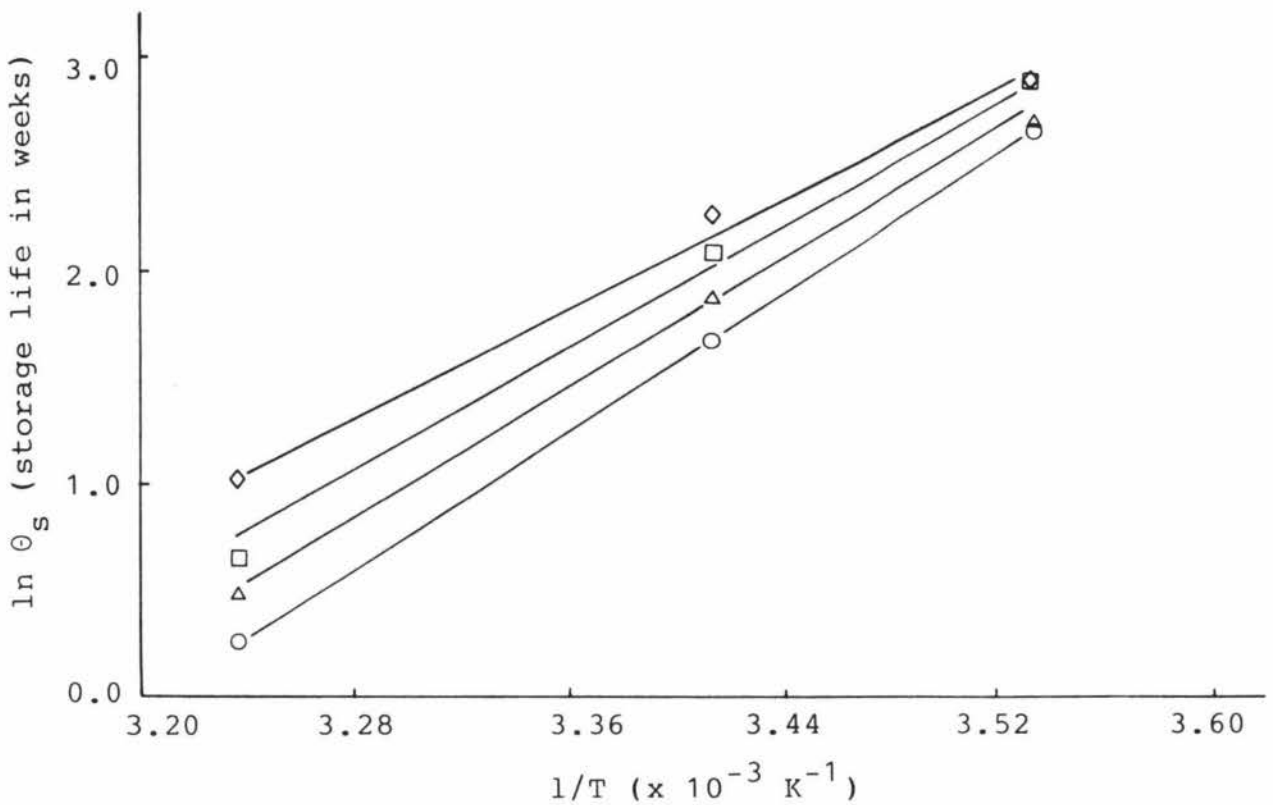

Figure 6.16: Plot of the predictive model (Equation 6-12) for storage life, showing the obtained storage life values (◇ - 20°Bx, □ - 30°Bx, △ - 40°Bx, ○ - 50°Bx) and the predicted regression lines for the lemon juice concentrates at 10 to 36°C.

Table 6.25: Predicted storage life in weeks of lemon juice and concentrates[1].

Temperature (°C)	Total Soluble Solids Content (°Bx)				
	9	20	30	40	50
5	23.36	28.1	26.4	24.8	23.0
10	16.5 (15.8)	19.2 (17.7)	17.4 (17.6)	15.9 (14.8)	14.1 (14.2)
20	8.5 (10.3)	8.7 (9.6)	7.4 (8.1)	6.4 (6.5)	5.3 (5.4)
36	3.3 (3.2)	2.7 (2.8)	2.1 (1.9)	1.7 (1.6)	1.2 (1.3)

[1] Values in brackets are the obtained storage life values given in Table 6.22.

