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Nonenzymatic Browning Studies
Using an Electrolytic Cell

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May 1995

A Thesis submitted to the Faculty of
Graduate Studies and Research in partial fulfilment
of the requirements for the degree of
Master of Science

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Abstract

The present study investigated the possibility of using an electrolytic cell to prevent nonenzymatic browning in lemon juice products. A single-strength, a double-strength and a triple-strength lemon juice were subjected to four different current densities (0.116, 0.231, 0.463 and 0.926 A/m^2) for 30 minutes using an electrolytic cell. Dissolved oxygen level, conductivity, redox potential, pH in juices and the voltage applied to the electrodes required to maintain constant current density through the cell were monitored. Juice samples were taken at 0, 5, 15 and 30 minutes and then stored for one month at 30^oC.

After one month of storage, juice samples were assessed for browning and quality alteration. The chemical indices used were the browning index and concentrations in furfural and 5-hydroxymethylfurfural (HMF). Although redox potentials and concentrations in dissolved oxygen were significantly reduced by the electrolytic cell treatment, none of the electrochemical treatments significantly retarded detrimental reactions in juices over the storage period. Initial levels in dissolved oxygen and redox potential do not seem to have any significant effect on browning or chemical quality deterioration in lemon juices. Thus, the present study suggests that nonenzymatic browning in lemon juice products may be due to factors other than oxidative reactions.

Résumé

Au cours de la présente étude, des jus de citron ont été soumis à différents traitements électrochimiques à l'aide d'une cellule électrolytique. En effet, trois jus de citron à des teneurs en solides totaux de 8.5, 17 et 25.5°Brix ont été soumis à quatre densités de courant (0.116, 0.231, 0.463 et 0.926 A/m^2) pour une période de 30 minutes. La concentration en oxygène dissout dans le jus, sa conductivité, son potentiel d'oxydo-réduction, son pH ainsi que le voltage appliqué aux electrodes nécessaire pour maintenir une densité de courant constante à travers la cellule ont été mesurés tout au long des traitements électrochimiques. Il a été observé que seuls le niveau d'oxygène dissout et le potentiel redox ont été significativement affectés par les traitements. Des échantillons de jus ont été pris à 0, 5, 15 et 30 minutes durant chaque traitement pour être par la suite entreposés à 30°C.

Après un mois d'entreposage, les différents échantillons de jus ont été analysés pour l'indice de brunissement et leur contenu en furfural et HMF. Les trois méthodes d'analyse révèlent qu'aucun des différents traitements électrochimiques a prévenu de façon significative la détérioration de la qualité des jus pendant la période d'entreposage. Le contenu initial en oxygène dissout du jus de citron ainsi que son potentiel redox n'auraient donc pas d'effet sur le développement des réactions indésirables. Ainsi, la présente étude suggère que les réactions d'oxydation ne seraient pas la principale cause du brunissement non-enzymatique des jus de citron.

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Many people have provided useful assistance and support throughout the course of this Master's thesis. First of all, I gratefully acknowledge the helpful support, encouragement, presence and guidance provided over the last two years by Dr. G.S.V. Raghavan, supervisor of this thesis. The numerous discussions, opportunities, advices and encouragements in the experimental phases given by Dr. François Lamarche, researcher at the Food Research and Development Centre in St-Hyacinthe, are also highly appreciated.

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List of Symbols

Symbols	Definition	Units
A	Ampere	-
ANOVA	analysis of variance	-
CC_t	change in conductivity at time t	% of K_0
CD	current density	A/m^2
$CE_{t-1/2}$	current efficiency at the average time of t and t-1	%
D	dimensions of the electrodes	m^2
DC	direct current	-
df	degrees of freedom	-
DO_0	initial level of dissolved oxygen	mg/L
DO_t	dissolved oxygen concentration at time t	mg/L
e^-	electron	-
F	Faraday's constant	A-hr/equiv
F value	MS / MS error	-
g	gram	-
GLM	general linear model	-
HMF	5-hydroxymethylfurfural	-
HPLC	high pressure liquid chromatograph	-
K_t	conductivity of the medium at time t	mmho
K_0	initial conductivity of the medium	mmho
m	mass	g
m	metre	-
M	molecular weight	g/mol
mg	milligram	-
mL	millilitre	-
mm	millimetre	-
mmho	millimho	-
mV	millivolt	-
MS	mean square	-
M^{x+}	metallic ion	-
n	number of moles per equivalent	mol/equiv
nm	nanometre	-
N	Newton	-
Pr > F	probability to have a greater F value	-
RDO_t	residual dissolved oxygen at time t	%
RT	residence time	minute
s	stoichiometric coefficient of the species	-
SAS	statistical analysis software	-
SS	sum of squares	-

Time $_{batch}$	time during which current was applied to the cell	minute
TSS	total soluble solids	°Brix
V_{cell}	internal volume of the electrolytic cell on the cathode side	mL
V_{juice}	amount of juice in the whole system at Time $_{batch}$	mL
v/v	volume by volume	-
w/w	weight by weight	-
µL	microlitre	-
µm	micrometre	-

I. Introduction

1.1 Background

The citrus industry is of prime importance in the world. The world citrus fruit production was 78 million metric tons in 1992 (FAO, 1992) and citrus production is economically important to various regions. Although some citrus fruits are consumed mainly as a fresh commodity, a major part of the crop is processed into chilled juices and concentrates. Juices are commonly sold on local markets whereas concentrates are sent all over the world to make juices and blends.

The citrus juice industry has been plagued by browning problems for several years. There are two types of browning occurring in citrus juices. Enzymatic browning results from the oxidation of juice constituents by enzymes present in the juice. This type of browning is not a major concern since the enzymatic activity can be minimized through thermal treatments such as pasteurization or sterilization. The second type is nonenzymatic browning and consists of a number of reactions that occur during storage. Nonenzymatic reactions cause deterioration of nutritive value, discolouration and development of off-flavours in citrus products. Although it is rather difficult to estimate the magnitude of the economical losses associated with nonenzymatic browning, the study undertaken by Lee and Nagy (1988) gives a good indication of the kinds of problems involved. They observed that single-strength grapefruit juice stored for 15 weeks at 40°C lost 75 percent of its vitamin C. Moreover, all natural sugars were hydrolysed, colour deteriorated and compounds indicating the development of off-flavours were in concentrations 100 times greater than in the original product.

Although such problems have been reduced by the use of chemical inhibitors, sulfites and sulfite derivatives being the most commonly used in the industry, all chemical food additives are under constant scrutiny by the Food and Drug Administration, and more and more chemicals are being banned. There is therefore strong motivation to develop natural or non-chemical alternatives to chemical food preservation.

Before developing such an alternative, it is important to understand the reaction mechanisms involved in quality degradation of citrus products. Although numerous research studies have been undertaken to elucidate browning precursors, there is still a lack of knowledge of the mechanisms responsible for detrimental reactions occurring in citrus juice products. However, the degradation of vitamin C is currently thought to be the most important source of nonenzymatic browning in citrus juice products.

Under aerobic conditions, oxygen takes on an electron from ascorbic acid to destabilize it, and the vitamin breaks down, yielding either furfural derivatives or brown pigments. Under anaerobic conditions, the oxidation-reduction (redox) reaction between oxygenated compounds in the juice and vitamin C leads to the same result. Although several techniques have been developed to minimize nonenzymatic browning in juice products, none of them has been fully successful, mainly because of their inability to both diminish dissolved oxygen concentrations to insignificant levels and reduce molecules and ions in their oxidized states, the reduced forms being not involved in vitamin C breakdown. For example, deaerators that are commonly found in the industry reduce oxygen levels to 2 mg/L without supplying electrons to oxygenated compounds. This is apparently not enough because both the remaining molecular oxygen and molecules having affinities for electrons can continue to destabilize the vitamin C.

The present work suggests the use of an electrolytic cell to minimize nonenzymatic browning reactions in citrus juice products. This newly-developed technology is not only capable of reducing dissolved oxygen concentrations to insignificant levels but also to provide electrons to oxygenated compounds within the juice. Thus, both aerobic and anaerobic degradations of vitamin C can theoretically be minimized, limiting discolouration and quality deterioration in citrus juice products.

1.2 Objective

The objective of the present study is to investigate the effects of electrochemical treatment on the rate of nonenzymatic quality degradation of single-strength, double-strength and triple-strength lemon juices made from concentrate.

1.3 Scope

The main focus is to determine whether or not an electrochemical process could be used to prevent nonenzymatic browning reactions in lemon juice products made from concentrate. Further research would be required to extend the conclusions of the present work to freshly-squeezed lemon juice and any other type of juice. Identification of possible side reactions occurring during the electrochemical treatments, effects of the type of cationic membrane separating the two compartments of the electrolytic cell, effects of electrode material and levels of turbulence at the electrode surface are beyond the scope of this study.

II. Literature Review

2.1 Importance of the citrus industry

Citrus fruit production is of prime importance throughout the world and rises from year to year. The world citrus fruit production passed from 57 in 1980 to more than 78 million metric tons in 1992 (FAO, 1992). According to the same source, Brazil and the United States are the two main producers of citrus fruit. Brazil produced slightly over 20 million metric tons of citrus fruit whereas the U.S. production was estimated at 12.5 million metric tons in 1992 (FAO, 1992; USDA, 1993).

Oranges are the most widely-grown citrus fruit, accounting for about 72 percent of the world's 1992 production. Grapefruit, lime, lemon, clementine, mandarine, tangerine and pomelo make up the remaining 28 percent (FAO, 1992). Brazil, the United States, China, Mexico and Spain accounted for 36 percent of the world orange fruit production that year. A major part of the production is processed mainly into chilled juices and concentrate. In 1991-92, less than 25 percent of the U.S. orange fruit production was freshly consumed, with almost 75 percent being processed mainly into chilled juices and frozen concentrate (USDA, 1993).

Lemons and limes are also an important part of the world citrus industry, together comprising 7 million metric tons in 1992 (FAO, 1992), or about 9 percent of the total citrus crop. Mexico, the Unites States, Argentina, India, Italy and Spain are the most important lemon and lime producing countries, accounting for 56 percent of the world production. Similar to oranges, a major part of the production is processed mainly into concentrate. According to USDA (1993), more than 40 percent of the U.S. lemon production was processed in 1991-92, the rest being either exported or sold on local markets as a fresh commodity.

Grapefruit also plays an important role in the citrus industry. The world grapefruit production is estimated at over 4.5 million metric tons in 1992, the United States accounting for about 43 percent of this total (FAO, 1992). More than 55 percent of the U.S. grapefruit production was freshly consumed, the remaining were processed mainly into chilled juices and concentrate (USDA, 1993).

As one can deduce, a major part of the world citrus fruit production is processed mainly into chilled juices and concentrate. Florida is a good example. This U.S. state accounts for about 70 percent of the orange production for the country. USDA (1993) reports that more than 94 percent of Florida's orange was processed mainly into concentrate and chilled juices in 1992. While more than 71 percent was used to make concentrate, 4.2 billion litres of single-strength orange juice were packed from Florida fruit that year.

2.2 Browning Problems

The citrus juice industry has been plagued by browning problems for a long time (Handwerk and Coleman, 1988). In fact, citrus juice products undergo browning reactions, leading to serious quality deterioration while in storage. Citrus juice products made from fresh fruit may be stored for 12 months or more before they reach the consumer. During that time, the juices may be exposed to conditions favouring the formation of brown pigments and development of off-flavours. According to the literature, nonenzymatic browning reactions are considered to be the major cause of quality loss in citrus juices during storage (Lee and Nagy, 1988a; Kacem et al., 1987a). These reactions are absolutely undesirable as they cause discolouration and adverse organoleptic properties.

Researchers have not fully identified the reactions responsible for browning in citrus juice products. Although extensive studies have been conducted on elucidating browning precursors, the reaction mechanisms remain unclear. Since browning causes the product to be rejected by consumers, a long list of methods has been suggested to control or prevent its occurrence in citrus juices. None of them has been fully successful due to the lack of knowledge of the chemical pathways to brown pigment formation. However, the following factors are thought to influence the rate and extent of nonenzymatic browning: storage temperature and period (Nagy et al., 1990; Lee and Nagy, 1988a; Reynolds, 1963), free oxygen concentration (Trammell et al., 1986;

Kacem et al., 1987a), amino acids (Kacem et al., 1987b), metal catalysts and pH (Alais and Linden, 1991), total soluble solids (Kanner et al., 1982; Robertson and Samaniego-Esguerra, 1990), ascorbic acid (Clegg, 1964; Kacem et al., 1987b), citric acid (Clegg, 1966) and packaging material (Mannheim et al., 1987; Nagy et al., 1990).

2.3 Causes of Nonenzymatic Browning

Even though the chemical pathways to brown pigment formation are still unclear, two main theories have been postulated to explain nonenzymatic browning in citrus juices; ascorbic acid breakdown and the Maillard reaction. These are thought to be associated to nonenzymatic quality adulteration in citrus juice products while in storage (Kaanane and Labuza, 1993).

2.3.1 Maillard Reaction

Maillard browning has been long identified as a browning pathway in citrus juices. Louis-Camille Maillard discovered the reaction that today bears his name, when he unsuccessfully attempted to synthesize polypeptides (Yaylayan, 1990). The Maillard reaction is a complex series of reactions occurring at different rates and to different extents, depending on many variables. The minimum reactant requirements are the presence of an amino-bearing compound, a reducing sugar and molecules of water (Whistler and Daniel, 1985). The first step in the Maillard browning is the condensation of a carbonyl compound (usually a reducing sugar) with an amino compound (usually an amino acid). The carbonyl carbon of the reducing sugar, in open-chain form, first undergoes nucleophilic attack by the amino nitrogen lone-pair electrons. This is followed by the loss of a molecule of water and ring closure, leading to the formation of a glycosylamine. After an Amadori rearrangement, a 1-amino-1-deoxy-ketose (or Amadori compound) is obtained in an acidic medium, such as in citrus juices. According to Alais and Linden (1991), three possible pathways are then possible for the continuation of the Maillard reaction as shown in Figure 1.

1) A splitting or scission of the Amadori compound into reactive carbonyl

molecules which subsequently react with amino-groups and polymerize to yield brown pigments.

2) A strong dehydration that leads to the formation of furfural, 5-hydroxymethylfurfural (HMF) and other furfural derivatives. According to Hodge et al. (1963), this is the most important of the possibilities. Furfural products tend to accumulate as by-products due to their relatively low reactivity. Although the compounds themselves would not contribute to detrimental changes (Marcy and Rouseff, 1984), their concentrations are often taken as index of quality deterioration in citrus juice products (Robertson and Samaniego, 1986).

3) A more moderate dehydration which gives rise to a mixture of reductones and dehydroreductones, known as reducing substances. These products further react with amino acids which decarboxylate and transform them into aldehydes.

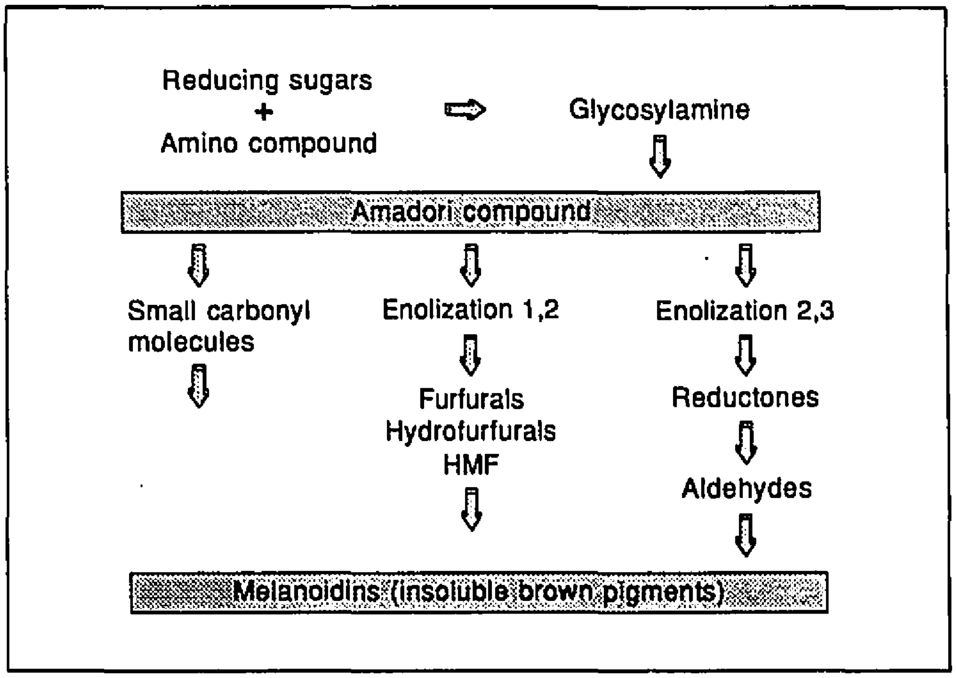

Figure 1: Simplified Scheme of the Maillard Reaction

Many factors may affect the course of the Maillard reaction in liquid systems.

Temperature and pH influence the rate and extent of sugar-amine condensation (Alais and Linden, 1991). Although Maillard browning occurs at low temperatures, it is stimulated by higher temperatures, particularly at those used for thermal processing. With respect to the acidity level of a liquid system, Alais and Linden (1991) reported that products having a pH in the range of 6 to 8 present the most favourable conditions for the development of the Maillard reaction.

Components of the juice product itself are also implicated in the enhancement of the Maillard browning. The presence of reactive sugars, amino acids, citric acid and metallic ions influence the rate and extent of the Maillard reaction in citrus juice products. Of the three major sugars (sucrose, glucose and fructose) found in citrus juices, fructose has been identified as the most reactive in the formation of breakdown products in single-strength grapefruit juice (Lee and Nagy, 1988b). Fructose was 36 times faster than glucose in forming HMF, and twice as fast as sucrose. The authors suggested that, due to its high reactivity, fructose may play an important role in nonenzymatic browning in citrus juices.

The contribution of amino acids to Maillard browning is also very important in citrus juice products. Kacem et al. (1987a) found a linear relationship between levels of amino acids and browning in single-strength orange juice. Curl (1949), Joslyn (1957) and Wolfrom et al. (1974) observed an accelerated sugar breakdown yielding brown pigments in the presence of amino acids, and Feather and Nelson (1984) found amine groups in a specific brown polymer. Similarly, Shaw and Berry (1977) showed that amine catalysis produced compounds of undesirable taste in model systems. As described by Handwerk and Coleman (1988), amino acids would interact with reactive carbonyl compounds, leading to the formation of brown pigments and objectionable flavours.

In addition, organic acids and their products promote sugar degradation in citrus juice products. Lee and Nagy (1988b) report that large amounts of organic acids and their salts, mainly citric, create favourable conditions for degradation of sugars in citrus juices during processing and subsequent storage. They suspect acid-catalyzed thermal decomposition of reducing sugars to be an important element in nonenzymatic browning of citrus juices.

Finally, there are many reports that certain metallic ions have an important effect on Maillard browning in liquid systems. Lee and Nagy (1988b) observed that the formation of HMF from sugar degradation is accelerated by the presence of some minerals (K^+, Ca^{2+}, Mg^{2+}). Alais and Linden (1991) report that certain cations such as Mn^{2+} and Sn^{2+} hinder the Maillard reaction whereas others such as Cu^{2+} and Fe^{3+} activate it. The valences of the latter two metallic ions have a great influence on their reactivity. Several researchers have noticed that iron and copper ions do not act as catalysers when they exist in reduced forms. Pollard and Timberlake (1971) report that iron-catalysed reactions in liquid systems are inhibited by the addition of bisulfite because it reduces the iron to its ferrous form.

Nevertheless, some research indicates that the Maillard reaction is likely of secondary importance in nonenzymatic browning of citrus juice products because of the media's acidity. Sugar-amino-acid reactions seem unlikely to be the main contributors to the formation of melanoidin pigments in citrus juice products due to the relatively high acidity level of the medium (pH 2-4) (Clegg and Morton, 1965; Kaanane and Labuza, 1993; Lee and Nagy, 1988b; Joslyn, 1957; Kaanane et al., 1988).

2.3.2 Ascorbic Acid Degradation

Ascorbic acid degradation, which involves both aerobic and anaerobic oxidative mechanisms, is currently thought to be the major source of nonenzymatic browning in citrus juice and citrus juice products. Ascorbic acid degradation not only implies nutritional quality loss (Vitamin C) but is also associated with other quality problems in citrus juices (Handwerk and Coleman, 1988). Oxidative breakdown of ascorbic acid yields reactive compounds such as carbonyls, which subsequently react with free amino acids and polymerize to yield brown pigments.

Ascorbic acid degradation has been reported under both aerobic and anaerobic conditions (Nagy, 1980). Figure 2 summarizes the different breakdown pathways.

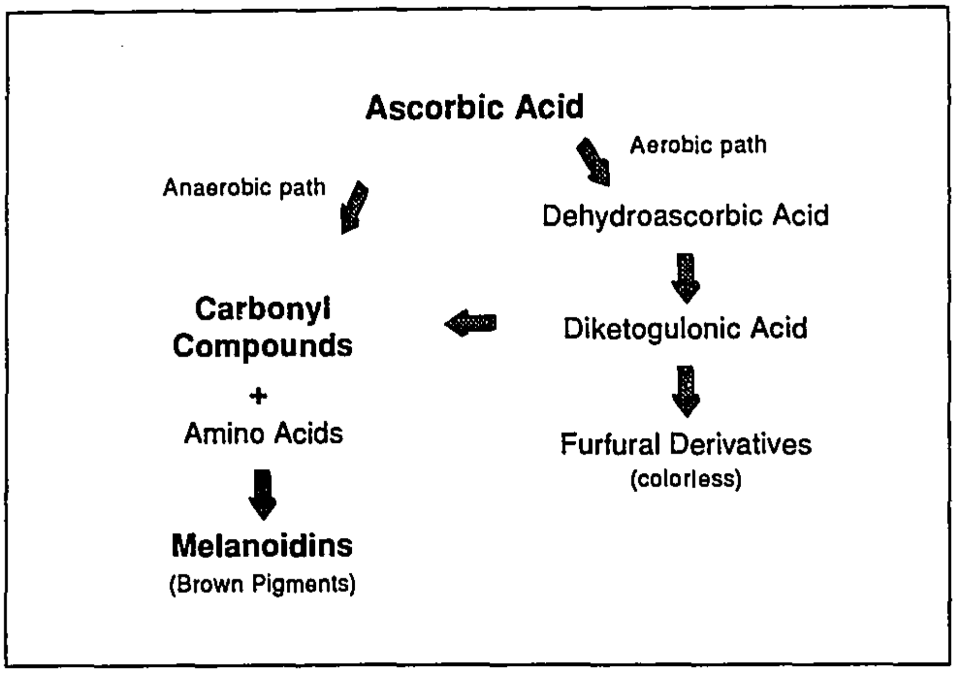

Figure 2: Simplified scheme of the different degradation paths of ascorbic acid

Ascorbic acid is regarded as being quite sensitive to oxygen (Trammell et al., 1986). Kefford et al. (1959) and Nagy and Smoot (1977) have shown an initial period of rapid ascorbic acid loss in single-strength orange juice caused by the presence of free oxygen. The oxidation-reduction (redox) reaction, or transfer of an electron from ascorbic acid to molecular oxygen, destabilizes the ascorbic acid. Although the aerobic redox pathway is much faster than the anaerobic pathway at ambient temperature (Tannenbaum et al., 1985), anaerobic breakdown of ascorbic acid has also been reported in liquid systems (Huelin, 1953) and citrus juices (Robertson and Samaniego, 1986). Once free oxygen is consumed, ascorbic acid continues to break down anaerobically by interaction with oxygenated compounds. The degradation rate is lower because the breakdown products have lower electron affinities than oxygen. The destabilized ascorbic acid is susceptible to undergo breakdown, yielding either furfural derivatives or brown pigments.

Vitamin C degradation seems to be influenced by several factors. Similar to the Maillard reaction, the nature and the rates of degradation mechanisms responsible for the breakdown of ascorbic acid are dependent upon many parameters. The redox potential of the medium, its acidity level, the presence of oxygen or other oxidizing agents (Bauernfeind and Pinkert, 1970), presence and valence of metals (especially copper and iron) (Pollard and Timberlake, 1971), temperature (Lee and Nagy, 1988a), citric acid (Clegg, 1966), reactive reducing sugars (Nagy, 1980), initial concentration of amino acids (Kacem et al., 1987a) and packaging material (Mannheim et al., 1987) are among the most important factors that may influence the course of ascorbic acid breakdown.

The oxidation-reduction potential of citrus juices may have an influence on the stability of the ascorbic acid they contain (Bauernfeind and Pinkert, 1970). In fact, the overall reactivity of the constituents of a given liquid system (more or less indicated by its redox potential) affects the stability of vitamin C. As a general rule, a system wherein molecules and ions are in their reduced states is more stable and characterized by a more negative redox potential. Thus, ascorbic acid should also be more stable in a system having a more negative redox potential (lower concentration of oxidizing agents).

The effect of oxygen on ascorbic acid breakdown in citrus juice products has been intensively studied by scientists. Most studies undertaken on the subject found that oxygen plays a significant role in the degradation of vitamin C. Studying the effect of oxygen on taste, ascorbic acid loss and browning in single-strength orange juice, Trammell et al. (1986) observed a linear relationship between ascorbic acid loss and initial levels of dissolved oxygen. Khan and Martell (1967) noticed the same tendency using model systems. Similarly, in studies of the vitamin composition of milks sterilized by different processes, Burton et al. (1970) found that the stability of ascorbic acid in sterilized milk was closely related to its content in dissolved oxygen. In agreement with the above studies, Johnson and Toledo (1975) noticed lower rates of ascorbic acid breakdown in orange juice concentrate in conditions of reduced head space oxygen.

Although deaeration and anaerobic storage resulted in increased retention of vitamin C in orange juice and drinks (Kacem et al., 1987b), a study on grapefruit concentrate conducted by Passy and Mannheim (1979) concluded that deaeration treatments do not significantly retard breakdown of ascorbic acid. Similarly, Robertson and Samaniego (1986) found that the rate of vitamin C degradation is not affected by the initial levels of dissolved oxygen in freshly-squeezed lemon juice.

Citric acid is also thought to play a role in the breakdown of ascorbic acid. Using a model system in simulating lemon juice, Clegg (1966) showed that specific brown pigment can be detected only when citric and ascorbic acids are both included. The author suggests possible pathways of formation of brown pigments in which citric acid may be involved. First, citric acid could act as a catalyst and enhance the rate of ascorbic acid oxidation to yield reactive carbonyl compounds. Second, citric acid itself might undergo fragmentation, yielding carbonyl compounds. Another possibility would be that citric acid reacts with some of the products resulting from the degradation of ascorbic acid. Similar to amino acids, it could be then incorporated into brown pigments.

Amino acids accelerate the breakdown of ascorbic acid in citrus juice products. In fact, Joslyn (1957), Clegg (1964) and Seck and Crouzet (1981) have demonstrated that amino acids increase the rate of vitamin C degradation. It was further shown that, in the presence of amine, dehydroascorbic acid is the most reactive intermediate in the pathway to furfural and brown pigment production (Hodge et al., 1953). In agreement with the above studies, Kacem et al. (1987a) observed that higher concentrations of amino acids result in higher degradation rates of ascorbic acid in studies of the effect of ascorbic acid, amino acid and oxygen on nonenzymatic browning in aseptically packaged orange drinks.

Fructose has been reported to enhance the breakdown of ascorbic acid (Nagy, 1980). Curl (1947) showed that higher levels of fructose in concentrated orange juice result in greater loss of vitamin C. Studies on the anaerobic decomposition of ascorbic acid in model systems undertaken by Huelin (1953) confirmed the degradative effects of fructose. Curl (1949) suggests that the carbonyl groups of fructose could be responsible for ascorbic acid breakdown.

The degradation of ascorbic acid would be the most important factor responsible for detrimental changes in citrus juice products. According to the literature, oxidation of ascorbic acid is thought to be the major source of nonenzymatic browning in these products (Clegg and Morton, 1965; Kaanane et al., 1988; Kacem et al., 1987b; Varsel, 1980; Berk and Korner, 1984; Marcy et al., 1984). This undesirable breakdown phenomenon not only reduces the nutritive value, but also causes the formation of brown pigments and gives adverse organoleptic properties to citrus juice products.

2.4 Chemical Inhibitors

Scientists have developed and tested chemical inhibitors to prevent browning reactions in citrus juice products. Although they have not completely elucidated the reaction mechanisms responsible for browning and development of off-flavours, numerous studies have been undertaken to investigate the protective effect of different chemical additives on browning. Bisulfites and sulfur dioxide are the most commonly used browning inhibitors in the citrus juice industry (Pollard and Timberlake, 1971; Davidek et al., 1990). Molnar-Perl and Friedman (1990) tested five other potential inhibitors in commercial grape, apple, pineapple, grapefruit and orange juices, namely: ascorbic acid, phosphoric acid derivatives, sodium sulfite, N-acetyl-L-cysteine, L-cysteine and reduced glutathione. Their results revealed that N-acetyl-L-cysteine and tripeptide reduced glutathione may be as effective as sodium sulfite in preventing nonenzymatic browning and the development of off-flavours. The authors suggest that their greater nucleophilic reactivity may explain their better effectiveness as browning inhibitors.

Tin retards detrimental reactions in citrus juice. Many studies have in fact shown that tin has the ability to significantly delay nonenzymatic browning reactions in citrus juice products, although it is not used as an additive. Steel cans used to store liquids are often tin-coated. Nagy et al. (1990) observed faster and more intensive browning in grapefruit juice stored in glass bottles than in tin-coated steel cans. An interaction would occur between the juice components and the material of the can (Nagy and Nikdel, 1986). According to Rouseff and Ting (1985), tin acts as a sacrificial anode, being oxidized preferentially to sensitive juice components such as vitamin C.

Chemical pathways for browning inhibition in citrus juice are still not entirely understood. It is still unclear as to how inhibitors prevent detrimental reactions. Friedman and Molnar-Perl (1990) suggest different inhibition mechanisms for sulfur-containing compounds. One involves the suppression of the free-radical formation whereas another suggests the combination of sulfhydryl compounds and intermediates formed during browning. Because of the strong nucleophilic reactivity of their sulfhydryl groups, sulfur-containing compounds can interact and trap either free radicals or intermediates formed during browning, thus preventing the formation of brown pigments. Similarly, Cheftel et al. (1985) reported that sulfites act as reducing agents and preserve ascorbic acid from degradation by reacting with carbonyl compounds that would otherwise destabilize the vitamin.

Although some chemical inhibitors have shown abilities to protect citrus juices from discolouration, and while most citrus juice companies add sulfite derivatives to prevent quality deterioration of their products, serious health problems and inconvenience have arisen from their usage (Brown, 1985; Gifon et al., 1989) and the Food and Drug Administration has severely restricted the use of chemical additives in foods. Alternatives are therefore needed.

2.5 Electrochemical Processes

Electrctechnologies might be an interesting alternative to chemical additives in preventing the occurrence of browning reactions in citrus juice products. Electrochemical processes have been used for over a century in industrial electrolysis, energy conversion and metal deposition (Prentice, 1991). More recent applications such as electrodialysis and electroreduction make use of electricity to separate different constituents and modify the valence of specific molecules of liquid systems. The last two techniques have paralleled the development of membrane technology as they make use of selective permeable membranes. At the present time, the most important applications of electrodialysis are the desalination of brackish water for the production of potable water, the production of table salt from sea-water and the desalination of cheese whey (Leiva, 1988). Electrotechnologies are certainly a growing field in the food industry wherein many applications are yet to be discovered.

2.5.1 Description of an electrolytic cell

The present work suggests that an electrochemical treatment based on an electrolytic cell may prevent the occurrence of nonenzymatic browning in citrus juice products. Figure 3 shows a simplified scheme of an electrolytic cell. It is made of an anode and a cathode compartments separated by a selective cationic membrane. The latter is designed in such a way that only cations, i.e. positively charged ions, are allowed to pass through. The liquid system to be treated is circulated through the cathode compartment whereas an electrolyte solution is passed through the anode

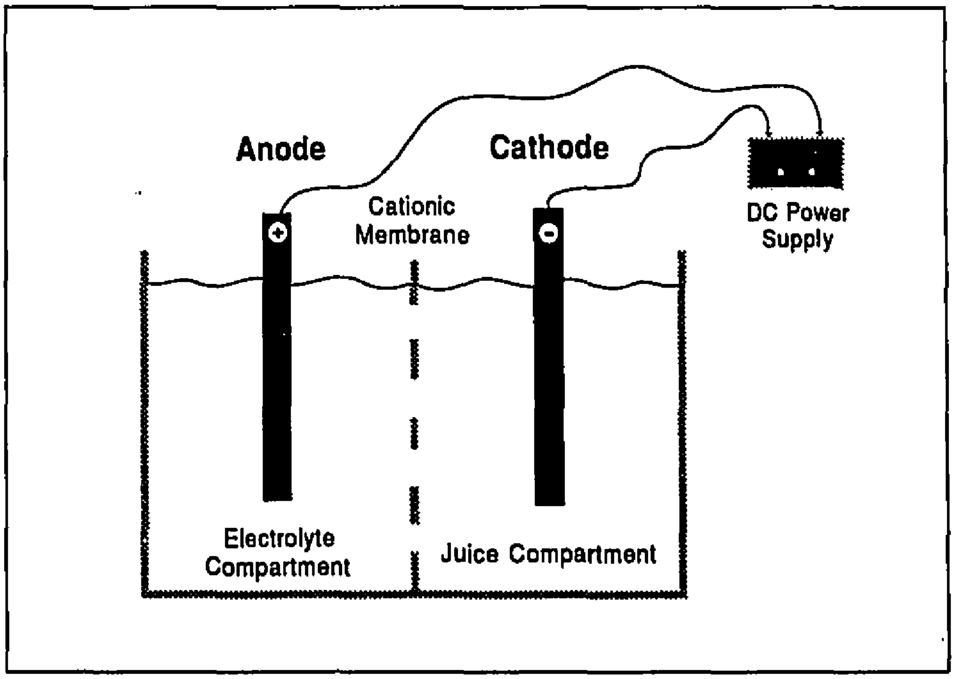

Figure 3: Simplified scheme of an electrolytic cell

compartment. The cell can be operated either on a batch or continuous basis whereby both anolyte and catholyte are pumped through their respective channels. Spacers usually surround the electrodes to maintain a high level of turbulence at the surface of the electrodes. The electrodes are made of non-toxic, electrically conductive material, the anode usually being a noble metal. While stainless steel is commonly used as cathode material, the anode is typically made of either platinum, platinum plated titanum, columbium, ruthenium or ruthenium plated titanium (Hekal, 1983). The electrodes are connected by conductive wiring to a direct current (DC) power supply which is used to apply a potential difference to the electrodes. Depending upon the voltage applied and the dimensions of the electrodes, both fluids are subjected to a current density as they are circulated through the cell.

2.5.2 Chemical Reactions at the Electrodes

As a voltage is applied to the electrodes, several chemical reactions occur at the surfaces of the anode and cathode. Figure 4 illustrates the different phenomena

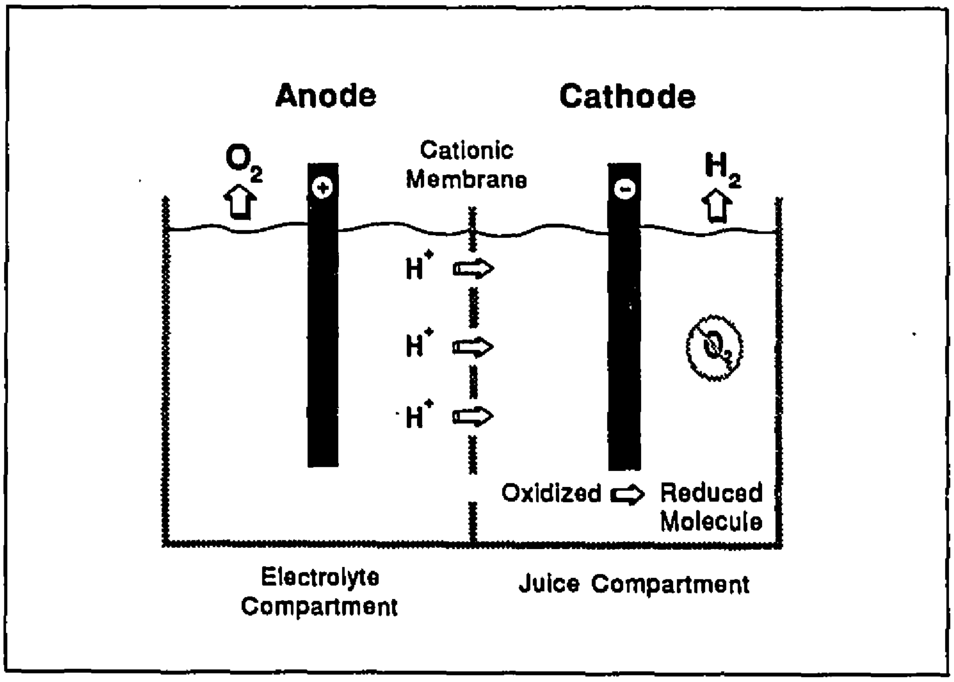

Figure 4: Phenomena in the electrolytic cell when subjected to a current density

occurring in the electrolytic cell when subjected to a current density. First of all, electrons are transferred from the anode compartment to the external circuit at the anode as shown in Equations (1) and (2) (Dick, 1978; Lacey, 1988). Hydrogen ions, electrons (e^-) and molecules of oxygen are thus generated from molecules of water contained in the anolyte. The hydrogen ions thus generated are later found in the cathode compartment since they are attracted by the negative electrode and are allowed to pass through the cationic membrane. Equation (2) indicates metal dissolution of the anode. Metallic ions (M^{x+}) and electrons are generated from this dissolution phenomenon. Similar to the hydrogen ions, the metallic ions thus generated might pass through the cationic membrane since they are attracted by the cathode.

$$6\ H_2O \quad \text{--->}\ 4\ H_3O^+ + 4\ e^- + O_2 \qquad (1)$$

$$M^0 \quad \text{--->}\ M^{x+} + x\ e^- \qquad (2)$$

On the other hand, Equations (3) to (6) show the reactions that would occur at the surface of the negative electrode (Dick, 1978; Lacey, 1988). At the cathode, electrons are transferred from the external circuit to ions and molecules present in the catholyte. For this reason, the cathode compartment is commonly called a reducing pool since electrons supplied by the external circuit reduce molecules and ions present in the system. Regarding the equations, the level of oxygen dissolved in the juice product is expected to decrease during the electrochemical process as shown in Equation (3). In fact, molecules of oxygen dissolved in the juice product will react with electrons coming from the external circuit and hydrogen ions available in the system to form molecules of water.

$$O_2 + 4\ H_3O^+ + 4\ e^- \text{--->}\ 6\ H_2O \qquad (3)$$

Hekal (1983) reduced levels of dissolved oxygen in citrus juice products using an electrochemical deoxidative treatment. In fact, using a similar electrolytic cell to the one to be used in the present work, Hekal (1983) significantly diminished the amount of dissolved oxygen in single-strength orange juice. While the juice was circulated through the cathode compartment, sulfuric acid (10%, w/w) was pumped through the anode compartment. When subjecting both fluids to a current density of 0.31 A/m^2 for

3.5 minutes, the dissolved oxygen concentration in the juice passed from 8 to 0 mg/L. Based on this, an electrochemical treatment would have the ability to significantly decrease the amount of dissolved oxygen in liquid systems.

On the other hand, the brewing industry has used electrochemical technologies to reduce oxygen levels in beer. Although few research studies have been published on the subject, brewers have investigated the use of a similar electrochemical treatment to prevent detrimental oxidation reactions during beer staling. Bamforth (1986) reports that oxygen is the single most important parameter causing flavour deterioration in beer, since it causes the breakdown of alcohol into aldehyde during beer staling. An electrochemical treatment to reduce the amount of dissolved oxygen in beer prior to the staling period is therefore used.