The predicted storage life for the 50 °Brix concentrate at 10°C (14.1 weeks) does not agree with the result of the sensory evaluation for colour (Table 6.21). The sensory panelists considered it as still acceptable after 16 weeks storage. As was discussed earlier, this discrepancy is probably due to the non-linearity between the sensory acceptability responses and the instrumental measurements of browning. Aside from this, small changes in browning were observed in the juices stored at 10°C, and the panelists were not sensitive enough to detect these changes.

All the other storage life values are consistent with the results of the sensory evaluation for colour.

Although the actual results of this experiment show that 40 and 50 °Brix concentrates can be stored for 16 weeks at 10°C without significant quality deterioration, the predicted storage life values suggest that for longer storage periods the 40 and 50 °Brix concentrates would lose acceptability first, based on browning, as compared to the 20 and 30 °Brix concentrates.

Table 6.25 shows that the highest storage life values could be attained at a soluble solids level of 20 °Brix at all four temperatures. However, this conclusion was made based solely on the browning reaction. Although browning was considered to be the main deteriorative reaction in lemon juice concentrates, the other parameters of deterioration (i.e. ascorbic acid retention and flavour), and costs have to be considered in determining the optimum storage conditions for the product.

Ascorbic acid retention has been shown in Section 6.3.2 to increase with increases in soluble solids content. The flavour of concentrates is also better retained with increasing juice concentrations (Navarro *et al*., 1980). Using these two parameters as criteria for determining storage life, it can then be concluded that it would be best to store lemon juice as a high Brix concentrate (50 °Brix). However, for long storage periods (greater than four months) at 10°C, quality loss due to the browning reaction may outweigh the advantages of higher ascorbic acid retention and better flavour quality.

The use of lower storage temperatures (0-5°C) would make it feasible to store high Brix lemon juice concentrates for extended periods, without significant colour deterioration.

Kanner *et al* . (1982) reported that 58 °Brix orange concentrate was stable in terms of colour at 5 and 12°C for 18 and 12 months, respectively. Products of lower concentration, such as 45 °Brix, could be stored at these temperatures for 24 and 18 months, respectively. Similar results were observed by Crandall and Graumlich (1982) on 72 °Brix orange concentrate stored at 4.4°C for 12 months and by Navarro *et al*. (1980) on 50 and 60 °Brix orange juice concentrate stored at 0-2°C over a 9-month storage period.

The predicted storage life values for lemon concentrates (40 and 50 °Brix) stored at 5 and 10°C are considerably lower than those obtained by Kanner *et al*. (1982) for 45 and 58 °Brix orange concentrate at 5 and 12°C. This is probably due to a higher rate of browning in lemon juice and the fact that colour changes are more noticeable in a light coloured product such as lemon juice than in orange juice. According to Swisher and Swisher (1980), low storage temperature is more essential for lemon juice, particularly concentrated juice, than for most citrus juices. Another possible reason to explain the differences in storage lives is that the orange concentrates in the study of Kanner *et al*. (1982) was aseptically packaged which is expected to result in better quality retention.

Other advantages of storing lemon juice as high Brix concentrates are reductions in storage and transportation costs and increased microbial stability. This can be illustrated by comparing the relative volumes of 9 and 50 °Brix juice. If 200 litres of single-strength lemon juice were concentrated to 50 °Brix, a final volume of 36 litres of concentrate would be obtained, which corresponds to approximately an 82% saving in storage space. This would mean a considerable reduction in storage and distribution costs.

Lemon juice could probably be concentrated to soluble solids levels higher than 50 °Brix, but the advantage of reduced storage and transportation costs has to be weighed against any losses in quality (particularly nonenzymic browning), the costs involved in the additional concentration and energy costs for lower temperature storage.

6.4 Conclusion

Temperature was observed to significantly affect the rate of ascorbic acid degradation, browning and furfural formation in lemon juice and concentrates, with higher rate constants obtained at higher temperatures. Temperature dependence of all three reactions followed the Arrhenius and linear models over the temperature range of 10-36°C.

The total soluble solids concentration also affected the rates of the different reactions but not to such a significant extent as the temperature effect. Ascorbic acid retention was observed to increase with an increase in soluble solids content.

The k values for the 9 °Brix juice at the three temperatures were considerably higher than those for the higher Brix concentrates (20-50 °Brix). Maximum retention of ascorbic acid (95.69%) was attained in the 50 °Brix juice stored at 10°C for 16 weeks.

The rate of the browning reaction increased with soluble solids content for the 20 to 50 °Brix juice samples at 20 and 36°C. At 10°C, for the same Brix concentrates, the rates did not differ significantly from each other. The k value for the 9 °Brix juice was the highest at 10°C, was higher than that for 20 °Brix at 20°C, and was higher than those for 20 to 30 °Brix at 36°C. An interaction effect between temperature and soluble solids content on the rate of browning was observed.