Metal deposition and hydrogen gas generation are expected at the cathode during the electrochemical process. Hydrogen ions should react with electrons coming from the external circuit to form molecules of water and hydrogen gas as shown in Equation (4). Wang et al. (1991) noticed the generation of hydrogen in gaseous form at the cathode when using a similar electrolytic cell. Equation (5) represents the metal deposition at the negative electrode. Since metal that dissolves at the anode is attracted by the negative electrode and may pass through the cationic membrane depending upon its selectivity, metallic ions generated at the anode might take on electrons and then deposit at the surface of the cathode.

$$2\ H_3O^+ + 2\ e^- \quad \text{--->}\ H_2 + 2\ H_2O \qquad (4)$$

$$M^{x+} + x\ e^- \quad \text{--->}\ M^0 \qquad (5)$$

Another chemical reaction expected to occur in the juice compartment is the reduction of molecules and ions in their oxidized states. As shown in Equation (6), the exposure of a liquid system to an electrical field allows the conversion of oxidized molecules into their reduced form. When a potential difference is applied to the electrodes, electrons are transferred from the anolyte to the catholyte during the process since the electrolytic cell is part of the electrical circuit. Species with high electron affinities, such as oxidized molecules and ions in their oxidized states, might then take on electrons provided at the cathode. This reduction phenomenon will favour the reduced form of species in the system.

$$\text{Oxidized Molecule} + 1\ e^- \dashrightarrow \text{Reduced Molecule} \qquad (6)$$

Many studies have reported the successful reduction of oxidized molecules and ions using an electrolytic cell. Wang et al. (1991) published their work on the conversion of cystine into cysteine (the reduced form of cystine) using an electrochemical membrane reactor. While a current density of 2.0 A/m^2 was applied to the system, cystine in HCl solution and 0.1 Normal H_2SO_4 solution were respectively circulated through the cathode and anode compartments, separated by a cation exchange membrane. After concentration and crystallization, they obtained an overall cysteine crystal recovery of over 91 percent. This indicates that an electrochemical treatment is quite effective in converting oxidized molecules into their reduced form.

In addition, Bazinet (1993) reduced disulfide bonds of whey protein using an electrolytic cell similar to the one to be used in this study. In studies of the effect of different electrochemical treatments on the allergenicity of whey protein, he successfully reduced disulfide bonds of whey protein into sulfhydryl groups. Although he found that this reduction reaction does not affect the allergenicity level of whey protein, he proved that an electrochemical treatment favours the reduced form of whey proteins.

On the other hand, Nojeim et al. (1981) modified the ferrous-ferric equilibrium ratio in model systems and foods using an electrolytic cell. In fact, they noticed that an electrochemical reduction treatment favours the lower valence of ionized iron. To achieve that, they designed an electrolytic cell to control the redox potential of model systems. The cell permitted the setting of the redox potential to any desired value between +300 and +650 millivolts (mV). Since they found a close relationship between redox potential and ferrous-ferric equilibrium ratio in model systems and foods, an electrochemical treatment could also favour the reduced form of ionized iron in liquid systems.

In agreement with the above study, Ito et al. (1990) improved the stability of natural colorant in sausages using an electrolytic treatment. Hemoglobin is an important heme-containing protein in animal blood and could be used as a natural colouring agent in sausages. However, its utilization is still rather limited due to the instability of its heme groups. Ito et al. (1990) improved the stability of heme protein solutions by reducing heme from ferric to ferrous state using an electrolytic cell. The instability of the protein was possibly attributable to heme containing iron in the oxidized state.

2.5.3 Why Electrochemical Treatment Could Prevent Browning?

Many reasons have led scientists to believe that an electrochemical treatment using an electrolytic cell could be an alternative to chemicals in limiting nonenzymatic browning in citrus juice products. An electrochemical reduction process should minimize both aerobic and anaerobic degradations of ascorbic acid, the major sources of quality deterioration in citrus juice products. First, since an electrolytic cell can decrease the initial dissolved oxygen level in liquid systems (Lacey, 1988; Hekal, 1983), it could minimize the aerobic breakdown of vitamin C in citrus juices. As seen previously (Equation 3), the amount of oxygen dissolved in liquid system can be significantly reduced through an electrochemical treatment. Since Trammel et al. (1986) observed that browning and loss of ascorbic acid are linearly related to the initial dissolved oxygen level in single-strength orange juice, an electrolytic cell could then possibly reduce detrimental aerobic reactions in citrus juice products.

Secondly, an electrochemical treatment could possibly minimize the anaerobic degradation of ascorbic acid in citrus juice products. The conversion of oxidized molecules into their reduced form (Equation 6) using an electrolytic cell could in fact diminish the detrimental oxidation of ascorbic acid under anaerobic conditions. Since molecules in their oxidized state can destabilize reduced-state molecules by taking on electrons, the destabilized species undergo further breakdown reactions. Vitamin C in citrus juice is particularly susceptible to this phenomenon since the force holding the electrons to the molecule is relatively low compared to other molecules found in citrus juices. Thus, ascorbic acid is an easy target for oxidized molecules. However, converting oxidants to their reduced state should prevent ascorbic acid breakdown. Since an electrolytic cell favours the reduced form of molecules in liquid systems (Wang et al., 1991), it should then minimize the anaerobic breakdown of vitamin C, which is partly responsible for browning in citrus juice products.

Similarly, an electrochemical treatment could diminish the reactivity of certain browning catalysers by converting them into their reduced form. Alais and Linden (1991) mentioned that certain metallic ions in their oxidized state such as Cu^{2+} and Fe^{3+} catalyse browning reactions in citrus juice products. However, it has been observed that these metallic catalysts should not be involved in the detrimental reactions when in their reduced forms (Pollard and Timberlake, 1971). Since an electrolytic cell favours the reduced state of molecules and ions in liquid systems (Nojeim et al., 1981), it could possibly inactivate metallic browning catalysts by reducing them, namely Cu^{+} and Fe^{2+}.

2.5.4 Actual Application

An electrochemical process has been used to retard detrimental reactions in citrus juice products. Hekal (1983) designed an electrolytic cell that significantly retards browning and ascorbic acid loss in single-strength orange juice. The proposed electrolytic deoxidative treatment should prevent the occurrence of oxidative reactions, mainly responsible for quality deterioration in citrus juice products. Although the experiments undertaken by Hekal (1983) are more or less preliminary, this study is nevertheless useful since it illustrates different aspects of the electroreduction process.

Hekal (1983) investigated the effect of several parameters of the electrochemical treatment. First of all, he tested the use of different anolytes and types of membrane separating the two compartments of the electrolytic cell. While cationic, anionic and red clay membranes were tested as partition-walls between the two compartments of the cell, different solutions were used as anolyte. These were phosphoric acid, sulfuric acid, potassium chloride and sodium chloride. Among the different anolytes and types of membrane tested, sulfuric acid and cationic membranes are the only ones that did not cause significant changes in acidity, taste, and ascorbic and citric acids contents.

The same researcher also reported some results on the storability of orange juice samples treated with the electrolytic cell. Using a solution of sulfuric acid (10%, w/w) as anolyte and a cationic membrane between the two compartments of the cell, single-strength orange juice was circulated in the cathode compartment while a current density of 0.31 A/m^2 was applied to the system. He observed better colour stability and ascorbic acid retention for the treated samples compared to the non-treated ones after 12 weeks of storage at 40°C. Therefore, the electrolytic deoxidative treatment proposed by Hekal (1983) seems to significantly retard detrimental reactions responsible for browning in citrus juice products.

2.5.5 Possible Limitations

Although an electrochemical treatment seems to be quite attractive to prevent nonenzymatic browning reactions in citrus juice products, some difficulties and limitations could raise from its usage. First of all, the electrode material is an important factor in directing the course of the electrode reactions (Lund, 1973). Although the material choice is more restricted by corrosion and sanitary considerations in the food processing industry, other factors should be considered when selecting the electrode materials. The course of the chemical reactions occurring at the electrodes will certainly be influenced by the constituent material since each of them has its own electrochemical properties. In addition, one should also take into account the type and level of uptake of metallic ions during the electrolytic process. Looking at Equation (2), a gain in metallic ions in the juice product throughout the electrolytic treatment might be expected. Although Wang et al. (1991) did not find any difference in metal content between treated and raw samples when converting cystine into cysteine, levels should be in accordance with current regulations and consumer acceptance.

The cationic membrane separating the two compartments of the cell is an important factor as well. The choice of the membrane will be of particular importance since each membrane has its own selectivity to ions and other molecules (Moutounet et al., 1993). One should keep in mind that the cationic membrane is the only parameter that controls exchanges between the anolyte and catholyte.

The acidity level of juice products might increase through the electrochemical process. In fact, migration of hydrogen ions through the cationic membrane during the process may induce a slight pH drop in citrus juice products to be treated. Although Hekal (1983) did not notice any pH difference between untreated single-strength orange juice and samples that have been electrolytically treated, a slight rise in acidity might be undesirable considering that citrus juice products are already acidic.

Current density and fluid velocity may affect the performance of the electrochemical treatment. Increasing current density could increase the reaction rate at both electrodes. However, flow rates of fluids and levels of turbulence in the cell should be sufficiently high to ensure that the reaction rate at the cathode does not exceed the rate of reactant diffusion toward the electrode. Current densities applied to the system should be limited to a certain level for a given product since a high operating current density, which requires a high potential difference at the electrodes, may induce undesirable side reactions at the cathode and impart objectionable flavour to the juice. Although no taste difference between untreated and treated single-strength orange juices was observed by Hekal (1983), subjecting the system to too high a current density may cause hydrolysis of sugars which could accelerate browning reactions in citrus juice products.

III. Materials and Methods

The experimental part of this research study was conducted at the Food Research and Development Centre in St-Hyacinthe (Québec). All electrochemical treatments, storage experiment and browning analysis of the different juice samples were performed using the equipments and resources available at the above Centre.

3.1 Apparatus

An electrolytic cell (Electrosynthesis Co Inc.) was used to subject lemon juice products to different electrochemical treatments. The different parts of the electrolytic cell used to perform the electrochemical treatments are shown in Figure 5, and a schematic diagram of the flows through the unit is given in Figure 6. Similar to the cell used by Hekal (1983), Bazinet (1993) and Wang et al.(1991), the electrolytic cell (552 mm high, 248 mm wide and 41 mm in thickness) is made of two compartments, each compartment containing an electrode. The anode and the cathode compartments are separated by a cationic membrane which allows only positively charged ions to pass through. The cell is operated on a continuous basis. The juice product and an electrolyte solution are respectively circulated through the cathode and anode compartments. The electrolytic cell has a capacity of 255 millilitres in the cathode compartment. Thus, the cell allows this amount of juice to be subjected to an electrical field at the same time. The cationic membrane used in this experiment (CMX membrane, Tokuyama Soda Neosepta) has the following characteristics: total cation transport number of 0.98, thickness of 0.17-0.19 mm, electrical resistance of 250-350 Ω per metre of thickness and bursting strength of 490,000 - 785,000 N/m^2. The cathode is made of stainless steel No. 40 and the anode is a Dimensionally Stable Anode (DSA-O_2) made of platinum-plated titanium. The dimensions of the electrodes are of prime importance since they directly influence the current density applied to the system. The electrodes used in this study are 0.146 m wide, 0.296 m high and have a thickness of 2.5 mm. Cross track sheet-flow type spacers surround both electrodes

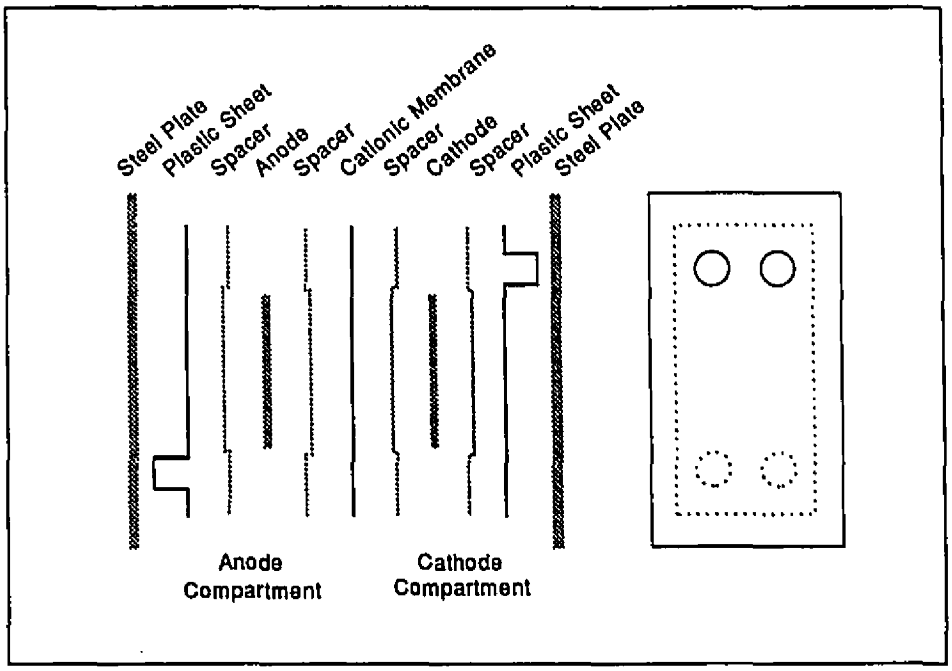

Figure 5: Parts of the electrolytic cell used to carry out the electrochemical treatments

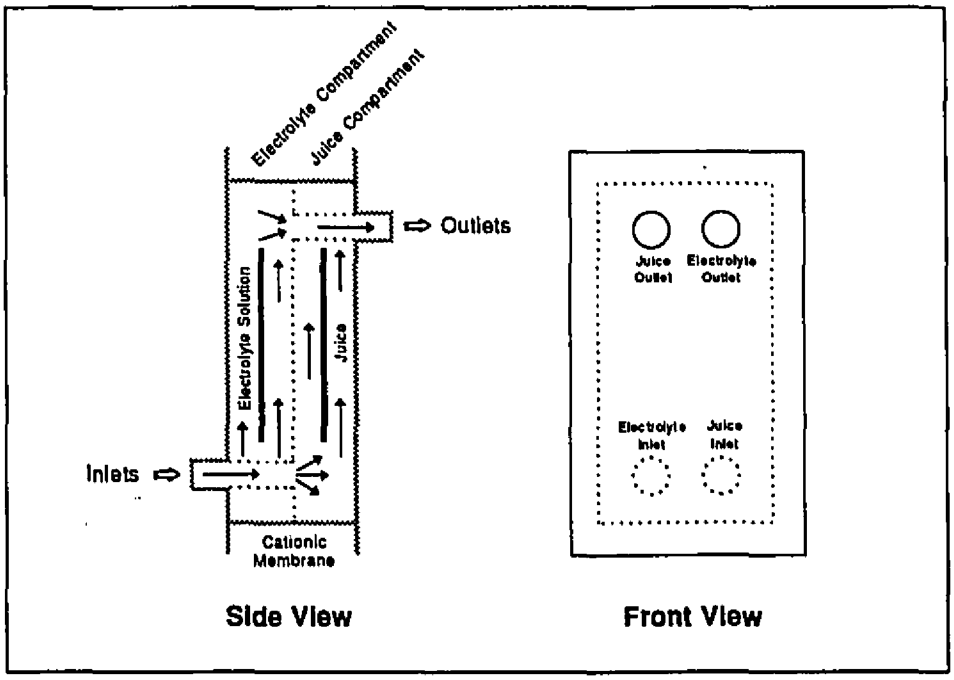

Figure 6: Schematic diagram of the flows through the electrolytic cell used

to maintain a high level of turbulence at the surface of the electrodes.

The experimental setup is illustrated in Figure 7, and a photograph of the system used is shown in Figure 8. Centrifugal pumps (March-MFG, BC-2CP-MD model) were used to circulate both anolyte and catholyte through their respective compartments. Although the electrolytic cell is operated on a continuous basis, both fluids are returned to their respective feed flasks after a single pass through the cell. A DC power supply (Hewlett Packard 6024) was used to subject the electrolytic cell to the desired current density and both fluids were pumped through at a constant flow rate of 1.9 litre/minute. The juice was maintained at constant temperature (25°C) in its flask during the electrochemical treatments using a water bath (Fisher Scientific, Haake G), thus eliminating the effect of temperature on the process. In addition, the juice container was flushed with nitrogen during the electrochemical process to prevent entry of atmospheric oxygen. Finally, a magnetic stirrer bar ensured a good mix in the juice flask during the treatment.

3.2 Experimental Design

Three lemon juice products were subjected to different electrochemical treatments in this study. A single-strength (8.5°Brix), a double-strength (17.0°Brix) and a triple-strength (25.5°Brix) lemon juice were subjected to four current densities. Different levels of total soluble solids were under study since each juice strength is characterized by its own physical and chemical properties (conductivity, viscosity, etc.). The rate and extent of the chemical reactions that can occur at the surface of the cathode are affected by the soluble solids content. Soluble solids higher than 25.5°Brix in lemon juice were not used because of pumping limitations.

While both lemon juice product and electrolyte were circulated through their respective compartments, constant currents of 0.5, 1, 2 and 4 Amperes (A) were applied to the system. These represented current densities of 0.116, 0.231, 0.463 and 0.926 A/m^2 at the electrodes. Preliminary tests revealed that these current densities provide a wide range of rates of changes in redox potential and dissolved oxygen levels in lemon

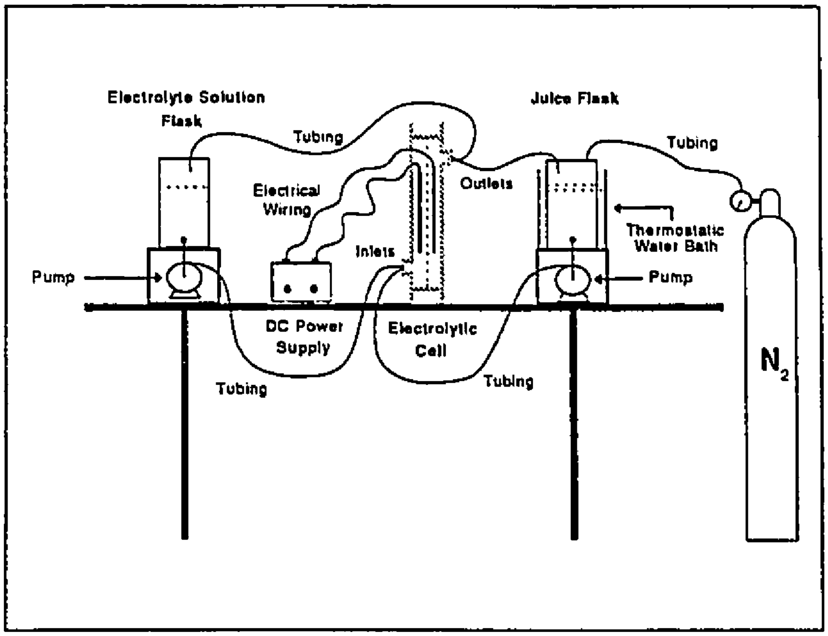

Figure 7: Schematic of the experimental setup used in this study

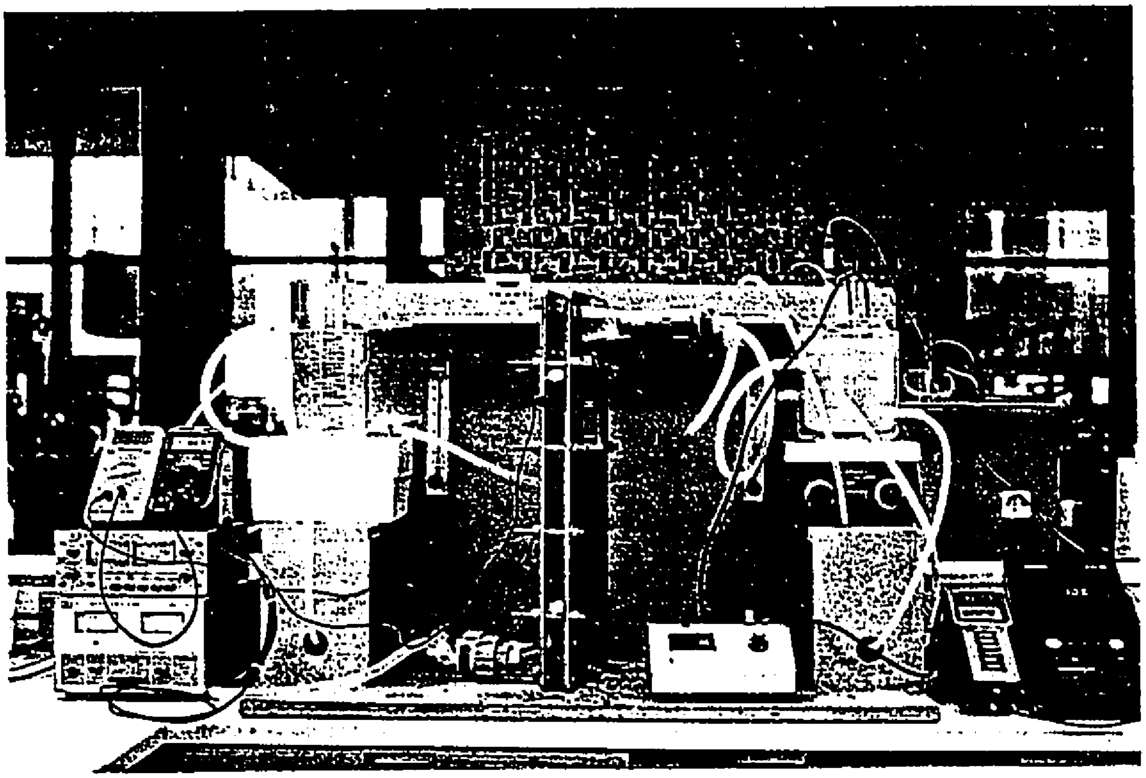

Figure 8: Photograph of the experimental setup of this study

juice products. It was also noticed that, of the four current densities used in the preliminary experiments, the higher one allowed a relatively rapid drop in redox potential and removal rate of dissolved oxygen in the juice products. Since Wang et al. (1991) suspect that high current densities may promote side reactions at the surface of both electrodes, the current density applied to the electrolytic cell was limited to 0.926 A/m^2.

Triplicates were performed for each current density and level of soluble solids in lemon juices. Since four different current densities and three levels of total soluble solids of lemon juices were under study, a total of 36 electrochemical treatments were performed. Each electrolytic treatment lasted 30 minutes since insignificant changes in redox potential and level of oxygen dissolved in the juice were observed for longer exposure to the electrical fields in preliminary runs.

Four juice samples were taken from the juice flask for each trial. Juice was sampled from its flask at times 0, 5, 15 and 30 minutes, the sample taken at time 0 being the control. This sampling procedure was adopted since preliminary tests revealed higher rates of changes in redox potential and dissolved oxygen content in the first 15 minutes of treatment. Since 36 electrochemical treatments were performed, a total of 144 juice samples were collected in the present study. A summary of the different treatment levels is given in Table 1.

Table 1. Experiment treatment levels

Treatments	Levels			
Total Soluble Solid (^{o}Brix)	8.5	17.0	25.5	
Current Density (A/m^2)	0.116	0.231	0.463	0.926
Time of Treatment (minutes)	0	5	15	30

The experiments were set up as a repeated measures design and the statistical analysis performed on the results obtained from the different treatments has to take into account its specific particularity. Such experiments have structures that involve more than one size of experimental unit (Milliken and Johnson, 1992). In the present case, there are two. The larger experimental unit is the electrochemical treatment itself. Its design is a two-way treatment structure (soluble solids content * current density) in a completely randomized design structure with three replications at each level of soluble solids and current densities. The smaller experimental unit is the time interval. The experimental design for time interval is a one-way treatment structure in a randomized complete block design structure in 36 blocks. The analysis of variance performed in order to test the significance of the main effects on the depending variables has to take into account the non-randomization of time intervals between measurements for each treatment. Thus, time intervals may be autocorrelated or not independent from one another.

3.3 Materials and Procedure

Lemon juice products were made from concentrate (45°Brix) supplied by Lassonde Technology Inc. Held at -18°C until needed, the concentrate was thawed overnight in a cold room maintained at 4°C and then used to make juice by the addition of demineralized water. An RFM 80 0-95% Sugar Digital Refractometer was used to adjust the amount of soluble solids to the required levels. For each electrochemical treatment, 2.4 litres of lemon juice products are introduced into the juice flask and circulated through the juice compartment of the electrolytic cell for 30 minutes. Even when no current density was applied to the system, excessive accumulation of foam in the juice flask was noticed when the juice was circulated through the electrolytic cell. To overcome this foaming problem (presumably caused by the high turbulence level in the cell), a drop of Antifoam A manufactured by Sigma Chemical (St-Louis, MO) was added to the juice prior to its circulation through the system. This silicone-containing chemical is widely used in the food industry and would not influence browning reactions.

While lemon juice products are circulated through the cathode compartment of the electrolytic cell, an electrolyte is pumped through the anode compartment. In general, the electrolyte solution used in electrochemical treatments depends on the purpose of the process. In the present case, the electrolyte should first promote the generation of hydrogen ions. In fact, hydrogen ions are necessary to reduce molecular oxygen dissolved in the juice products (Equation 3). It would also be desirable that the electrolyte solution have a high electrical conductivity. Since the electrolyte is part of the electrical circuit, a higher electrical conductivity implies a lower resistance to the flow of electrons, thus a lower power input for a given current density. For these reasons, a solution of sulfuric acid was chosen as electrolyte since it dissociates readily and has a high electrical conductivity. However, preliminary experiments revealed that higher pH drops occur in the juice products when solutions of sulfuric acid at higher concentrations are used as electrolyte. Since lemon juice products are already quite acidic, solutions of low concentrations in sulfuric acid would be more suitable as electrolyte. For this reason, sulfuric acid at a concentration of 0.1 Normal was used as the anolyte. Wang et al. (1991) used 0.1 Normal H_2SO_4 solution as well as anolyte when converting cystine into cysteine using a similar electrolytic cell to that used in this study. A volume of 1.7 litre of electrolyte was circulated through the anode compartment of the electrolytic cell throughout the electrochemical treatments.

Four different juice samples were taken from the juice flask in each trial. Using a plastic syringe, 125 mL universal glass bottles were filled under nitrogen flushing and sealed with a metallic cap as shown in Figure 9. This sampling procedure minimized the possible contamination of juice samples with ambient oxygen. Furthermore, the literature indicates that oxygen contained in the head space may play an important role in nonenzymatic browning of lemon juices (Robertson and Samaniego, 1986). Therefore, possible differences in head space between juice samples were eliminated by filling bottles to the top.

Juice samples were then pasteurized in boiling water for 8 minutes to prevent any enzymatic activity. This pasteurization procedure was chosen based on the work done by Berk and Korner (1984) who observed that the rate and extent of browning for single-strength lemon juice samples stored at 35°C were independent of pasteurization

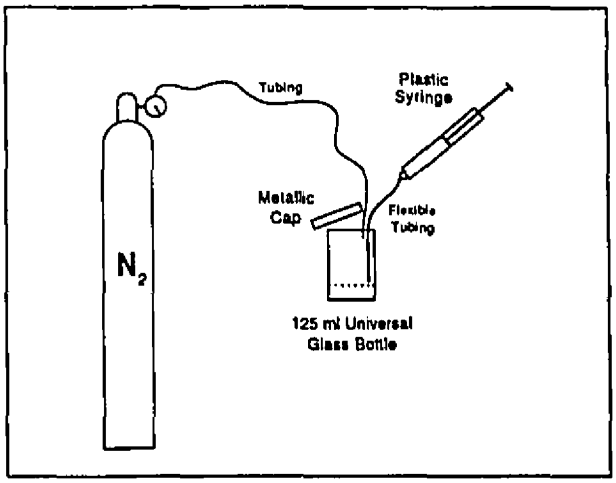

Figure 9: Procedure used to fill juice bottle under nitrogen flushing

time for the range of 2 to 30 minutes. Thus, juice samples were pasteurized by holding bottles in boiling water for 8 minutes. The bottles were rapidly cooled down with cold water and stored for four weeks in a Forma Scientific reach-in incubator maintained at 30°C. Several samples were frozen and kept at -18°C, as controls.

3.4 Parameters Measured throughout the Electrolytic Processes

Several physical and chemical characteristics of the juice were monitored during the electrochemical treatments. While the cell was subjected to constant current densities, the voltage applied to the electrodes, the level of oxygen dissolved in the juice product, its pH, conductivity and redox potential were measured in the juice flask. The current passing through the cell was adjusted to the desired levels using an A.W. Sperry DM-8600 multimetre whereas a Micronta high speed sampling digital multimetre was used to measure the corresponding voltage applied to the electrodes. Levels of dissolved oxygen in the juice was measured with a YSI model 55 Handheld Dissolved Oxygen System previously calibrated using two solutions of known dissolved oxygen concentration. Water saturated in oxygen at a given temperature and sodium sulfite were used for this purpose to ensure the accuracy of the readings given by the equipment. The pH of juice was measured using a Corning pH metre 140. Prior to utilization, the apparatus was calibrated with both neutral and acidic standard solutions. The conductivity of juice was measured in millimho (mmho) with a YSI model 35 Conductance metre, previously calibrated using 1 and 10 mmho standard solutions. Finally, the last parameter under control during the process was the redox potential measured in millivolts. It was monitored using a Corning pH metre 140 with a Metrohm AG Herisau electrode. A solution of dithiothreitol (0.05%) corresponding to a redox potential of -330 mV (Gerhard and Murray, 1981) and a redox standard PM +250 mV were used to verify the accuracy of the readings given by the apparatus.

As the pumps were turned on and a given current density applied to the system, the parameters were recorded at every 30 seconds for the first five minutes, at every minute for the following five minutes and then at every two minutes for the remaining 20 minutes. Such a strategy was adopted since preliminary experiments showed higher rates of changes in the first part of treatments. However, the pH of juice products was measured at the beginning and at the end of the process. Preliminary experiments revealed no significant change in acidity in juice products throughout the electrolytic treatment when sulfuric acid at a concentration of 0.1 Normal was used as electrolyte.

3.5 Browning Analysis

After four weeks of storage at 30°C, the different juice samples were analyzed for colour. Several methods are proposed in the literature to quantify discolouration and other detrimental changes occurring during storage in citrus juice products (Robertson and Samaniego, 1986). In the present study, three different methods were used to analyze the extent of nonenzymatic browning in the lemon juice samples: browning index, furfural and HMF contents. These are described below.

3.5.1 Browning Index

First of all, the browning index of the different juice samples was determined by the method suggested by Klim and Nagy (1988). The method used in this experiment entailed the following steps:

Step 1. 15 mL of juice was centrifuged at 1000*g* for 15 minutes using a GS-6 Beckman centrifuge.

Step 2. 3 mL of the supernatant fluid was removed and placed in a 15-mL centrifuge tube. 3 mL of methanol was added. The solution was then mixed and placed in an ice bath for 15 minutes to accelerate flocculation of finely suspended, colloidal particles.

Step 3. The samples were recentrifuged at 1000*g* for 15 minutes using the same equipment as mentioned above.

Step 4. The supernatant fluid was then read at 420 nm with a Beckman DU-7 Spectrophotometre using 13-mm cuvettes against a solution of distilled water and methanol (50:50, v/v).

It should be noted that double-strength and triple-strength lemon juices were diluted to 8.5°Brix (single-strength) prior to step 1 for comparison purposes. Triplicate measurements were performed for each juice sample. Some authors recommend an additional filtration step between steps 3 and 4 of the above procedure (Nagy et al., 1990). However, it was found unnecessary in this study since absorbances obtained for unfiltered supernatant fluids were not significantly different from those obtained using a 0.45 μm 13-mm disposable syringe filter (Nylon Acrodisc 13; Gelman Sciences).

3.5.2 Furfural and HMF Contents

Similar to browning index, concentrations in furfural and HMF are suitable chemical indices of quality deterioration in lemon juices (Robertson and Samaniego, 1986). Furfural is an end product of ascorbic acid degradation in the pathway proposed by Bauernfeind and Pinkert (1970). Its formation parallels that of brown pigments in citrus juices and for that reason, has been recommended by several researchers as an index of storage temperature abuse in citrus juice products (Dinsmore and Nagy, 1972; Kanner et al., 1982; Robertson and Samaniego, 1986). Similarly, HMF has also been reported as a suitable index of quality deterioration (Robertson and Samaniego, 1986). For these reasons, concentrations in furfural and HMF were determined in the different juice samples and taken as indices of deterioration.

Concentrations in both furfural and HMF were determined in juice samples by a method similar to that of Cuzzoni et al. (1988). Triplicate determinations were performed for each of the 144 juice samples. Sample preparation differs depending upon the amount of soluble solids in the juice. The procedure for both single-strength and double-strength lemon juices entailed the following steps:

Step 1. 15 mL of juice was centrifuged at 1000*g* for 15 minutes using a GS-6 Beckman centrifuge.

Step 2. Supernatant fluids were glass-wool filtered to remove any suspended particles.

Step 3. The clear solution was then analyzed by reverse-phase High Pressure Liquid Chromatography (HPLC) in a Waters 490 liquid chromatograph. For this purpose, a RP-18 guard column (30 * 4.6 mm) and a ZORBAX C8 4.6 mm * 250 mm column was eluted with a mobile phase of methanol-water (15:85, v/v) at a flow rate of 1 mL/minute with an injection volume of 15 µL. The eluate was monitored at a wavelength of 280 nm.

An additional dilution step was required to measure furfural and HMF contents in triple-strength lemon juice. In fact, preliminary experiments revealed that an additional dilution step between step 1 and step 2 of the above procedure is required to prevent rapid fouling of the guard column. Supernatant fluids of triple-strength lemon juice obtained from step 1 were then diluted 1:4 (v/v) with water prior to filtration (step 2). This dilution factor was of course considered when calculating the actual concentrations of furfural and HMF in triple-strength lemon juices.

Different standard solutions were analyzed as well through HPLC. Solutions of known concentration in furfural and HMF were passed through the HPLC to establish the correlation between the response given by the apparatus and the actual concentrations in juice samples. The chromatographic peak area provided by the HPLC was considered in establishing the relationship.

IV. Results and Discussion

The analysis of the results obtained in this study is segregated into two major parts. First, the evolution of the different parameters under measurement throughout the electrochemical treatments is analyzed and discussed. Secondly, results obtained from the browning analysis of the different juice samples are presented and discussed. Based on colour stability of juice samples, conclusions are drawn for the use of an electrolytic cell to prevent the occurrence of nonenzymatic browning in lemon juice products.

Results obtained from the experiments were analyzed for statistical validity using the General Linear Model (GLM) Procedure of Statistical Analysis Software (SAS). The entire data set was analyzed as a repeated measures design to test the linear, quadratic and cross-product effects of the three factors (total soluble solids, current density and residence time) on dependent variables. The ANOVA Tables thus obtained are given in Appendix 1. In addition, regression analyses were performed in order to establish the relationships between the three independent variables and the dependent ones under study. The models used initially contained 18 terms, including all possible combinations of the three independent variables for which the sum of the exponents is 3 or less. The regression (REG) procedure with the stepwise option in SAS was then used to retain the terms that were significant at the .05 level. The estimates of regression coefficients and the standard errors of the statistical model for each dependent variable are presented in Tables in Appendix 2.

4.1 Parameters Measured

Five parameters were monitored with respect to time during each of the electrochemical treatments: dissolved oxygen in juice product, redox potential, acidity level (pH), conductivity and the potential difference required at the electrodes to maintain a given current density.

4.1.1 Oxygen

Initial levels of dissolved oxygen ranged from 3.02 to 7.42 mg/L. Since it is rather difficult to discuss or comment on the evolution of dissolved oxygen concentrations when initial values are not the same, data were transformed for comparison purposes. This led to the parameter:

$$RDO_t = (DO_t / DO_0) * 100 \qquad (7)$$

where;

RDO_t is the residual dissolved oxygen in juice product at time t (%)

DO_t represents the actual concentration in dissolved oxygen at time t (mg/L)

DO_0 is the initial level of oxygen dissolved in juice (mg/L).

The time the juice spent in the electrolytic cell needed to be calculated as well. Since each of the electrochemical treatments was operated as a batch-type process, the actual time the juice product was subjected to the electric current had to be determined in order to establish a possible relationship between residual dissolved oxygen in juice product and its residence time in the electrolytic cell. Equation (8) was used for this purpose.

$$RT = Time_{batch} * V_{cell} / V_{juice} \qquad (8)$$

where;

RT	is the residence time of juice product in the electrolytic cell (minute)
$Time_{batch}$	represents the time during which current was applied to the electrolytic cell (minute)
V_{cell}	is the internal volume of the electrolytic cell on the cathode side (mL)
V_{juice}	is the amount of juice in the whole system (electrolytic cell, juice flask and tubing) at $Time_{batch}$ (mL).

Although the cell capacity on the cathode side has a constant value of 255 mL, the amount of juice in the whole system changes since juice samples were taken from the juice flask throughout the electrochemical treatments.

The amount of oxygen dissolved in juice products changed considerably during the treatments. Figures 10 to 12 respectively show the residual dissolved oxygen in a single-strength, a double-strength and a triple-strength lemon juice with respect to their residence times in the electrolytic cell. Notice that each curve represents the average of three replicates. First, the amount of oxygen dissolved in lemon juices tends to decrease with an increase in residence time. Such behaviour was expected since, as the process goes on, electrons are transferred from the external circuit to juice constituents at the cathode, thus promoting the conversion of molecular oxygen into molecules of water (Equation 3). As discussed in the literature review, Hekal (1983) observed the same trend with single-strength orange juice using a similar electrolytic cell. According to the statistical analysis, residence time has both significant linear and quadratic effects on residual dissolved oxygen at the .05 level. It is rather surprising to obtain a significant quadratic effect since Faraday's law establishes a linear relationship between the amount of oxygen that can be removed from a solution and the time it is subjected

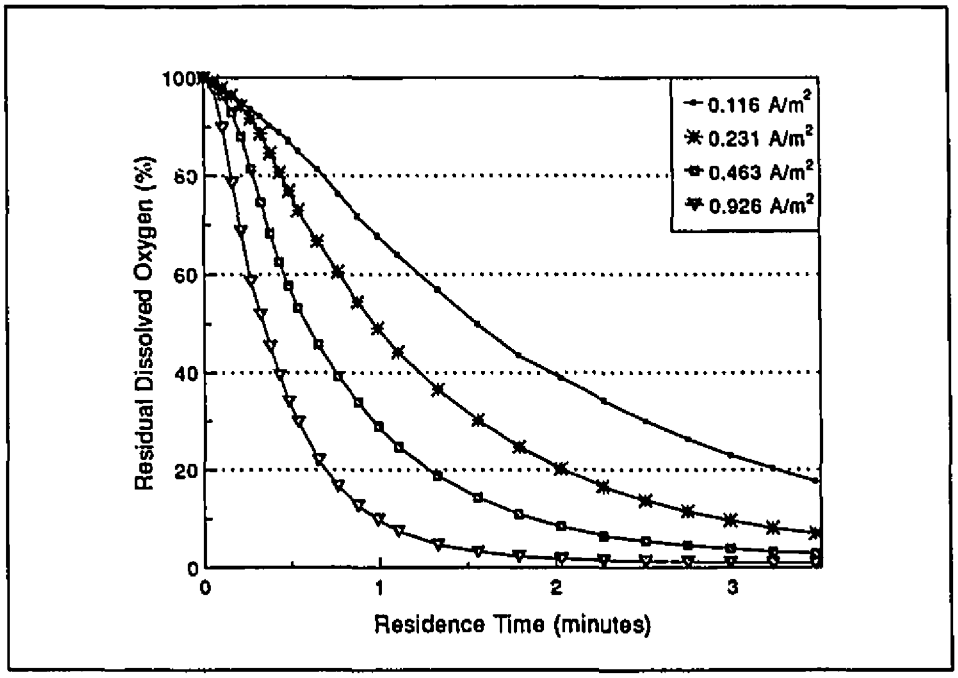

Figure 10: Residual dissolved oxygen in a single-strength lemon juice with respect to its residence time in the electrolytic cell