The rate of furfural formation consistently increased with increase in soluble solids level at 36°C, but was not as consistent at 10 and 20°C. The rate was observed to decrease for the 50 °Brix juice at both temperatures.

A close correlation among all the quality parameters was demonstrated with the 9 °Brix juice samples at the three storage temperatures. However, with the higher Brix concentrates (20-50 °Brix), ascorbic acid retention had a poor correlation with browning and furfural formation.

This observation and the considerably higher k and E_a values that were obtained for the 9 °Brix juice, suggests that the mechanism of the reactions or the reactions themselves that predominated in the single-strength lemon juice differed from those that predominated in the concentrates.

Browning and furfural formation could be attributed to ascorbic acid degradation in the 9 °Brix juice. However, in the concentrates (20-50 °Brix), these may be due to other reactions predominating at high soluble solids levels, such as acid-catalysed sugar breakdown and Maillard browning.

In single-strength lemon juice, furfural could serve as an index of quality deterioration. Furfural formation and decomposition occurs simultaneously in lemon juice concentrates. Thus it does not seem suitable as an index of quality change in these products.

Significant changes in colour were observed prior to changes in flavour, as perceived by a sensory panel. The browning reaction should thus be the main criterion in determining storage life.

The important factor to consider for optimum storage stability of lemon juice and concentrates is temperature. Low temperature storage is essential. Over a 16-week storage period at 10°C, it is suggested that lemon juice be stored as a 50 °Brix concentrate. At this level, there was maximum retention of ascorbic acid, and colour and flavour remained acceptable through the whole storage period. Other possible advantages of using high Brix

concentrates are reduced storage and distribution costs and increased microbial stability.

The lemon juice samples at all five soluble solids levels were unacceptable based on colour after four weeks storage at 36°C and 8 weeks at 20°C (except 9 °Brix juice which was unacceptable at this temperature after 12 weeks storage).

To extend the storage life of high Brix lemon juice concentrates beyond four months, storage temperatures lower than 10°C would be necessary so that the extent of browning would not reach unacceptable levels.

CHAPTER 7

SUMMARY AND CONCLUSIONS

All the parameters (ascorbic acid retention, nonenzymic browning, sensory quality, and furfural and HMF formation) used to measure quality deterioration in single-strength lemon juice stored at 55°C, were observed to be highly correlated with one another.

The spectrophotometric method for measuring browning was found to be a more suitable method compared to the use of the Neotec Du-Colorimeter because it was simpler and less time consuming. Furfural was chosen as the chemical index rather than HMF for the same reasons. Furfural formation had a slightly higher correlation coefficient with the other quality parameters compared to HMF.

Ascorbic acid degradation and HMF formation were adequately described by a first-order reaction kinetic model while browning and furfural formation followed a zero-order model.

The initial dissolved oxygen content (0.41, 1.44 and 3.74 mg/L) did not significantly affect the rate of ascorbic acid degradation and furfural formation in single-strength lemon juice stored at 36°C. However, browning and HMF formation were significantly higher in the juice with 3.74 mg/L dissolved oxygen content than in the samples of the two other treatments. This indicates that oxygen would be an important factor leading to quality deterioration in lemon juice, when present at high levels.

Present commercial operations of deaerating and hot-filling citrus juices, would reduce the oxygen content dissolved in the juice and in the headspace to a level sufficiently

low to make oxygen a non-critical factor in the loss of quality in lemon juice during storage.

Temperature dependence of ascorbic acid degradation browning and furfural formation followed the linear and Arrhenius models over the temperature range of 10 to 36°C.

The total soluble solids concentration also affected the rates of the different reactions but not to such a significant extent as the temperature effect. Ascorbic acid retention was observed to increase with an increase in soluble solids content. Maximum retention of ascorbic acid (95.69%) was attained in the 50 °Brix juice stored at 10°C for 16 weeks.

The rate of the browning reaction increased with soluble solids content for the 20 to 50 °Brix juice samples at 20 and 36°C. At 10°C, the rates for the higher Brix concentrates did not significantly differ from each other. The k values for the 9 °Brix juice at all three temperatures were unexpectedly high. An interaction effect between temperature and soluble solids content on the rate of browning was observed.

The rate of furfural formation consistently increased with increase in soluble solids level at 36°C, but was not as consistent at 10 and 20°C.

A close correlation among all the quality parameters was demonstrated with the 9 °Brix juice samples at the three storage temperatures. This was not so for the higher Brix concentrates (20-50 °Brix).

This observation and the considerably higher k and E_a values that were obtained for the 9 °Brix juice, suggests that the mechanism of the reactions or the reactions themselves that predominated in the single-strength lemon juice differed from those that predominated in the concentrates.

Furfural could serve as an index of quality deterioration in single-strength lemon juice but not in concentrates (20-50 °Brix) due to its simultaneous formation and decomposition at these high soluble solids levels.

The sensory panel perceived significant changes in colour in the juices prior to changes in flavour. The browning reaction should thus be the main criterion in the determination of storage life.

Low temperature storage is essential for optimum storage stability. Over a 16-week storage period at 10°C, it is suggested that lemon juice be stored as a 50 °Brix concentrate. High retention of ascorbic acid and flavour properties, and reduction in storage and distribution costs are the advantages of storing lemon juice at such high Brix concentrations. To extend the storage life of lemon juice concentrates beyond four months, storage temperatures lower than 10°C would be necessary so that the extent of browning could be maintained at acceptable levels.

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Appendix 1: Correction table for total soluble solids determined by means of the refractometer in sucrose solutions containing citric acid.[1]

% Anhy Citric Acid	Add (%)	% Anhy Citric Acid	Add (%)	% Anhy Citric Acid	Add (%)
0.5	0.11	13.0	2.55	25.5	4.77
1.0	0.21	13.5	2.65	26.0	4.86
1.5	0.31	14.0	2.73	26.5	4.93
2.0	0.40	14.5	2.83	27.0	5.02
2.5	0.50	15.0	2.92	27.5	5.11
3.0	0.60	15.5	3.01	28.0	5.19
3.5	0.68	16.0	3.10	28.5	5.26
4.0	0.78	16.5	3.19	29.0	5.36
4.5	0.88	17.0	3.28	29.5	5.45
5.0	0.98	17.5	3.37	30.0	5.52
5.5	1.08	18.0	3.47	30.5	5.60
6.0	1.17	18.5	3.55	31.0	5.69
6.5	1.27	19.0	3.64	31.5	5.77
7.0	1.37	19.5	3.73	32.0	5.85
7.5	1.47	20.0	3.82	32.5	5.94
8.0	1.57	20.5	3.91	33.0	6.02
8.5	1.67	21.0	4.00	33.5	6.10
9.0	1.77	21.5	4.08	34.0	6.19
9.5	1.87	22.0	4.17	34.5	6.27
10.0	1.97	22.5	4.26	35.0	6.35
10.5	2.07	23.0	4.34	35.5	6.44
11.0	2.17	23.5	4.43	36.0	6.52
11.5	2.27	24.0	4.52	36.5	6.60
12.0	2.37	24.5	4.59		
12.5	2.47	25.0	4.69		

[1] From McAllister (1980).

Appendix 2A: Scoresheet for flavour evaluation of lemonade samples.

Name: Set No.
Date:

SCORESHEET FOR LEMON JUICE

INSTRUCTIONS: Please evaluate the following samples of lemon juice based on flavour. Check the descriptive terms which best describe the product. Do nothesitate to give comments.

		SAMPLE CODE			
Fresh Lemon Flavour					
very pronounced	5				
pronounced	4				
recognizable	3				
slightly noticeable	2				
none	1				
Off-Flavour					
very pronounced	1				
pronounced	2				
recognizable	3				
slightly noticeable	4				
none	5				

(please describe the off-flavour if detected _____)

THANK YOU

Appendix 2B: Scoresheet for the colour evaluation of lemon juice.[1]

Name: Set No.
Date:

You will be presented with a series of lemon juice samples in irregular order. Your task is to tell how intense the brown colour appear by assigning numbers to them. Give the first sample any number that seems appropriate to you. Then assign numbers to the other samples. Try to make each number match the colour intensity as you perceive it. There is no limit to the range of numbers that you may use. You may use whole numbers, decimals, or fractions.

After rating the sample, please answer the question, "Do you find this colour acceptable for lemon juice?" Place a check mark under the appropriate answer.

SAMPLE CODE	RATING	ANSWER TO QUESTION	
		YES	NO
_____	_____	___	___
_____	_____	___	___
_____	_____	___	___
_____	_____	___	___
_____	_____	___	___
_____	_____	___	___

THANK YOU

[1] Second paragraph was only added for experiment 6.