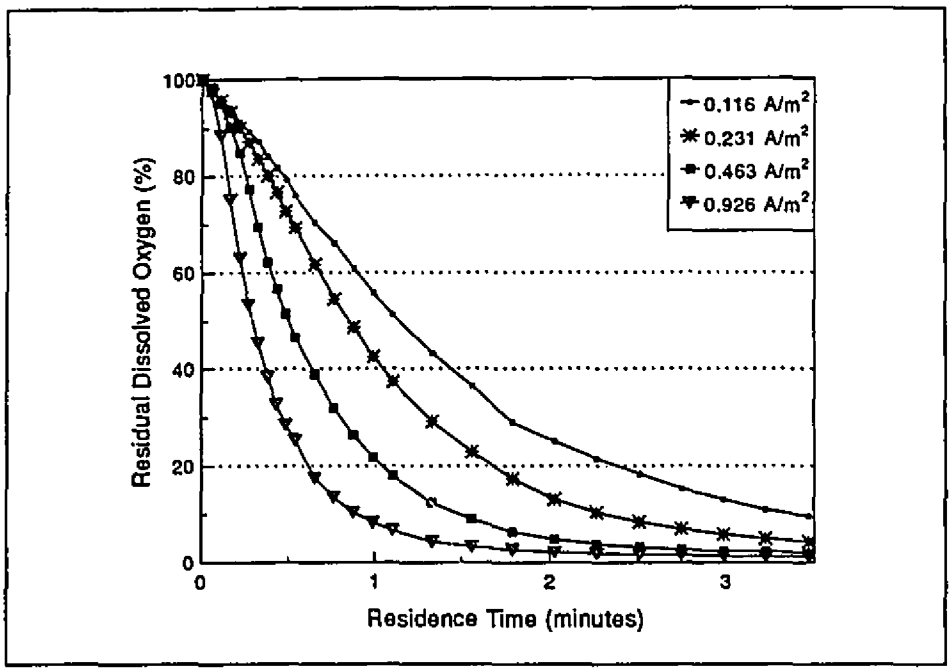

Figure 11: Residual dissolved oxygen in a double-strength lemon juice with respect to its residence time in the electrolytic cell

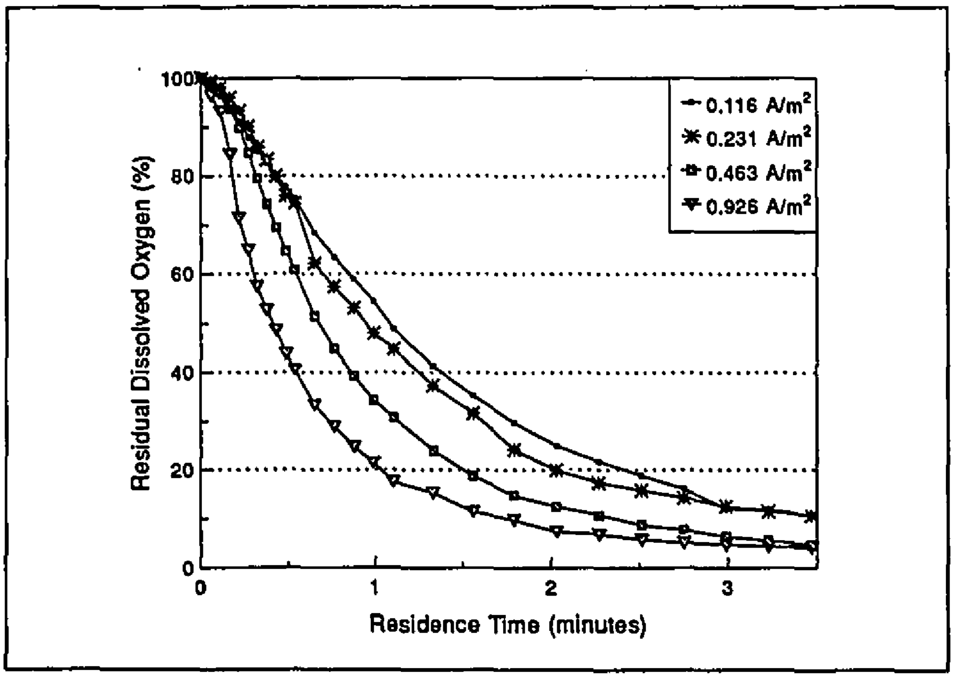

Figure 12: Residual dissolved oxygen in a triple-strength lemon juice with respect to its residence time in the electrolytic cell

to a current density. Although not expected, the quadratic effect might be attributable to lower efficiency in removing dissolved oxygen as residence time increases. This hypothesis will be confirmed later on when analyzing current efficiencies related to the removal of dissolved oxygen with respect to residence time.

On the other hand, faster removal rates of dissolved oxygen can be noticed when juices are subjected to higher current densities. As shown, residual dissolved oxygen tends toward 0 percent rapidly for higher current densities. Such a phenomenon was expected since higher current densities are associated with a greater transfer of electrons to juice constituents at the cathode. As shown in the repeated measures ANOVA Table in Table 1 of Appendix 1, the statistical analysis reveals both significant linear and quadratic effects of current density on residual dissolved oxygen at the .05 level. Although, a linear relationship was expected based on Faraday's law, the quadratic effect would be attributable to lower efficiencies in reducing molecular oxygen at higher current densities as will be confirmed later on.

Looking at Figures 10 to 12, it can be seen that dissolved oxygen in a double-strength lemon juice seems to disappear faster than in a single-strength and a triple-strength lemon juice. The statistical analysis supports the above statement since it indicates both significant linear and quadratic effects of soluble solids content on residual dissolved oxygen at the .05 level. At first sight, such a phenomenon seems rather difficult to explain. However, it becomes clearer when considering the intrinsic properties of juices, especially conductivity and viscosity. The amount of soluble solids in juice directly influences its conductivity and viscosity; an increase in soluble solids levels brings a rise in conductivity until the viscosity of juice becomes a limiting factor for electron circulation. Regarding the electrochemical treatment itself, both conductivity and viscosity will play a major role on the rates of reaction occurring at the cathode surface; *i)* the higher the conductivity of juice surrounding the cathode, the easier will be the electron flow; *ii)* the lower its viscosity, the better the electron transfer. Since double-strength lemon juice has a greater conductivity than single-strength lemon juice and a lower viscosity than triple-strength lemon juice, it would presumably present the best compromise between conductivity and viscosity among the soluble solids contents tested regarding the acceptance and transportation of electrons generated at the cathode.

Theoretical curves of residual dissolved oxygen were computed for conditions similar to the ones used in the experiment. Considering that electrons transferred from the external circuit to juice constituents at the cathode are entirely used to remove dissolved oxygen, residual dissolved oxygen levels were calculated with respect to residence time using Faraday's law. Figure 13 shows the theoretical residual dissolved oxygen in a 2.4 litre solution containing 5.5 mg/L of dissolved oxygen initially. These conditions simulate the same ones as in the experiment undertaken in this study, 2.4 litres being the initial amount of juice in the whole system and 5.5 mg/L being the average initial dissolved oxygen concentration in juices. Based on Faraday's law (Equation 9) and the reduction of molecular oxygen into molecules of water (Equations

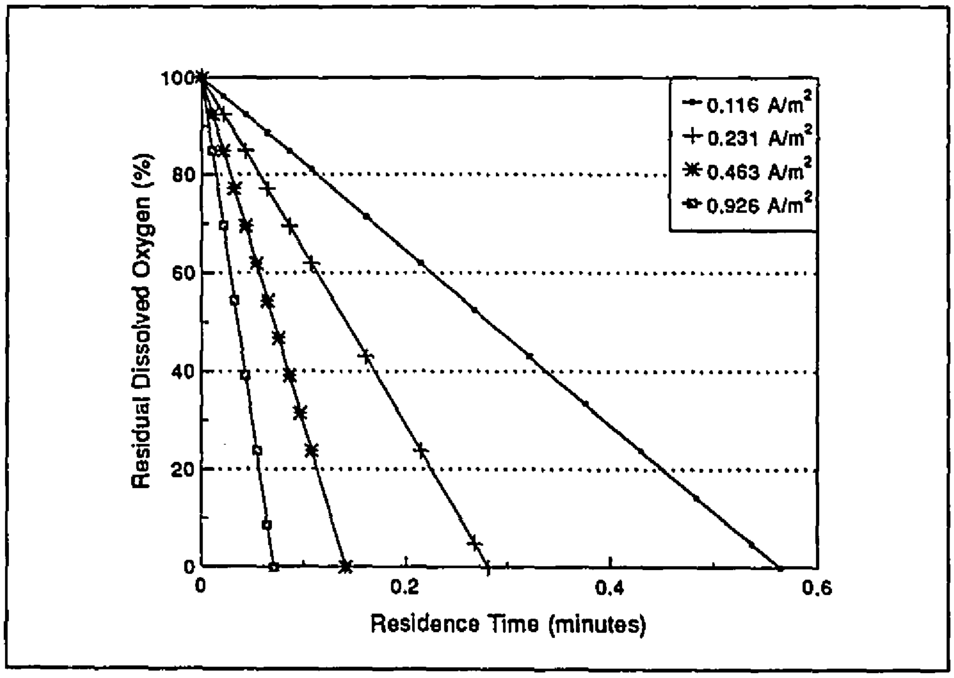

Figure 13: Theoretical residual dissolved oxygen in a 2.4 litre solution containing initially 5.5 mg/L of dissolved oxygen with respect to its residence time in the electrolytic cell

3 and 10), the maximum amount of oxygen that can be removed with respect to time for a given current density was computed. As shown in Figure 13, the greater the current density, the faster the rate of oxygen removal. When compared to experimental values obtained in this study, theoretical removal rates are much larger than those obtained experimentally. Notice that even for the lowest current density, oxygen would have been completely removed before the time to first sample the juice in the experiment ($\text{Time}_{\text{batch}} = 5$ minutes).

$$m = \frac{s * \mathrm{M} * \mathrm{CD} * \mathrm{D} * \mathrm{RT}}{n * \mathrm{F}} \tag{9}$$

$$1/2\ \mathrm{O_2} + 2\ \mathrm{e^-} \dashrightarrow \mathrm{O^{2-}} \tag{10}$$

where;

m	is the theoretical mass of molecular oxygen converted into molecules of water (g)
s	is the stoichiometric coefficient of the species (1/2 mol)
M	is the molecular weight of oxygen (32 g/mol)
CD	is the current density applied ($\mathrm{A/m^2}$)
D	is the dimensions of the electrodes ($\mathrm{m^2}$)
RT	is the time the solution is subjected to the current density (minute)
n	is the number of moles per equivalent (2 mol/equivalent)
F	is Faraday's constant (1608 A-minute/equivalent).

Current efficiencies for the removal of dissolved oxygen in lemon juice products were computed as well. Based on Equation (9) and the experimental values obtained in this study, Figures 14 to 16 respectively show the proportion of electrons generated at the cathode used to remove dissolved oxygen in a single-strength, a double-strength and a triple-strength lemon juice. Expressed in percent, the current efficiency of the system to reduce molecular oxygen into molecules of water at the average residence time of t and t-1 ($\mathrm{CE}_{t-1/2}$) was calculated as follows:

$$\mathrm{CE}_{t-1/2} = (1/1000m) * (\mathrm{DO}_t - \mathrm{DO}_{t-1}) * V_{\text{juice}} * 100\% \tag{11}$$

where;

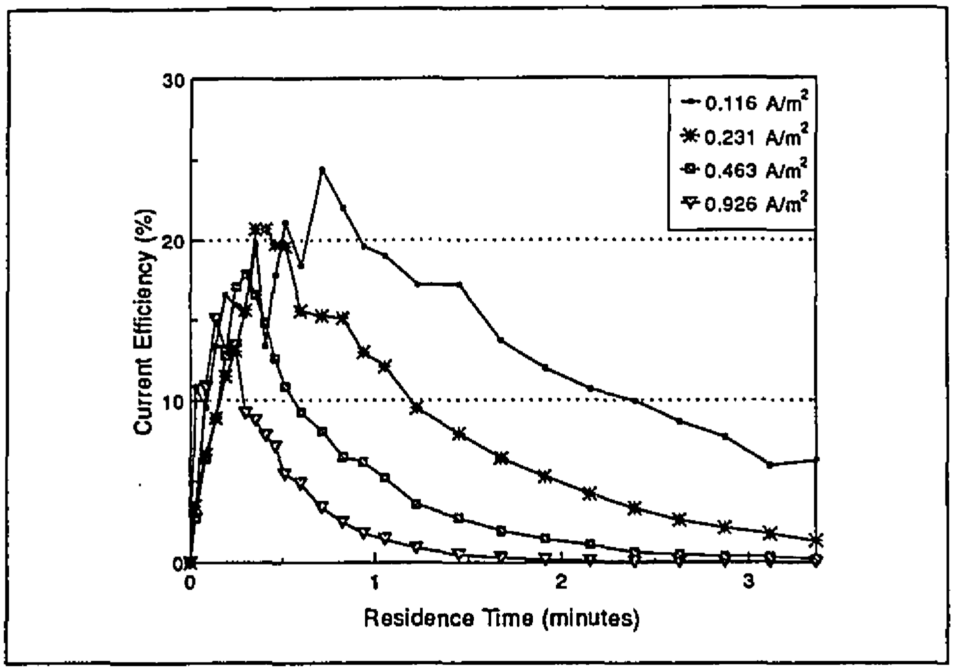

Figure 14: Current efficiency for the removal of dissolved oxygen in single-strength lemon juice with respect to its residence time in the electrolytic cell

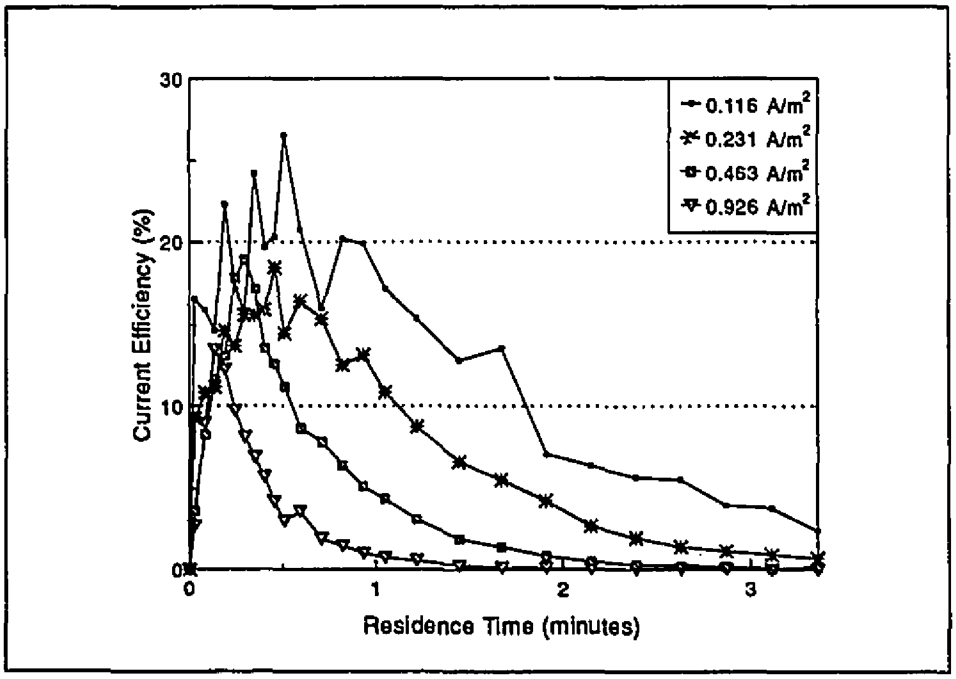

Figure 15: Current efficiency for the removal of dissolved oxygen in double-strength lemon juice with respect to its residence time in the electrolytic cell

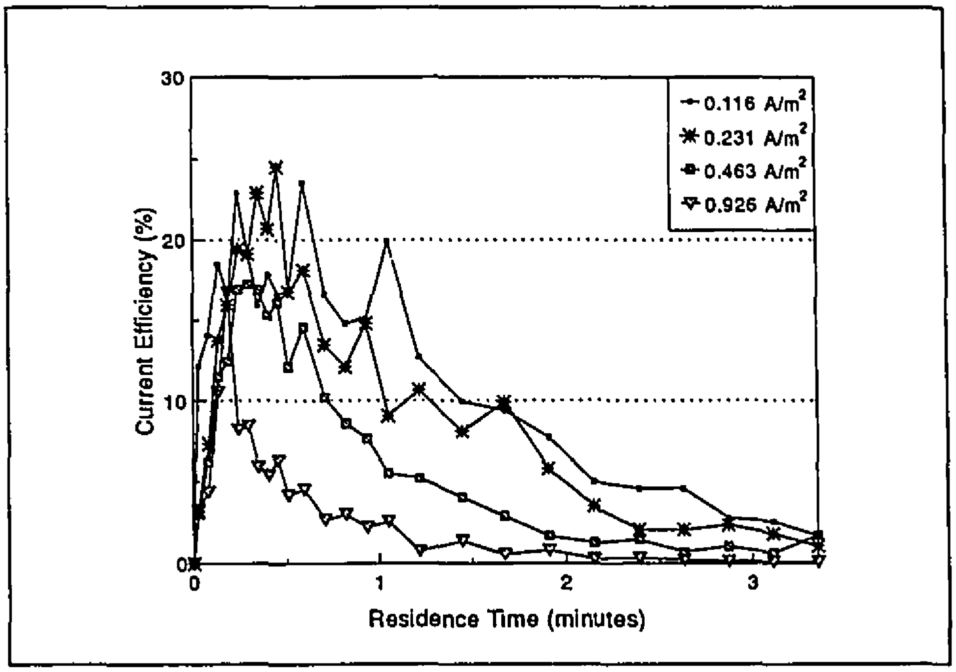

Figure 16: Current efficiency for the removal of dissolved oxygen in triple-strength lemon juice with respect to its residence time in the electrolytic cell

m is the theoretical mass of molecular oxygen converted into molecules of water (g) (Equation 9)

DO_t is the dissolved oxygen concentration in juice at time t (mg/L)

DO_{t-1} is the dissolved oxygen concentration in juice at time t-1 (mg/L)

V_{juice} is the amount of juice in the whole system at time t (L).

As shown in the graphs, there is a rapid and highly significant increase in the use of electrons to reduce oxygen in the first step of electrochemical processes, regardless of the current density. The maximum current efficiency attained is about 25%, occurring at a residence time of around 0.5 minute. At this moment, about 25% of electrons generated at the cathode are used to convert molecular oxygen dissolved in the juice into molecules of water (Equation 3). The remaining 75% may be used either to convert oxidized molecules or ions into their reduced forms (Equation 6), to generate hydrogen gas (Equation 4) or to precipitate metallic ions coming from the anode (Equation 5). Once the maximum current efficiency is reached, it decreases quadratically with respect to residence time. Soluble solids content does not seem to have any influence on current efficiency. Accordingly, the repeated measures ANOVA Table shown in Table 2 of Appendix 1 reveals no significant effect of soluble solids content on current efficiency at the .05 level, neither linearly nor quadratically. On the other hand, the current density applied to the electrolytic cell has an important effect on current efficiency. Although higher removal rates of dissolved oxygen are observed at higher current densities (Figures 10 to 12), higher current efficiencies are achieved with lower current densities. The statistical analysis indicates both significant linear and quadratic effects of current density on current efficiency at the .05 level, lower current densities being more efficient in reducing levels of dissolved oxygen compared to higher ones. This suggests that once there are electrons available in the system, the conversion of molecular oxygen into molecules of water would be a question of time rather than of the quantity of electrons available.