Appendix 3: Correlation coefficients at the 5 percent and 1 percent levels of significance.[1]

Degrees of Freedom	5 Per cent	1 Per cent	Degrees of Freedom	5 Per cent	1 Per cent
1	.997	1.000	24	.388	.496
2	.950	.990	25	.381	.487
3	.878	.959	26	.374	.478
4	.811	.917	27	.367	.470
5	.754	.874	28	.361	.463
6	.707	.834	29	.355	.456
7	.666	.798	30	.349	.449
8	.632	.765	35	.325	.418
9	.602	.735	40	.304	.393
10	.576	.708	45	.288	.372
11	.553	.684	50	.273	.354
12	.532	.661	60	.250	.325
13	.514	.641	70	.232	.302
14	.497	.623	80	.217	.283
15	.482	.606	90	.205	.267
16	.468	.590	100	.195	.254
17	.456	.575	125	.174	.228
18	.444	.561	150	.159	.208
19	.433	.549	200	.138	.181
20	.423	.537	300	.113	.148
21	.413	.526	400	.098	.128
22	.404	.515	500	.088	.115
23	.396	.505	1,000	.062	.081

[1] From Kramer and Twigg (1970)

Appendix 4: Anova for flavour and browning changes in lemon juice in experiment 4.2.

FRESH LEMON FLAVOUR

Source	D.F.	SS	MS	F_c [1]	F_t (.05)
Factor	4	11.767	2.942	5.32*	2.54
Error	55	30.417	0.553		
Total	59	42.183			

[1] F_c is the calculated F-value while F_t is the F-value at $p = 0.05$ taken from a statistical table.

OFF-FLAVOUR

Source	D.F.	SS	MS	F_c	F_t (.05)
Factor	4	19.40	4.85	4.08*	2.54
Error	55	65.33	1.19		
Total	59	84.73			

BROWN COLOUR

Source	D.F.	SS	MS	F_c	F_t (.05)
Factor	4	100.11	25.03	24.14*	2.54
Error	55	57.02	1.04		
Total	59	157.12			

* Significant difference exist at 5% probability level.

Appendix 5: Anova for flavour and browning changes in lemon juice in experiment 4.3.

FRESH LEMON FLAVOUR

Source	D.F.	SS	MS	F_c	F_t (.05)
Factor	4	2.833	0.708	0.95^{ns}	2.54
Error	55	41.167	0.748		
Total	59	44.000			

OFF-FLAVOUR

Source	D.F.	SS	MS	F_c	F_t (.05)
Factor	4	1.933	0.483	1.02^{ns}	2.54
Error	55	26.000	0.473		
Total	59	27.933			

BROWN COLOUR

Source	D.F.	SS	MS	F_c	F_t (.05)
Factor	4	54.88	13.72	12.79*	2.54
Error	55	59.01	1.07		
Total	59	113.89			

* Significant difference exist at 5% probability level

^{ns} Not significant at the 5% probability level

Appendix 6A: Anacova for ascorbic acid retention

ANACOVA TABLE					
SOURCE OF VARIANCE	D.F.	SS	MS	F_c	F_t (.05)
Equality of adj. means[1]	2	0.0221	0.0111	0.8830^{ns}	3.59
Zero slope	1	1.0634	1.0634	84.8424*	4.45
Error	17	0.2131	0.0125		
Equality of slopes	2	0.0028	0.0014	0.0994	3.68
Error	15	0.2103	0.0140		

ns Not significant at p = 0.05

* significant at p = 0.05

1 The adjusted means are the predicted values of Y (% ascorbic acid retention) when evaluating the separate regression equations using $\bar{X}$ (the combined mean of the covariate time)

Appendix 6B: Anacova and T-test for browning
(Absorbance at 420 nm)

ANACOVA TABLE

SOURCE OF VARIANCE	D.F.	SS	MS	Fc	F_t (.05)
Equality of Adj. means	2	0.0005	0.0002	4.8752*	3.98
Zero slope	1	0.0053	0.0053	109.1300*	4.84
Error	11	0.0005	0.0000		
Equality of slopes	2	0.0000	0.0000	0.735	4.26
Error	9	0.0005	0.0001		

T-test for adjusted group means (degrees of freedom = 11)

Dissolved O_2 Level (mg/L)	0.41	1.44	3.74
0.41	0.0000		
1.44	-0.4535	0.0000	
3.74	2.4488*	2.9023*	0.0000

* Significant at p = 0.05

Appendix 6C: Anacova and T-test for browning (Neotec-Z) .

ANACOVA TABLE

Source of Variance	D.F.	SS	MS	F_c	F_t (.05)
Equality of adj. means	2	4.8455	2.4227	7.4739*	4.10
Zero slope	1	54.4643	54.4643	168.0178*	4.96
Error	10	3.2416	0.3242		
Equality of slopes	2	0.2206	0.1103	0.2921	4.46
Error	8	3.0210	0.3776		

T-test for adjusted group means (degrees of freedom = 10)

Dissolved O_2 Level (mg/L)	0.41	1.44	3.74
0.41	0.0000		
1.44	0.4443	0.0000	
3.74	-3.1904*	-3.6048*	0.0000

* Significant at p = 0.05

Appendix 6D: Anacova for furfural formation .

ANACOVA TABLE

Source of Variance	D.F.	SS	MS	F_c	F_t (.05)
Equality of adj. means	2	0.1378	0.0689	0.8948^{ns}	3.59
Zero slope	1	74.0344	74.0344	961.1433*	4.45
Error	17	1.3095	0.0770		
Equality of slopes	2	0.0856	0.0428	0.5244	3.68
Error	15	1.2239	0.0816		

ns Not significant at p = 0.05

* Significant at p = 0.05

Appendix 6E: Anacova and T-test for HMF formation .

ANACOVA TABLE

Source of Variance	D.F.	SS	MS	F_c	$F_t(.05)$
Equality of adj. means	2	0.0490	0.0245	3.6017*	3.59
Zero slope	1	4.1766	4.1766	614.6217*	4.45
Error	17	0.1155	0.0068		
Equality of slopes	2	0.0021	0.0011	0.1407	3.68
Error	15	0.1134	0.0076		

T-test for adjusted group means (degrees of freedom = 17)

Dissolved O_2 Level (mg/L)	0.41	1.44	3.74
0.41	0.0000		
1.44	-0.8219	0.0000	
3.74	1.8017	2.6236*	0.0000

* Significant at p = 0.05

Appendix 7: Composition of lemon juice.[1]

Constituent	Content per 100g Range		Average	
Protein (total N x 6.25)	0.26-0.77	g	0.42	g
Amino nitrogen	0.019-0.046	g	0.035	g
Fat (ether extract)	None-0.6	g	0.2	g
Soluble solids, total (°Brix)	7.1-11.9	g	9.3	g
Acid, total, as anhyd, citric	4.20-8.33	g	5.97	g
Malic acid	0.15-0.41	g	0.26	g
Sugar, total, as invert	0.77-4.08	g	2.16	g
Reducing sugar	0.78-2.63	g	1.67	g
Sucrose	0.03-0.63	g	0.18	g
Minerals, total ash	0.15-0.35	g	0.25	g
Calcium	5.6-27.9	mg	9.88	mg
Phosphorus	5.3-16.6	mg	9.35	mg
Iron	0.14-0.69	mg	0.23	mg
Magnesium	5.8-11.3	mg	6.7	mg
Potassium	99-128	mg	103	mg
Sodium	1.0-5.0	mg	1.3	mg
Sulfur	2.0-8.0	mg	3.36	mg
Chlorine	2.3-4.0	mg	3	mg
Vitamin A (as carotene)	None or trace		None	
Thiamine (B-1)	0.004-0.125	mg	0.043	mg
Riboflavin (B-2)	0.005-0.073	mg	0.0183	mg
Niacin	0.056-0.196	mg	0.089	mg
Inositol	56-76	mg	66.5	mg
Folic acid	0.00082-0.00094	mg	0.00091	mg
Flavanones	46-54	mg	50	mg
Ascorbic acid (vitamin C)	31-61	mg	45	mg
pH	2.11-2.48		2.30	

[1] From Swisher and Swisher, 1980

Appendix 8: Tukey test for additivity.[1]

The 'Tukey Test for additivity' is a quantitative approach toward the measurement of interaction in a two-factor model without replication, which was developed by Tukey (1949).

This procedure assumes that any interaction γ_{ij} that may exist is a multiplicative function of the main effects of the two factors so that

$$\gamma_{ij} = DA_iB_j \tag{1}$$

Thus the null and alternative hypotheses can be stated as

$H_0 : D = 0$ (no interaction is present)

$H_1 : D \neq 0$ (interaction is present)