4.1.2 Redox Potential

Figures 17 to 19 respectively show the oxidation-reduction potential of a single-strength, a double-strength and a triple-strength lemon juice with respect to their residence times in the electrolytic cell. Initial redox potential values average 160 mV, indicating that there is a lot of species in their oxidized states in juice products. As shown in the graphs, there is a rapid and significant drop of redox potential in the first part of the electrolytic treatments regardless of soluble solids content and current density. Such a phenomenon was expected since a great number of electrons coming from the external circuit are suddenly available to species with high electron affinity in juice products. By taking on electrons, these species are then reduced, lowering the redox potential of the system. The extent of this rapid drop seems to be influenced by the current density applied. In fact, it can be noticed that lower redox potentials are attained at higher current densities. As the process goes on, redox potential decreases quadratically with respect to residence time. After the initial rapid drop, the rate at which redox potential diminishes is independent of the current density. Accordingly,

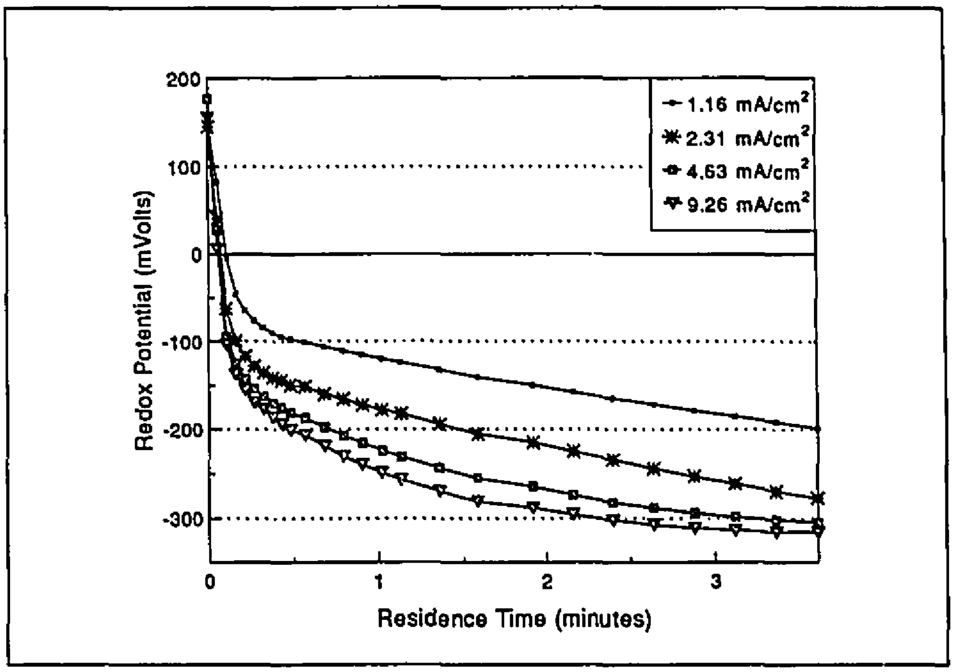

Figure 17: Redox potential of a single-strength lemon juice with respect to its residence time in the electrolytic cell

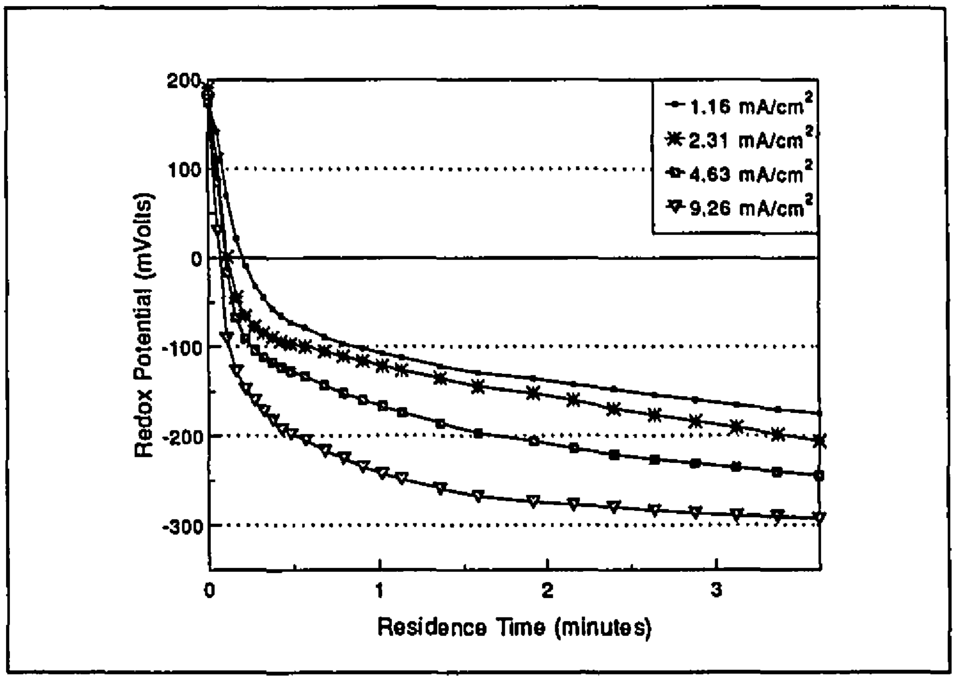

Figure 18: Redox potential of a double-strength lemon juice with respect to its residence time in the electrolytic cell

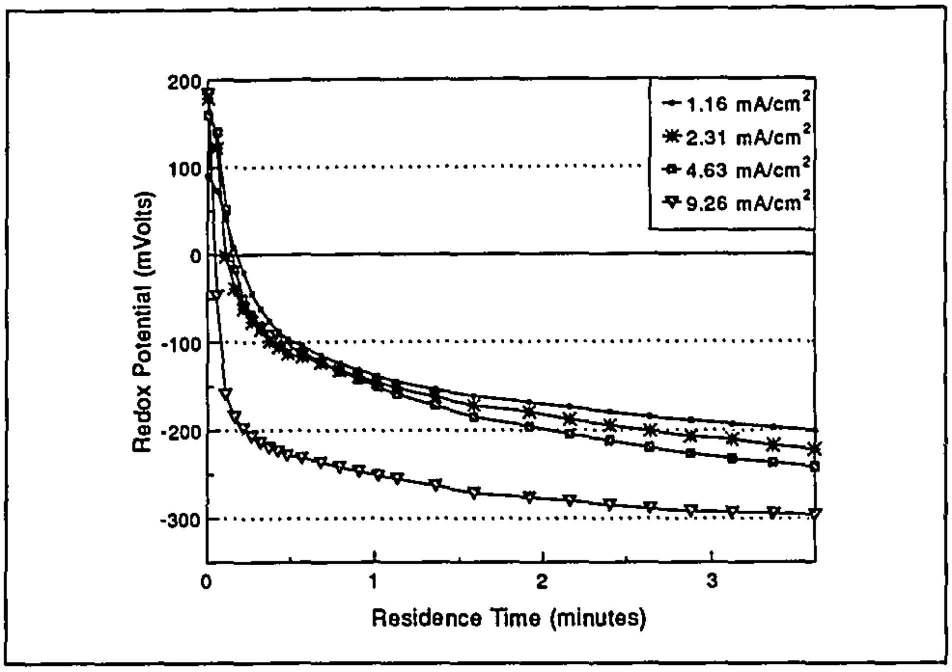

Figure 19: Redox potential in a triple-strength lemon juice with respect to its residence time in the electrolytic cell

the repeated measures ANOVA Table shown in Table 3 of Appendix 1 reveals no significant effect of current density on redox potential at the .05 level. This suggests that once species with electron affinity are reduced in the first step of the treatment, changes in redox potential seem to be a question of time rather than of the number of electrons available in the system.

4.1.3 Conductivity

Conductivity in juice products was measured during each of the electrochemical treatments. Initial conductivities for a single-strength, a double-strength and a triple-strength lemon juice ranged from 4.85 to 5.16, 6.29 to 6.51 and 6.12 to 6.73 mmho respectively. For comparison purposes, changes in conductivity throughout the treatments were considered for analysis and discussion. Figures 20 to 22 show conductivity changes in a single-strength, a double-strength and a triple-strength lemon juice with respect to their residence times in the electrolytic cell; each curve

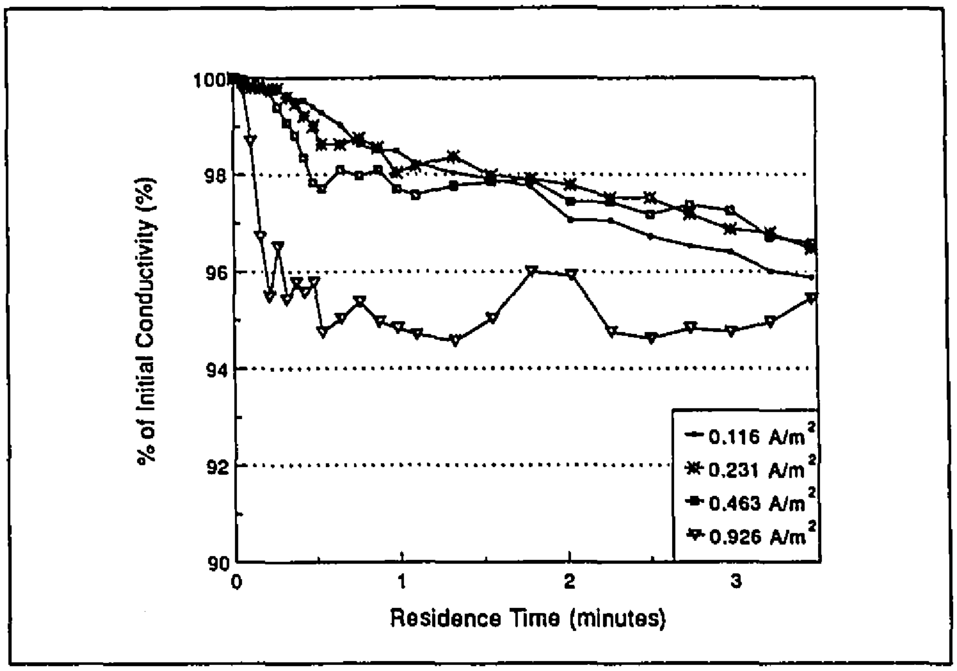

Figure 20: Change in conductivity in a single-strength lemon juice with respect to its residence time in the electrolytic cell

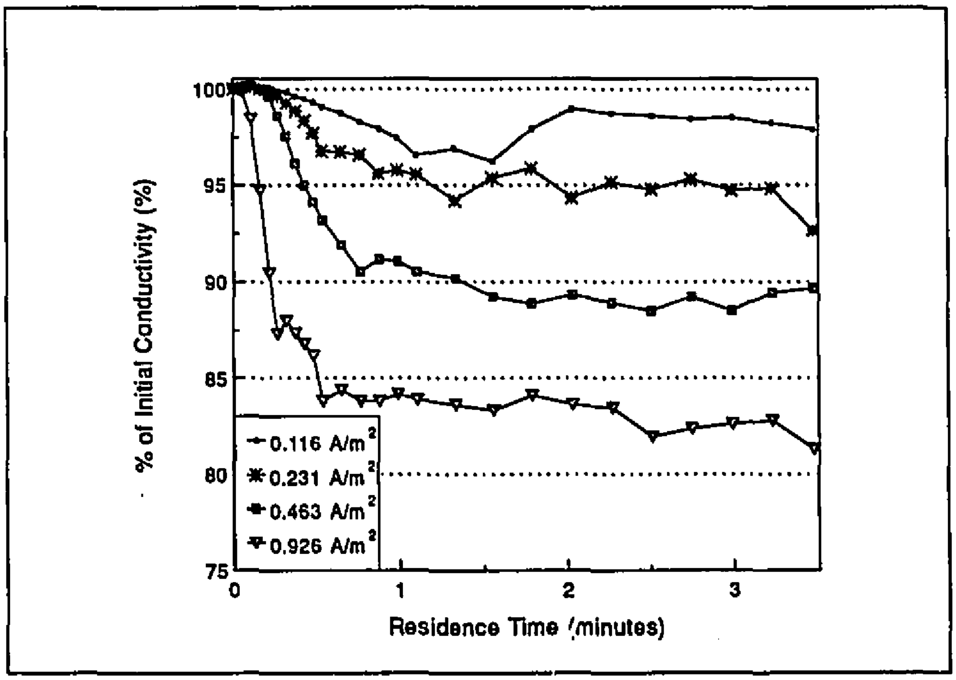

Figure 21: Change in conductivity in a double-strength lemon juice with respect to its residence time in the electrolytic cell

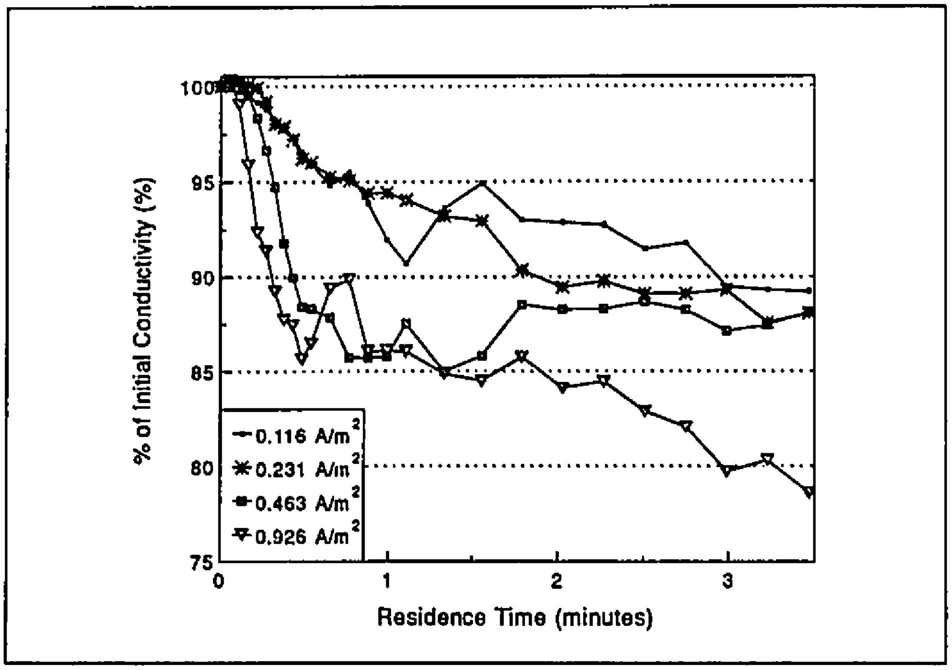

Figure 22: Change in conductivity in a triple-strength lemon juice with respect to its residence time in the electrolytic cell

representing the average of three replicates. Expressed in percent of initial conductivity, changes in conductivity at time t (CC_t) were computed from the initial and actual values throughout the process as follows:

$$CC_t = (K_t / K_0) * 100 \qquad (12)$$

where;

K_t is the actual conductivity of the medium at time t (mmho)

K_0 is the initial conductivity of juice product (mmho).

Looking at the charts, a slight drop in conductivity may be noticed throughout the electrochemical process and its extent seems to depend on both amount of soluble solids and current density applied to the system. However, according to the statistical analysis shown in Table 4 of Appendix 1, conductivity is independent of residence time, soluble solids content, current density and the cross-products at the .05 level. Although insignificant, this drop in conductivity is rather difficult to explain. In fact, a rise was theoretically expected since hydrogen ions migrate through the cationic membrane during the electrochemical process, thus increasing both conductivity and acidity of juice products. Such a drop in conductivity might be explained by the accumulation of conductive species on the negative electrode along the process. In fact, when a voltage is applied to the electrodes, conductive species (positively charged) are attracted by the cathode and could stick to it during the electrochemical treatment, thus reducing juice conductivity. Greater drops in conductivity observed at higher current densities support the above hypothesis.

4.1.4 Voltage

Figures 23 to 25 show the voltage applied to the electrodes to maintain a given current density through the electrolytic cell with respect to the residence time of a single-strength, a double-strength and a triple-strength lemon juice. Each curve represents the average of triplicates. As shown, a slight increase in voltage may be noticed over the electrochemical treatments. Although the repeated measures ANOVA

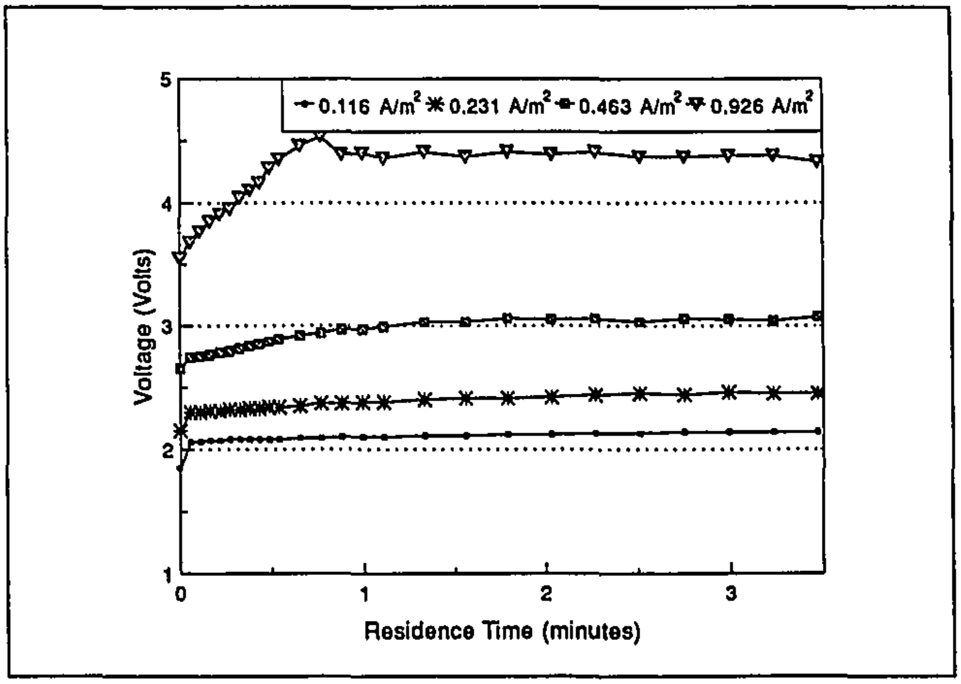

Figure 23: Voltage applied to the electrodes for a single-strength lemon juice with respect to its residence time in the electrolytic cell

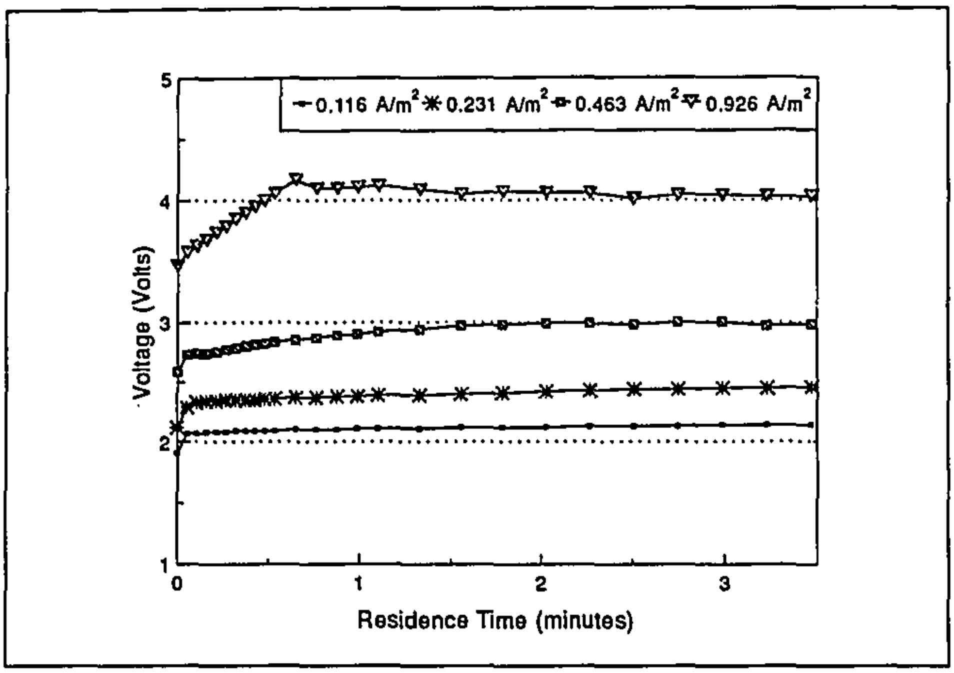

Figure 24: Voltage applied to the electrodes for a double-strength lemon juice with respect to its residence time in the electrolytic cell

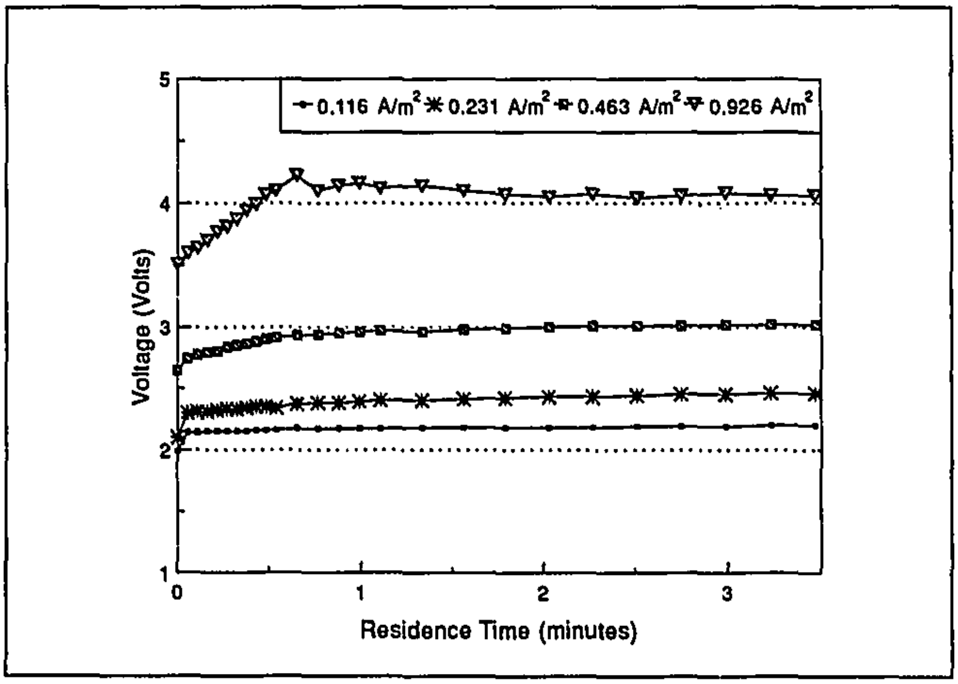

Figure 25: Voltage applied to the electrodes for a triple-strength lemon juice with respect to its residence time in the electrolytic cell

Table shown in Table 5 of Appendix 1 indicates that the rise in voltage with respect to residence time is not significant at the .05 level. This observation is in agreement with the progressive decrease in conductivity of juice products during the process since voltage is inversely proportional to the conductivity of the medium (Ohm's law).