To perform this test of additivity, the error sum of squares given in the two-way Anova must be partitioned into two components, 'nonadditivity' and pure error, so that

$$SSE = SSNA + SSPE \tag{2}$$

where:

$$SSNA = \left[\frac{\sum_{i=1}^{r}\sum_{j=1}^{c} Y_{ij}(\bar{Y}_{i.} - \bar{Y}..)(\bar{Y}_{.j} - \bar{Y}..)}{[(SSA)(SSB]/rc}\right]^2 \tag{3}$$

and

$$SSPE = SSE - SSNA \tag{4}$$

The nonadditivity component has one degree of freedom while the pure error component has $rc-r-c$ degrees of freedom. The appropriate test statistic is given by

$$F = \frac{SSNA}{SSPE/(rc-r-c)} \sim F_{1,rc-r-c} \tag{5}$$

and the decision rule is to reject the null hypothesis of additivity (i.e., no interaction) if

$$F \geq F_{1-\alpha;\ 1,\ rc-r-c}$$

---

1 From Berenson *et al.* (1983)

Appendix 9: Changes in citric acid concentration (mg/mL) in lemon juice and concentrates during storage.[1,2]

Time (months)	Temp. (°C)	Total Soluble Solids (°Bx) 9	20	30	40	50
	10	62.1	55.8	60.3	64.5	62.4
2	20	65.1	55.4	60.9	59.4	60.9
	36	52.8	64.5	60.9	61.8	60.3
	10	51.3	56.1	52.8	54.0	57.0
3	20	55.5	51.3	51.9	51.6	51.3
	36	54.6	54.0	55.8	57.0	55.2
	10	56.3	56.3	58.0	58.0	57.2
4	20	50.3	53.2	52.4	54.1	55.3
	36	52.4	51.3	50.6	49.4	49.6

[1] Initial concentration of citric acid in single-strength lemon juice was 63.5 mg/mL.

[2] An analysis of variance ($p = 0.05$) of the results indicated that citric acid concentration significantly decreased with an increase in storage time. No significant differences were attributable to storage temperature and soluble solids content.

Appendix 10: Changes in citric acid concentration (mg/mL) of single-strength lemon juice stored at 55°C.[1]

Sample No.	Storage Time (days)						
	0	1	2	3	4	5	6
1	51.3	52.0	52.5	50.1	51.0	51.0	51.6
2	55.5	53.8	53.1	51.0	53.2	51.3	53.4
3	51.6	53.1	52.4	53.1	52.2	50.7	50.4
4	-	53.4	52.9	52.9	50.2	52.4	51.8
5	-	53.1	51.0	53.2	48.4	52.4	52.5
6	-	52.8	49.4	53.2	52.0	51.6	52.0
$\bar{X}$	52.80	53.0	51.9	52.3	51.2	51.6	52.0

[1] No significant differences exist among the samples based on an analysis of variance of the data ($p = 0.05$).

Appendix 11: Correlation coefficients among the different parameters measured in the 9 °Brix lemon juice (as averaged value of the correlation coefficients for the three storage temperatures).[1]

	Ascorbic Acid	Browning (abs)	Furfural	Browning (sen. eval.)	Lemon Flavour
Browning (absorbance)	-0.945				
Furfural	-0.971	0.959			
Browning (sen. eval.)	-0.904	0.876	0.900		
Lemon Flavour	0.548*	-0.719*	-0.660*	-0.719*	
Off-flavour	0.808	-0.920	-0.873	-0.920	0.878

[1] The correlation with the parameters determined by sensory evaluation (browning; flavour) were determined using the data obtained every four weeks for the objective parameters.

* Not significant at p = 0.05.

Appendix 12: Correlation coefficients among the different parameters measured in lemon juice and concentrates (9-50 °Brix) at the three storage temperatures

Temperature (°C)	Parameter	Ascorbic Acid	Browning (abs)	Furfural	Browning (sen.eval.)	Lemon Flavour
10	Browning (absorbance)	-0.181				
	Furfural	0.099	0.800**			
	Browning (sen. eval)	-0.579	0.448	0.070		
	Lemon Flavour	0.617	0.081	0.468	-0.328	
	Off-flavour	0.826**	-0.110	0.303	-0.459	0.780
20	Browning (absorbance)	-0.013				
	Furfural	-0.169	0.874*			
	Browning (sen. eval.)	-0.126	0.865**	0.793		
	Lemon Flavour	0.634	-0.008	-0.037	-0.043	
	Off-flavour	0.763	-0.132	-0.122	-0.211	0.815**
36	Browning (absorbance)	-0.089				
	Furfural	0.023	0.888*			
	Browning (sen. eval.)	-0.062	0.768	0.744		
	Lemon Flavour	0.584	0.250	0.425	-0.036	
	Off-flavour	0.800**	0.101	0.364	0.165	0.693

* Significant at p = 0.05 ** Significant at p = 0.10

Appendix 13: Computer output for the predictive model of browning (Equation 6-7).