On the other hand, soluble solids content has a major influence on the potential difference applied to the electrodes required to maintain a given current density through the cell. Voltage was found to be both linearly and quadratically affected by the amount of soluble solids in juice products at the .05 level. Such a relationship was expected since conductivity of juice products increases with soluble solids content until viscosity becomes a limiting factor to electron circulation. Similarly, current density was found to have both significant linear and quadratic effects on voltage at the .05 level. Contrary to the quadratic one, the linear effect was expected since Ohm's law reveals a direct correlation between voltage and the current passing through a system.

4.1.5 pH

Initial acidity levels ranged from 2.18 to 2.30 for single-strength lemon juices, from 1.94 to 2.19 for double-strength lemon juices and from 1.85 to 2.14 for triple-strength lemon juices. After 30 minutes of treatment, pH values were recorded for each electrochemical process. Although not graphically illustrated, a slight pH drop was noticed during the treatments. Such a rise in acidity in juice products was expected since hydrogen ions in the electrolyte solution pass through the cationic membrane during the electrochemical process. The pH drop over the treatment is not however significant at the .05 level. Based on the difference between initial and final pH values, the statistical analysis shown in Table 6 of Appendix 1 indicates that neither soluble solids content, current density nor cross-products have significant effects on pH change at the .05 level. In other words, subjecting a single-strength, a double-strength or a triple-strength lemon juice to the current densities used for 3.5 minutes does not seem to significantly change the acidity level in juice products. These findings are in agreement with the work published by Hekal (1983) who did not observe any pH change when treating a single-strength orange juice for 3.5 minutes at a current density of 0.31 A/m^2.

4.2 Browning Analysis

Results obtained from the browning analysis of juice samples subjected to different electrochemical treatments and stored for one month at 30°C will be discussed in the following paragraphs. Three different methods were used to measure quality alteration in lemon juice products over the storage period. As suggested by Robertson and Samaniego (1986), browning index, furfural content and HMF content in juices were used to evaluate quality deterioration in lemon juices during storage.

4.2.1 Browning Index

Figure 26 shows the browning indices of a single-strength, a double-strength and a triple-strength lemon juice with respect to their residence times in the electrolytic cell after one month of storage at 30°C. Each curve represents the average of triplicates. Notice that browning indices are expressed on a single-strength basis for comparison purposes. It should be noted that lemon juices used to perform the electrochemical treatments had browning indices averaging of 0.097 on a single-strength basis. Looking at the graph, the times the juice products spent in the electrolytic cell did not seem to influence their browning indices. Accordingly, the repeated measures ANOVA Table shown in Table 7 of Appendix 1 indicates no significant effect of residence time on browning index at the .05 level. Similarly, the current density applied to the system was found to have insignificant effect in preventing discolouration in lemon juices at the .05 level, neither linearly nor quadratically. Therefore, none of the electrochemical treatments significantly retarded discolouration in lemon juice products, regardless of the current density applied and the time the juice spent in the electrolytic cell.

Such results were rather unexpected based on what it has been published in the literature. The results obtained in this study in fact suggest that colour stability in lemon juices is not significantly influenced by dissolved oxygen concentration and redox

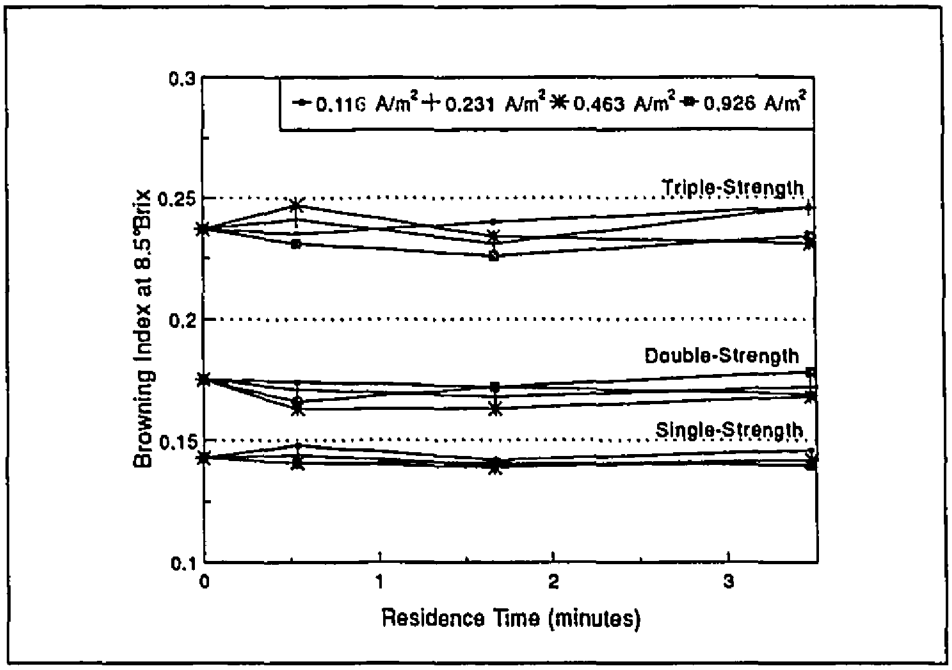

Figure 26: Browning indices of a single-strength, a double-strength and a triple-strength lemon juice after 1 month of storage at 30°C with respect to their residence times in the electrolytic cell

potential. Since juice products subjected to different current densities for various residence times cover a wide range of dissolved oxygen concentrations and redox potentials (Figures 10 to 12, 17 to 19), it would have been expected to observe significant colour differences between samples. Although not much has been published on the effect of redox potential on colour stability in citrus juice products, many researchers have noticed the adverse effect of oxygen on browning. Storing single-strength orange juice for 5 months at 22°C, Trammell and coworkers (1986) observed that browning was linearly related to the initial oxygen concentration. In addition, Robertson and Samaniego (1986) reported that the initial dissolved oxygen level influenced the lag period before browning became evident in single-strength lemon juice, shorter lag periods occurring at higher levels of dissolved oxygen. Despite the differences in lag period, they found the rate of browning to be the same during the post-lag period for all oxygen levels studied. One should however notice the rather narrow range of oxygen levels used in their experiment (0.41 to 3.74 mg/L). On the other hand, Kacem and coworkers (1987b) observed results that are in agreement with the ones obtained in the present study. In fact, storing orange juice products for 16 weeks at 24°C, they noticed insignificant change in browning between samples stored under aerobic and anaerobic conditions.

Looking at the graph, soluble solids content in juice products was found to have a major effect on discolouration in lemon juices. According to the statistical analysis, browning index increases quadratically with respect to the level of soluble solids in juices. Studying the effect of soluble solids content on browning index in lemon juices, Berk and Korner (1984) observed the same trend and suggested that the mechanism of browning may be different at higher concentrations. Similar results were reported by Kanner et al. (1982) with orange juices.

4.2.2 Concentrations in Furfural and HMF

Figures 27 and 28 respectively show furfural content and HMF content in a single-strength, a double-strength and a triple-strength lemon juice with respect to their residence times in the electrolytic cell after one month of storage at 30°C. Each curve represents the average of triplicates. It should be noted that insignificant levels of both furfural and HMF were initially found in juice products used to perform the electrochemical treatments. Looking at the graphs, soluble solids content has a significant effect on the accumulation of both furfural and HMF in juices, higher furfural and HMF contents being found in more concentrated juices. Accordingly, the repeated measures ANOVA Tables shown in Tables 8 and 9 of Appendix 1 respectively indicate that the level of soluble solids in juices has both significant linear and quadratic effects on furfural content and a significant quadratic effect on HMF concentration at the .05 level. Since furfural and HMF concentrations are suitable chemical indices of quality deterioration in lemon juices (Robertson and Samaniego, 1986), more concentrated juices showed greater quality loss. The same conclusion was drawn from the results obtained from browning index. Saguy et al. (1978) reported that the degradation rate of ascorbic acid increases with soluble solids content in grapefruit

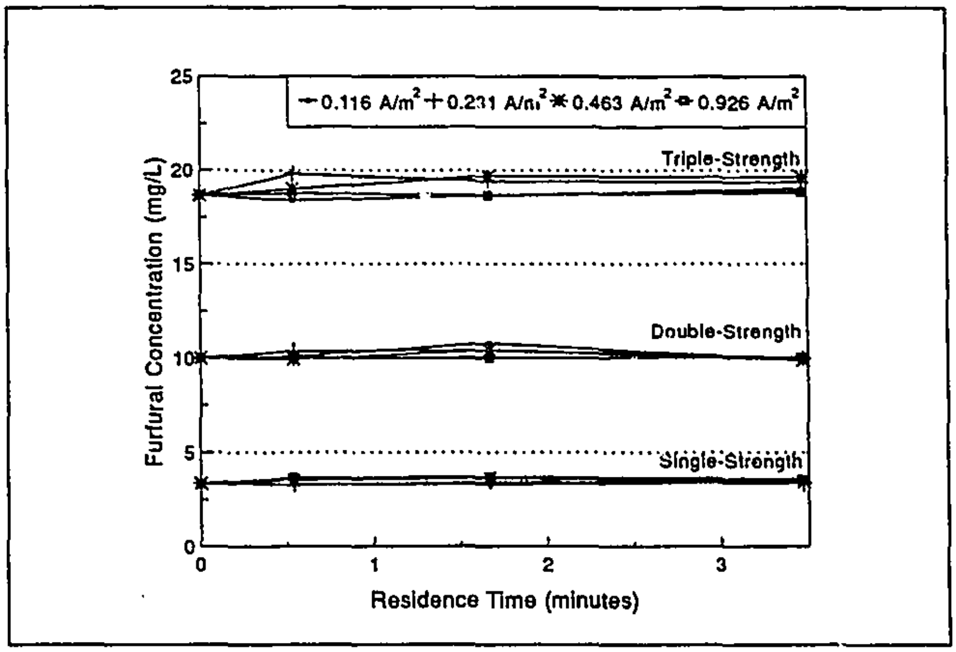

Figure 27: Concentrations in furfural in a single-strength, a double-strength and a triple-strength lemon juice after 1 month of storage at 30°C with respect to their residence times in the electrolytic cell

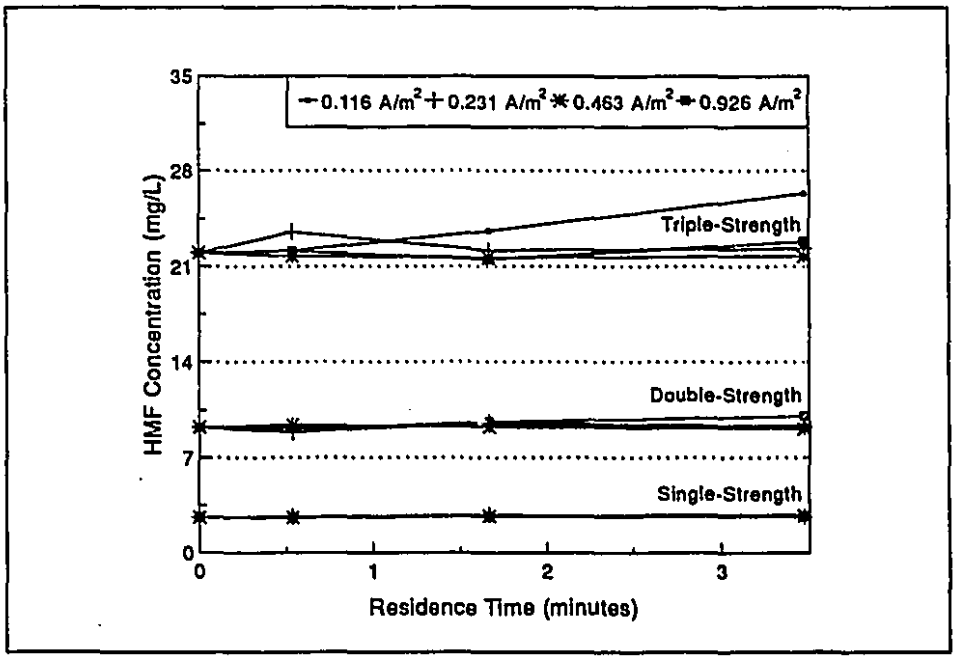

Figure 28: Concentrations in HMF in a single-strength, a double-strength and a triple-strength lemon juice after 1 month of storage at 30°C with respect to their residence times in the electrolytic cell

juices. This is in agreement with the results obtained in the present study considering that furfural is a breakdown product of vitamin C. Similar results were obtained by Kanner et al. (1982) for orange juice products. On the other hand, Robertson and Samaniego-Esguerra (1990) observed smaller ascorbic acid loss as soluble solids content increases in lemon juices.

Looking at the graphs, concentrations in both furfural and HMF seem to be independent of the time the juice spent in the electrolytic cell. Accordingly, the statistical analysis reveals neither significant linear nor quadratic effect of residence time on furfural and HMF contents at the .05 level. Similarly, the current density applied to the system was found to have no significant effect on the accumulation of both furfural and HMF in lemon juices at the .05 level. Therefore, none of the electrochemical treatments significantly retarded the accumulation of either furfural or HMF in lemon juices. The accumulation of furfural and HMF in stored lemon juices would then be independent of the initial dissolved oxygen concentration and redox potential.

Differences in accumulations of furfural and HMF were expected to be found among samples subjected to different electrochemical treatments. Although the effect of redox potential on furfural and HMF accumulations in citrus juice products has not been intensively studied, initial level of dissolved oxygen has been reported in the literature to play a major role in the formation of furfural derivatives. First, Trammell et al. (1986) observed a linear relationship between losses in ascorbic acid and initial oxygen concentrations in single-strength orange juice. Similarly, Khan and Martell (1967) indicated that destruction rates of vitamin C in model systems were directly proportional to the initial oxygen level. Since furfural is a breakdown product of vitamin C (Kanner et al., 1982), dissolved oxygen level would be expected to have a marked effect on the accumulation of furfural. In addition, Kacem and coworkers (1987b) observed that deaeration resulted in increased retention of ascorbic acid in single-strength orange juice. On the other hand, Robertson and Samaniego (1986) reported results that are in agreement with the ones obtained in the present work. They in fact noticed that furfural accumulation and the rate of ascorbic acid degradation in single-strength lemon juice is not related to its initial oxygen level.

Results obtained from the browning analysis suggest that the electrochemical treatments performed did not prevent quality alteration in lemon juice products. After one month of storage at 30°C, no significant difference in colour stability and accumulation of furfural derivatives was in fact noticed between controls and samples subjected to the various electrochemical treatments. Such a conclusion is rather surprising considering that juice samples taken during the electrochemical processes cover a wide range of redox potentials and initial dissolved oxygen levels. Redox potential and initial dissolved oxygen concentration do not seem to have any effect on colour stability and furfural derivatives accumulation in lemon juice products while in storage. Therefore, nonenzymatic browning in lemon juices may be due to factors other than oxidative reactions.

V. Summary and Conclusions

Citrus juice products undergo nonenzymatic browning, leading to serious deterioration in quality. Chemical inhibitors have been developed by researchers to prevent the occurrence of detrimental reactions in citrus juices. However, alternatives are still needed since serious health problems have arisen from their usage and recent bans established by the Food and Drug Administration have restricted the use of chemical additives in foods.

The present study investigated the potential of an electrochemical process to prevent nonenzymatic browning in citrus juice products. A set of experiments was performed in which single-strength, double-strength and triple-strength lemon juices were subjected to four different current densities for various periods of time using an electrolytic cell.

All electrochemical treatments significantly reduce the amount of oxygen dissolved in juice products. Dissolved oxygen concentration progressively diminished during the electrochemical treatments, reaching values as low as 0.1 mg/L for current densities of 0.926 A/m^2. It was also noticed that higher current densities resulted in faster removal rates of oxygen in lemon juices. However, the proportion of electrons used to remove oxygen was found to be lower for higher current densities, suggesting that lower current densities are more efficient than higher ones in reducing molecular oxygen in molecules of water. It should be noted that the highest current efficiency achieved throughout the process was about 25% and occurred at a residence time of about 0.5 minute. This suggests that most of the electrons generated at the cathode are either used to reduce oxidized molecules, generate hydrogen gas or precipitate metallic ions coming from the anode.

It was also found that the redox potential of lemon juices was significantly affected by the electrochemical treatments. An initial rapid drop was noticed, the extent being proportional to the current density applied to the system, followed by a more or less constantly decreasing rate, regardless of current density. This suggests that once molecules with electron affinity are reduced, the conversion of oxidized molecules into their reduced states seems to be time dependent rather than dependent on the availability of electrons in the system.

As a result of the electrochemical process, intrinsic properties of lemon juice were slightly affected, although not significantly. Conductivity of lemon juice products decreases slightly throughout the treatment. In agreement with this drop in conductivity, the voltage required at the electrodes to maintain a constant current density increased slightly during the process. This is rather difficult to explain since hydrogen ions from the electrolyte solution migrate through the cationic membrane during the treatment, which would contribute to diminishing the electrical resistance of lemon juices. This transport phenomenon must have occurred since a slight rise in acidity was noticed in lemon juice. The drop in conductivity might be due to the fact that conductive species (positively charged) in lemon juices are attracted by the cathode and could stick to it when a voltage is applied to the system.

Regarding the storability of lemon juice products that have been subjected to different electrochemical treatments, the three methods used to quantify deterioration in quality in lemon juices (browning index and concentrations in furfural and HMF) revealed that detrimental reactions in lemon juices are independent of the current density applied to the system as well as of the time the juice spent in the electrolytic cell. The only parameter found to have a significant effect was the amount of soluble solids in juices. Quality deterioration increases with higher concentrations in solids. Since lemon juices that have been subjected to different electrochemical treatments are characterized by a wide range of initial dissolved oxygen levels and redox potentials, these two parameters do not seem to have any effect on browning and quality preservation. Thus, the present study suggests that quality adulteration in lemon juice products would not be predominantly due to oxidative reactions.