The Regression Equation is

$\ln k = 5.80 + 0.302\ Bx - 3117\ 1/T - 84.6\ Bx/T$

Column	Coefficient	St. Dev. of Coef.	T-Ratio = Coef/S.D.
	5.803	1.902	3.05
Bx	0.30179	0.05272	5.72
1/T	-3117.2	559.9	-5.57
Bx/T	-84.58	15.52	-5.45

S = 0.07542

R-Squared = 99.4 percent

R-Squared = 99.1 percent, adjusted for D.F.

Analysis of Variance

Due to	DF	SS	MS
Regression	3	6.9708	2.3236
Residual	8	0.0455	0.0057
Total	11	7.0163	

Further Analysis of Variance
SS Explained by Each Variable When Entered In The Order Given

Due to	DF	SS	MS	F_c
Regression	3	6.9708	2.3236	407.65*
Bx	1	0.3421	0.3421	60.02*
1/T	1	6.4598	6.4598	1133.30*
Bx/T	1	0.1689	0.1689	29.63*

Appendix 13: Cont'd

Row	Bx	Y ln k	Pred. Y Value	St. Dev. Pred.	Residual	St. Res.
1	19.1	-5.0640	-5.1559	0.0545	0.0919	1.76
2	19.1	-4.6274	-4.5850	0.0363	-0.0425	-0.64
3	19.1	-3.6929	-3.7483	0.0587	0.0554	1.17
4	29.0	-5.1904	-5.1272	0.0363	-0.0632	-0.96
5	29.0	-4.5756	-4.4556	0.0242	-0.1200	-1.68
6	29.0	-3.4770	-3.4716	0.0391	-0.0054	-0.08
7	38.5	-5.0162	-5.0994	0.0353	0.0833	1.25
8	38.5	-4.3583	-4.3308	0.0235	-0.0275	-0.38
9	38.5	-3.1966	-3.2044	0.0380	0.0078	0.12
10	50.2	-5.0313	-5.0654	0.0566	0.0341	0.68
11	50.2	-4.2336	-4.1779	0.0377	-0.0558	-0.85
12	50.2	-2.8353	-2.8772	0.0610	0.0419	0.94

Durbin-Watson Statistic = 2.15

---

* Significant at p = 0.05

Appendix 14: Computer output for the predictive model of storage life (Equation 6-12)

The Regression Equation is

lnSL = -16.6 - 0.192 Bx + 5576 1/T + 51.6 Bx/T

Column	Coefficient	St. Dev. of Coef.	T-Ratio = Coef/S.D.
	-16.561	1.930	-8.58
Bx	-0.19206	0.05351	-3.59
1/T	5575.6	568.3	9.81
Bx/T	51.58	15.76	3.27

S = 0.07656

R-squared = 99.5 percent

R-squared = 99.4 percent, adjusted for D.F.

Analysis of Variance

Due to	DF	SS	MS
Regression	3	10.1557	3.3852
Residual	8	0.0469	0.0059
Total	11	10.2026	

Further Analysis of Variance

SS Explained by Each Variable When Entered in the Order Given

Due to	DF	SS	MS	F_c
Regression	3	10.1557	3.3852	573.76*
Bx	1	0.4566	0.4566	77.39*
1/T	1	9.6364	9.6364	1633.29*
Bx/T	1	0.0628	0.0628	10.64*

Appendix 14: Continued

Row	Bx	Y ln SL	Pred.Y Value	St.Dev. Pred.Y	Residual	St. Res.
1	19.1	2.8736	2.9538	0.0553	-0.0802	-1.51
2	19.1	2.2618	2.1624	0.0369	0.0994	1.48
3	19.1	1.0296	1.0028	0.0596	0.0269	0.56
4	29.0	2.8679	2.8572	0.0368	0.0107	0.16
5	29.0	2.0919	2.0045	0.0246	0.0874	1.20
6	29.0	0.6419	0.7550	0.0397	-0.1131	-1.73
7	38.5	2.6946	2.7639	0.0358	-0.0693	-1.02
8	38.5	1.8718	1.8520	0.0239	0.0198	0.27
9	38.5	0.4700	0.5157	0.0386	-0.0457	-0.69
10	50.2	2.6532	2.6497	0.0574	0.0035	0.07
11	50.2	1.6864	1.6653	0.0383	0.0211	0.32
12	50.2	0.2624	0.2227	0.0619	0.0397	0.88

Durbin-Watson Statistic = 2.16

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* Significant at p = 0.05