VI. Recommendations

Several recommendations can be formulated to guide further research on the subject. Although the use of an electrolytic cell was not found to significantly retard quality deterioration in lemon juices made from concentrate, conclusions might be different for other types of juices. Accordingly, it might be interesting to repeat the experimental work performed in this study for different types of juice, especially freshly-squeezed juices. Irreversible detrimental reactions that were possibly initiated during the concentration process might have played a major role in the development of nonenzymatic browning reactions. Starting with freshly-squeezed juices, this hypothesis would be elucidated.

Regarding the electrochemical treatment itself, the effect of several operational conditions on intrinsic properties of juices would have to be determined. In fact, the contribution of turbulence level at the electrode surface on redox potential, pH, conductivity and the removal rate of dissolved oxygen should be looked at. Since only one type of spacer was used and since the flow rates of both fluids were kept constant for all electrochemical treatments, possible relations of level of turbulence and reaction rate at the electrode surface remain unknown. In addition, the effects of juice viscosity and operating temperature should be investigated to provide a better understanding of the reaction kinetics at the electrodes. It might also be interesting to look at the effect of different electrode materials and types of cationic membrane separating the two compartments of the electrolytic cell. Since each material has its own electrochemical properties, the electrode material would certainly affect the generation rate of electrons at the cathode. On the other hand, the type of partition-wall between the anode and cathode compartments should have an impact on the mass transfer between the two since each membrane has its own selectivity.

Finally, the rate of production of hydrogen gas on the cathode side might be of particular interest. This measurement would give more details on the use of electrons generated at the cathode.

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Appendices

Appendix 1: ANOVA Tables obtained from the Statistical Analysis of Dependent Variables

Table 1: Repeated Measures Analysis of Variance for Residual Dissolved Oxygen

Source*	df	SS	MS	F	Pr > F
TSS	1	5501.26	5501.26	20.60	0.0001
TSS^2	1	3857.18	3857.18	14.44	0.0009
CD	1	16390.10	16390.10	61.38	0.0001
CD^2	1	3156.67	3156.67	11.82	0.0021
TSS * CD	1	3285.61	3285.61	12.30	0.0018
Error #1	24	6408.93	267.04		
RT	25	10264.36	410.57	59.51	0.0001
RT * TSS	25	980.69	39.23	5.69	0.0018
RT * CD	25	6957.50	278.30	40.34	0.0001
RT * TSS * CD	25	833.11	33.32	4.83	0.0047
RT * TSS^2	25	656.98	26.28	3.81	0.0151
RT * CD^2	25	4204.62	168.19	24.38	0.0001
Error #2	600	4139.49	6.90		

*TSS : Amount of total soluble solids in juice product
CD : Current density applied to the electrolytic cell
RT : Residence time the juice product spent in the electrolytic cell
df : Degrees of freedom
SS : Sum of squares
MS : Mean square
F : MS/MS error
Pr > F : Probability to have a larger F value

Table 2: Repeated Measures Analysis of Variance for Current Efficiency

Source[*]	df	SS	MS	F	Pr > F
TSS	1	303.52	303.52	0.84	0.3684
TSS^2	1	346.11	346.11	0.96	0.3374
CD	1	11186.99	11186.99	30.97	0.0001
CD^2	1	6926.47	6926.47	19.18	0.0002
TSS * CD	1	97.47	97.47	0.27	0.6082
Error #1	24	8668.43	361.18		
RT	24	3733.74	155.57	1.86	0.1310
RT * TSS	24	2234.37	93.10	1.11	0.3529
RT * CD	24	25610.21	1067.09	12.77	0.0001
RT * TSS * CD	24	6529.83	272.08	3.26	0.0187
RT * TSS^2	24	1536.95	64.04	0.77	0.5380
RT * CD^2	24	20189.35	841.22	10.07	0.0001
Error #2	576	48134.09	83.57		

[*] TSS : Amount of total soluble solids in juice product
CD : Current density applied to the electrolytic cell
RT : Residence time the juice product spent in the electrolytic cell
df : Degrees of freedom
SS : Sum of squares
MS : Mean square
F : MS/MS error
Pr > F : Probability to have a larger F value

Table 3: Repeated Measures Analysis of Variance for Redox Potential

Source*	df	SS	MS	F	Pr > F
TSS	1	242900.95	242900.95	4.92	0.0354
TSS^2	1	180378.00	180378.00	3.66	0.0669
CD	1	34337.88	34337.88	0.70	0.4117
CD^2	1	224.46	224.46	0.00	0.9467
TSS * CD	1	8606.94	8606.94	0.17	0.6796
Error #1	24	1282463.27	49325.51		
RT	25	55134.88	2205.40	4.53	0.0085
RT * TSS	25	10262.91	410.52	0.84	0.4603
RT * CD	25	40415.80	1616.63	3.32	0.0310
RT * TSS * CD	25	34293.94	1371.76	2.82	0.0534
RT * TSS^2	25	3843.43	153.74	0.32	0.7839
RT * CD^2	25	30250.28	1210.01	2.48	0.0768
Error #2	650	316504.33	486.93		

*TSS : Amount of total soluble solids in juice product
CD : Current density applied to the electrolytic cell
RT : Residence time the juice product spent in the electrolytic cell
df : Degrees of freedom
SS : Sum of squares
MS : Mean square
F : MS/MS error
Pr > F : Probability to have a larger F value

Table 4: Repeated Measures Analysis of Variance for Change in Conductivity

Source*	df	SS	MS	F	Pr > F
TSS	1	397.87	397.87	1.47	0.2363
TSS^2	1	181.96	181.96	0.67	0.4198
CD	1	102.08	102.08	0.38	0.5446
CD^2	1	19.20	19.20	0.07	0.7921
TSS * CD	1	313.45	313.45	1.16	0.2918
Error #1	26	7040.21	270.78		
RT	25	200.61	8.02	1.11	0.3561
RT * TSS	25	152.03	6.08	0.84	0.4964
RT * CD	25	234.82	9.39	1.30	0.2785
RT * TSS * CD	25	145.59	5.82	0.80	0.5178
RT * TSS^2	25	83.83	3.35	0.46	0.7489
RT * CD^2	25	244.64	9.79	1.35	0.2591
Error #2	650	4712.53	7.25		

*TSS : Amount of total soluble solids in juice product
CD : Current density applied to the electrolytic cell
RT : Residence time the juice product spent in the electrolytic cell
df : Degrees of freedom
SS : Sum of squares
MS : Mean square
F : MS/MS error
Pr > F : Probability to have a larger F value

Table 5: Repeated Measures Analysis of Variance for Voltage

Source*	df	SS	MS	F	Pr > F
TSS	1	0.302	0.302	6.08	0.0201
TSS^2	1	0.583	0.583	11.72	0.0019
CD	1	16.609	16.609	333.85	0.0001
CD^2	1	0.287	0.287	5.77	0.0232
TSS * CD	1	1.681	1.681	33.79	0.0001
Error #1	28	1.393	0.050		
RT	25	0.042	0.002	1.47	0.1990
RT * TSS	25	0.041	0.002	1.44	0.2099
RT * CD	25	0.471	0.019	16.56	0.0001
RT * TSS * CD	25	0.256	0.010	8.97	0.0001
RT * TSS^2	25	0.060	0.002	2.09	0.0634
RT * CD^2	25	0.313	0.013	11.00	0.0001
Error #2	700	0.796	0.001		

*TSS : Amount of total soluble solids in juice product
CD : Current density applied to the electrolytic cell
RT : Residence time the juice product spent in the electrolytic cell
df : Degrees of freedom
SS : Sum of squares
MS : Mean square
F : MS/MS error
Pr > F : Probability to have a larger F value

Table 6: Analysis of Variance for pH Difference between initial and final values

Source*	df	SS	MS	F	Pr > F
Corrected Total	35	0.040097			
TSS	2	0.001356	0.000678	0.59	0.5633
CD	3	0.003942	0.001314	1.14	0.3531
TSS * CD	6	0.007133	0.001189	1.03	0.4295
Error	24	0.027667	0.001153		

*TSS : Amount of total soluble solids in juice product
CD : Current density applied to the electrolytic cell
df : Degrees of freedom
SS : Sum of squares
MS : Mean square
F : MS/MS error
Pr > F : Probability to have a larger F value

Table 7: Repeated Measures Analysis of Variance for Browning Index

Source[*]	df	SS	MS	F	Pr > F
TSS	1	0.00125	0.00125	10.03	0.0035
TSS^2	1	0.01098	0.01098	487.99	0.0001
CD	1	0.00028	0.00028	2.21	0.1475
CD^2	1	0.00037	0.00037	2.95	0.0960
TSS * CD	1	0.00005	0.00005	0.43	0.5166
Error #1	30	0.00374	0.00012		
RT	3	0.00009	0.00003	0.64	0.5914
RT * TSS	3	0.00023	0.00008	1.57	0.2112
RT * CD	3	0.00013	0.00004	0.86	0.4649
RT * TSS * CD	3	0.00009	0.00003	0.63	0.5963
RT * TSS^2	3	0.00025	0.00008	1.70	0.1720
RT * CD^2	3	0.00022	0.00007	1.49	0.2239
Error #2	90	0.00437	0.00005		

[*] TSS : Amount of total soluble solids in juice product
CD : Current density applied to the electrolytic cell
RT : Residence time the juice product spent in the electrolytic cell
df : Degrees of freedom
SS : Sum of squares
MS : Mean square
F : MS/MS error
Pr > F : Probability to have a larger F value

Table 8: Repeated Measures Analysis of Variance for Furfural Concentration

Source*	df	SS	MS	F	Pr > F
TSS	1	22.728	22.728	28.81	0.0001
TSS^2	1	39.101	39.101	39.10	0.0001
CD	1	2.528	2.528	3.20	0.0835
CD^2	1	1.748	1.748	2.22	0.1470
TSS * CD	1	0.778	0.778	0.99	0.3286
Error #1	30	23.666	0.789		
RT	3	0.583	0.194	0.96	0.4171
RT * TSS	3	0.853	0.284	1.40	0.2482
RT * CD	3	0.103	0.034	0.17	0.9174
RT * TSS * CD	3	0.110	0.037	0.18	0.9094
RT * TSS^2	3	1.015	0.338	1.67	0.1801
RT * CD^2	3	0.147	0.049	0.24	0.8671
Error #2	90	18.28	0.203		

*TSS : Amount of total soluble solids in juice product
CD : Current density applied to the electrolytic cell
RT : Residence time the juice product spent in the electrolytic cell
df : Degrees of freedom
SS : Sum of squares
MS : Mean square
F : MS/MS error
Pr > F : Probability to have a larger F value

Table 9: Repeated Measures Analysis of Variance for HMF Concentration

Source*	df	SS	MS	F	Pr > F
TSS	1	14.543	14.543	2.65	0.1142
TSS^2	1	336.476	336.476	61.23	0.0001
CD	1	5.538	5.538	1.01	0.3235
CD^2	1	11.133	11.133	2.03	0.1650
TSS * CD	1	3.942	3.942	0.72	0.4037
Error #1	30	164.859	5.495		
RT	3	0.819	0.273	0.32	0.8140
RT * TSS	3	0.710	0.237	0.27	0.8443
RT * CD	3	3.808	1.269	1.47	0.2287
RT * TSS * CD	3	2.506	0.835	0.97	0.4125
RT * TSS^2	3	1.535	0.512	0.59	0.6222
RT * CD^2	3	5.726	1.909	2.21	0.0927
Error #2	90	77.841	0.865		

*TSS : Amount of total soluble solids in juice product
CD : Current density applied to the electrolytic cell
RT : Residence time the juice product spent in the electrolytic cell
df : Degrees of freedom
SS : Sum of squares
MS : Mean square
F : MS/MS error
Pr > F : Probability to have a larger F value

Appendix 2: Statistical Models obtained for the Different Dependent Variables

Table 1: Estimates of Regression Coefficients and Standard Errors of the Statistical Model obtained for Residual Dissolved Oxygen in Lemon Juices

i	Term*	Estimate	Standard Error
0	Intercept	114.05016	0.77538
1	TSS * CD	-3.27010	0.38000
2	TSS^2 * CD	0.10820	0.01110
3	CD	-6.34310	3.12270
4	CD * RT	-46.58220	3.10857
5	CD * RT^2	11.33500	0.70200
6	CD^2 * RT	15.63200	1.91776
7	RT	-84.07829	1.94362
8	RT^2	32.07620	1.22717
9	RT^3	-4.73519	0.23476

* TSS : Amount of soluble solids in juice (°Brix)
CD : Current density applied to the electrolytic cell (A/m^2)
RT : Residence time the juice spent in the electrolytic cell (minute)

The residual dissolved oxygen (RDO) in lemon juice can be calculated from the following equation:

$$\mathbf{RDO}_{\text{TSS, CD, RT}} = \text{Intercept} + \sum_{i=1}^{9} \mathbf{(Estimate)}_i * \mathbf{(Term)}_i$$

The above statistical model has a r^2-value of 0.9685 and coefficient of variation of 21.17.

Table 2: Estimates of Regression Coefficients and Standard Errors of the Statistical Model obtained for Current Efficiency in Removing Dissolved Oxygen in Lemon Juices

i	Term*	Estimate	Standard Error
0	Intercept	18.30364	1.29744
1	CD * RT	-57.14710	11.87625
2	TSS^2 * CD	-13.01230	1.72030
3	CD * RT^2	4.79930	0.53943
4	CD^2	98.61900	29.67988
5	CD^3	-55.40000	19.84499
6	RT	18.41184	1.39355
7	RT^2	-14.98385	0.93084
8	RT^3	2.57130	0.18543

* TSS : Amount of soluble solids in juice (^{o}Brix)
CD : Current density applied to the electrolytic cell (A/m^2)
RT : Residence time the juice spent in the electrolytic cell (minute)

The current efficiency for the removal of molecular oxygen (CE) in lemon juice can be calculated from the following equation:

$$\mathbf{CE}_{\text{TSS, CD, RT}} = \text{Intercept} + \sum_{i=1}^{8} \mathbf{(Estimate)}_i * \mathbf{(Term)}_i$$

The above statistical model has a r^2-value of 0.6368 and coefficient of variation of 26.19.

Table 3: Estimates of Regression Coefficients and Standard Errors of the Statistical Model obtained for Redox Potential in Lemon Juices

i	Term*	Estimate	Standard Error
0	Intercept	-75.15381	15.61800
1	TSS	16.62811	2.03212
2	TSS * CD^2	-11.40545	1.46276
3	TSS^2	-0.47448	0.06358
4	TSS^2 * CD	0.29632	0.06033
5	CD * RT	-216.82797	26.61200
6	CD * RT^2	17.03731	6.35488
7	CD^2 * RT	145.63658	22.09952
8	RT	-366.94722	18.10508
9	RT^2	209.54981	11.97492
10	RT^3	-35.17741	2.30110

* TSS : Amount of soluble solids in juice (^{0}Brix)
CD : Current density applied to the electrolytic cell (A/m^2)
RT : Residence time the juice spent in the electrolytic cell (minute)

The redox potential in lemon juice can be calculated from the following equation:

$$\textbf{Redox potential}_{\text{TSS, CD, RT}} = \text{Intercept} + \sum_{i=1}^{10} \textbf{(Estimate)}_i * \textbf{(Term)}_i$$

The above statistical model has a r^2-value of 0.7329 and coefficient of variation of 45.85.

Table 4: Estimates of Regression Coefficients and Standard Errors of the Statistical Model obtained for Change in Conductivity in Lemon Juices

i	Term*	Estimate	Standard Error
0	Intercept	102.65167	0.46547
1	$TSS * CD$	-2.82253	0.23713
2	$TSS * CD * RT$	-0.14160	0.04466
3	$TSS * RT^2$	0.07232	0.01524
4	$TSS^2 * CD$	0.07815	0.00702
5	$TSS^2 * RT$	-0.00941	0.00125
6	CD	16.11954	1.99484
7	$CD * RT$	-3.99219	1.59392
8	$CD * RT^2$	1.53953	0.42144
9	RT	-6.99706	1.22524
10	RT^2	4.71051	0.78189
11	RT^3	-1.07293	0.14092

* TSS : Amount of soluble solids in juice (°Brix)
CD : Current density applied to the electrolytic cell (A/m^2)
RT : Residence time the juice spent in the electrolytic cell (minute)

The change in conductivity (CC) in lemon juices can be calculated from the following equation:

$$CC_{TSS, CD, RT} = \text{Intercept} + \sum_{i=1}^{11} (\textbf{Estimate})_i * (\textbf{Term})_i$$

The above statistical model has a r^2-value of 0.6570 and coefficient of variation of 45.75.

Table 5: Estimates of Regression Coefficients and Standard Errors of the Statistical Model obtained for Voltage applied to the electrodes

i	Term*	Estimate	Standard Error
0	Intercept	1.69442	0.03564
1	TSS	0.01358	0.00407
2	TSS * CD	-0.08081	0.00919
3	TSS * CD * RT	-0.00351	0.00058
4	TSS * CD^2	-0.01605	0.00501
5	TSS^2	-0.00028	0.00011
6	TSS^2 * CD	0.00238	0.00022
7	CD	2.14646	0.12202
8	CD * RT	0.84291	0.04502
9	CD * RT^2	-0.15986	0.00797
10	CD^2	0.73181	0.10006
11	CD^2 * RT	-0.15812	0.03281
12	RT	0.22683	0.02249
13	RT^2	-0.20105	0.01393
14	RT^3	0.04260	0.00266

* TSS : Amount of soluble solids in juice (^{o}Brix)
CD : Current density applied to the electrolytic cell (A/m^2)
RT : Residence time the juice spent in the electrolytic cell (minute)

The voltage applied to the electrodes can be calculated from the following equation:

$$\textbf{Voltage}_{\text{TSS, CD, RT}} = \text{Intercept} + \sum_{i=1}^{14} \textbf{(Estimate)}_i * \textbf{(Term)}_i$$

The above statistical model has a r^2-value of 0.9917 and coefficient of variation of 22.12.

Table 6: Estimates of Regression Coefficients and Standard Errors of the Statistical Model obtained for Browning Index in Lemon Juices

i	Term*	Estimate	Standard Error
0	Intercept	0.150417	0.005206
1	TSS	-0.003124	0.000695
2	TSS^2	0.000260	0.000020
3	$TSS^2 * CD$	-0.000012	0.000001

* TSS : Amount of soluble solids in juice (°Brix)
CD : Current density applied to the electrolytic cell (A/m^2)

The browning index (BI) in lemon juices after one month of storage at 30°C can be calculated from the following equation:

$$\mathbf{BI}_{TSS,\,CD} = \text{Intercept} + \sum_{i=1}^{3} \mathbf{(Estimate)}_i * \mathbf{(Term)}_i$$

The above statistical model has a r^2-value of 0.9592 and coefficient of variation of 4.16.

Table 7: Estimates of Regression Coefficients and Standard Errors of the Statistical Model obtained for Content in Furfural in Lemon Juices

i	Term*	Estimate	Standard Error
0	Intercept	-1.00510	0.37180
1	TSS	0.39383	0.04967
2	TSS^2	0.01530	0.00144

* TSS : Amount of soluble solids in juice (^{0}Brix)

Furfural concentration (mg/L) in lemon juices after one month of storage at 30°C can be calculated from the following equation:

$$\textbf{Furfural Content}_{TSS} = \text{Intercept} + \sum_{i=1}^{2} \textbf{(Estimate)}_i * \textbf{(Term)}_i$$

The above statistical model has a r^2-value of 0.9916 and coefficient of variation of 16.13.

Table 8: Estimates of Regression Coefficients and Standard Errors of the Statistical Model obtained for Content in HMF in Lemon Juices

i	Term*	Estimate	Standard Error
0	Intercept	2.47729	0.87682
1	TSS	-0.36025	0.11714
2	TSS^2	0.04420	0.00342
3	$TSS^2 * RT$	0.00048	0.00021

* TSS : Amount of soluble solids in juice (°Brix)
RT : Residence time the juice spent in the electrolytic cell (minute)

HMF concentration (mg/L) in lemon juices after one month of storage at 30°C can be calculated from the following equation:

$$\textbf{HMF Content}_{\text{TSS, RT}} = \text{Intercept} + \sum_{i=1}^{3} (\textbf{Estimate})_i * (\textbf{Term})_i$$

The above statistical model has a r^2-value of 0.9729 and coefficient of variation of 10.08.